stimulation (which on its own has little or no effect on synaptic plasticity) and then to test whether this changes the response to a second period of conditioning stimulation which produces LTP or LTD when given alone (Abraham, 2008). Many human studies have used designs in which both priming and conditioning are applied over the primary motor cortex (M1) (Iyer *et al.* 2003; Siebner *et al.* 2004; Lang *et al.* 2004; Müller *et al.* 2007; Hamada *et al.* 2008*a*). Others showed that metaplastic effects can be elicited by finger movements or voluntary muscle contraction, in place of priming stimulation, which might be associated with activity changes in cortical circuits within various motor-related areas (Ziemann *et al.* 2004; Gentner *et al.* 2008; Huang *et al.* 2008). More recently, Ragert *et al.* (2009) demonstrated the effects of priming stimulation over the right M1 on subsequent rTMS-induced plasticity of left M1 (Ragert *et al.* 2009). Of note is the fact that the effects of priming stimulation over motor-related areas other than ipsilateral or contralateral M1 have not been well documented, despite important anatomical and functional interplay between those areas and M1.

Among the motor-related areas, the lateral premotor cortex (PM) such as dorsal and ventral PM (PMd and PMv) has been extensively studied by means of TMS in humans to shed light on the interaction between M1 (see review by Reis *et al.* 2008). The supplementary motor area (SMA) also has dense cortico-cortical connections with M1 in animals (Dum & Strick, 1991; Luppino *et al.* 1993; Tokuno & Nambu, 2000) and plays a substantial role in higher motor control and learning (Tanji & Shima, 1994; Hikosaka *et al.* 1999; Nachev *et al.* 2008). However, as SMA is located in the interhemispheric fissure, it is a more difficult site to stimulate than the lateral PM (Reis *et al.* 2008). Thus, not much has been done in SMA as compared to PMd; two studies revealed that TMS over SMA can modulate the cortical excitability of M1 via cortico-cortical synaptic connections (Civardi *et al.* 2001; Matsunaga *et al.* 2005). In light of this accruing evidence, we aimed to explore the effects of preceding stimulation over SMA on subsequent rTMS-induced plasticity of the M1 in order to test the hypothesis that priming over the SMA modulates some cortical neurons within M1 via cortico-cortical connections, and that such prior history of neuronal activity alters subsequent rTMS-induced plasticity.

For priming and subsequent conditioning, we employed a new rTMS protocol, quadripulse stimulation (QPS) (Hamada *et al.* 2007, 2008*a*). QPS consists of repeated trains of four monophasic TMS pulses separated by inter-stimulus intervals (ISI) of 1.5–1250 ms, inducing bidirectional motor cortical plasticity in an ISI-dependent, non-linear form that are compatible with changes in synaptic plasticity. In addition, we showed that QPS interventions could interact in a metaplastic manner such that priming over M1 using QPS at short ISIs, which did not induce any plastic changes by itself, occluded subsequent LTP-like plasticity, whereas priming using QPS with long ISIs tended to do the reverse and increased the probability that facilitatory effects would be produced by a subsequent period of stimulation. The data support a BCM-like model of priming that shifts the crossover point at which the synaptic plasticity reverses from LTD to LTP. We proposed that such a broad range of after-effects produced by QPS facilitates detailed examinations of metaplasticity theories in humans (Hamada *et al.* 2008*a*). In the present study, we evaluated how LTD-like and LTP-like QPS-induced plasticity was altered by a preceding period of priming stimulation over SMA to understand metaplastic interplay between SMA and M1.

Methods

Subjects

Subjects were nine healthy volunteers (one woman, eight men; 27–45 years old, mean ± S.D., 34.5 ± 6.7 years) who gave their written informed consent to participate in the experiments. No subjects had neurological, psychiatric or other medical problems, or had any contraindication to TMS (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*. The procedures are in compliance with *The Journal of Physiology*'s guidelines for experimentation on humans (Drummond, 2009).

Recordings

Subjects were seated on a comfortable chair. The electromyogram (EMG) activity was recorded from the right first dorsal interosseous muscle (FDI) and the right tibialis anterior muscle (TA) using a belly-tendon arrangement. 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

Focal TMS was given using a hand-held figure-of-eight coil (9 cm external diameter at each wing; The Magstim Co. Ltd, Whitland, Dyfed, UK). Single monophasic TMS pulses were delivered by a magnetic stimulator (Magstim 200; The Magstim Co. Ltd). Quadripulse stimuli were delivered by four magnetic stimulators (Magstim 200^2; 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 from the right FDI muscle (i.e. hot spot for FDI) was determined before each experiment and considered to be the primary motor cortex for FDI muscle ($M1_{FDI}$). A figure-of-eight coil was placed tangentially over the scalp with the handle pointing backwards at about 45 deg laterally. We stimulated several positions in 1 cm increments in the anterio-posterior and medio-lateral direction from each other using the same intensity and determined $M1_{FDI}$ as the site at which the largest responses were elicited. This position was marked with a blue pen on the scalp for repositioning the coil. The resting motor threshold for FDI (RMT_{FDI}) was defined as the lowest intensity that evoked a response of at least 50 μV in the relaxed FDI in at least 5 of 10 consecutive trials (Rossini *et al.* 1994). The active motor threshold for FDI (AMT_{FDI}) was defined as the lowest intensity that evoked a small response (>100 μV) when the subjects maintained a slight contraction of the right FDI (~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 (MSO).

SMA stimulation was given with a coil centred at a point 3 cm anterior to the optimal site for eliciting MEPs in the right TA muscle (i.e. hot spot for TA) according to previous studies (Hikosaka *et al.* 1996; Lee *et al.* 1999; Terao *et al.* 2001; Matsunaga *et al.* 2005). The hot spot for TA was determined by moving the coil in 1 cm steps along the sagittal mid-line through the vertex (Cz) with the handle pointing to the right until we detected the position which evoked the largest MEP. This was considered to be the primary motor cortex for the TA muscle ($M1_{TA}$). We then used this position to determine the AMT for TA (AMT_{TA}). On average, the site of SMA stimulation (i.e. the point 3 cm anterior to $M1_{TA}$) was 2–3 cm anterior to Cz, in line with data from previous studies (Hikosaka *et al.* 1996; Lee *et al.* 1999; Matsunaga *et al.* 2005).

Measurement of motor cortical excitability

Motor cortical excitability was assessed by measuring the peak-to-peak amplitude of MEPs from the relaxed right FDI muscle elicited by single pulse TMS over the left $M1_{FDI}$ for all experiments. The stimulus intensity was adjusted to produce MEPs of about 0.5 mV in the right FDI muscle. During the experiments, EMG activity of the FDI was monitored with an oscilloscope monitor. Trials contaminated with voluntary EMG activities were discarded from analyses.

Quadripulse stimulation

The QPS protocol consisted of trains of TMS pulses with an inter-train interval (ITI) of 5 s (i.e. 0.2 Hz). Each train consisted of four magnetic pulses separated by inter-stimulus intervals (ISI) of 1.5, 5, 10, 30, 50 or 100 ms. These conditioning types were designated as QPS-1.5 ms to QPS-100 ms, respectively.

Experiment 1: Effects of SMA priming on subsequent QPS-induced plasticity

Six of nine subjects were enrolled. The experimental sessions were separated by 1 week or longer in the same subject. The order of the experiments was randomized and balanced among subjects.

Experiment 1a: QPS-induced plasticity without priming over SMA (Fig. 1). To investigate QPS-induced plasticity without any priming, conditioning stimulation was applied over the left $M1_{FDI}$ using QPS at various ISIs (QPS-1.5 ms, QPS-5 ms, QPS-10 ms, QPS-30 ms, QPS-50 ms and QPS-100ms) for 30 min (Fig. 1). The stimulus intensity of each pulse for QPS conditioning was set at 90% AMT_{FDI} (Hamada *et al.* 2008*a*). During the conditioning, no MEPs were observed.

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 0.5 mV in the right FDI muscle at baseline (B1 in Fig. 1); the intensity was kept constant throughout the same experiment. After QPS conditioning, MEPs were measured every 5 min for 30 min. At each time point of the measurements, MEPs were collected in the same manner as baseline measurements.

Experiment 1b: QPS-induced plasticity with QPS-5 ms priming over SMA (Fig. 1). Priming stimulation was applied over SMA using 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). Importantly, priming over SMA using QPS-5 ms for 10 min induces no substantial effects on MEP sizes (see Results). The aim of the experiment was to test whether this priming stimulation would have effects on subsequent QPS-induced plasticity according to previous animal studies, which showed that prior induction of LTP is not prerequisite for metaplasticity (Abraham & Tate, 1997; Abraham, 2008). The stimulus intensity of each pulse for priming was set at 90% AMT_{TA}, which was identical to about 130% AMT_{FDI} (i.e. 90% RMT_{FDI}) (Table 1). The stimulus intensity used for priming over SMA was calculated relative to AMT_{TA} in order to secure effective stimulation of SMA, given that its depth is almost the same as that of the M1 for foot muscles in the

interhemispheric fissure. Additionally, 130% AMT of hand muscle over SMA has been considered not to spread to the PMd or M1 (Matsunaga *et al.* 2005).

Subsequent conditioning was applied over the left $M1_{FDI}$ using QPS at various ISIs (QPS-1.5 ms, QPS-5 ms, QPS-10 ms, QPS-30 ms, QPS-50 ms and QPS-100ms) for 30 min (i.e. 360 trains, 1440 pulses in total) (Fig. 1). The stimulus intensity of each pulse for this subsequent conditioning was set at 90% AMT_{FDI}.

Before the priming stimulation (B0 in Fig. 1), 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 0.5 mV in the right FDI muscle at B0 and kept constant throughout the experiment. After priming over SMA, 20 MEPs were again obtained in the same manner as measurements at B0 (B1 in Fig. 1). Following this measurement at B1 (i.e. immediately after priming), QPS conditioning of various types over the left $M1_{FDI}$ was performed. After each QPS, MEPs were measured every 5 min for 30 min (Fig. 1).

Experiment 1c: QPS-induced plasticity with QPS-50 ms priming over SMA (Fig. 1). 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) was selected as another priming stimulation over SMA to reveal the opposite priming effects to QPS-5 ms priming. The stimulus intensity for each pulse was set at 90% AMT_{TA}. Subsequent conditioning types after QPS-50 ms priming over SMA were QPS-5 ms, QPS-10 ms, QPS-30 ms and QPS-100 ms over the left $M1_{FDI}$ for 30 min. We have previously shown that QPS-50 ms priming over M1 produced substantial changes in subsequent QPS-induced plasticity at ISIs of

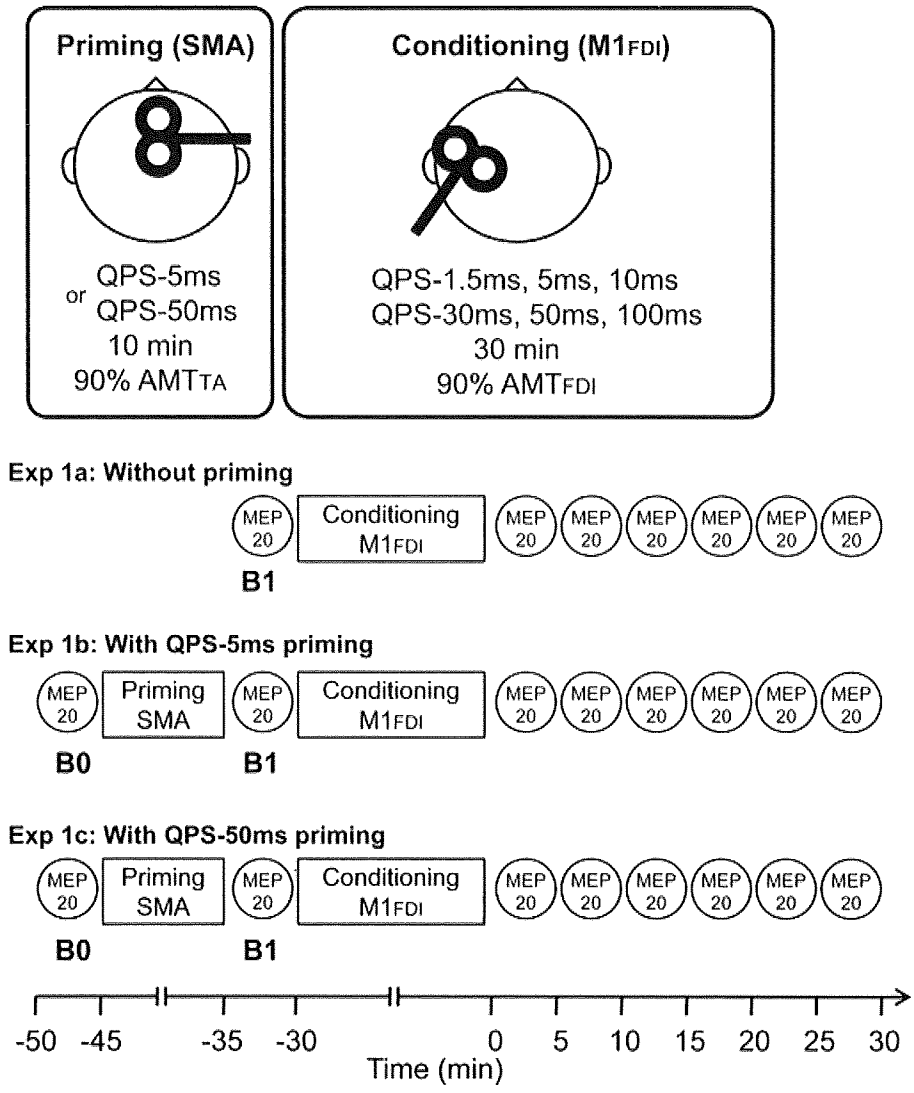

Figure 1. Timelines of experiments
(See Methods.)

Table 1. Physiological parameters (mean ± S.D.)

	RMT_{FDI}	AMT_{FDI}	AMT_{TA}	Stimulus intensify for SMA priming (relative to RMT_{FDI})	MEP size (mV), right FDI	
Experiment 1a: Without priming		Baseline (B1)				B1
QPS-1.5 ms	57.1 ± 9.8%	40.8 ± 6.9%	–	–	–	0.46 ± 0.11
QPS-5 ms	58.5 ± 9.5%	43.6 ± 5.1%	–	–	–	0.51 ± 0.13
QPS-10 ms	61.8 ± 10.9%	41.0 ± 6.6%	–	–	–	0.48 ± 0.16
QPS-30 ms	61.2 ± 11.6%	40.6 ± 5.8%	–	–	–	0.49 ± 0.09
QPS-50 ms	60.5 ± 14.1%	42.6 ± 5.4%	–	–	–	0.51 ± 0.18
QPS-100 ms	60.1 ± 12.1%	40.8 ± 5.8%	–	–	–	0.55 ± 0.06
Experiment 1b: With QPS-5 ms priming over SMA		Baseline (B1)		Mean ± S.D. (range)	B0	B1
QPS-1.5 ms	61.8 ± 9.2%	40.3 ± 1.9%	60.2 ± 8.0%	89 ± 8% (76–98%)	0.51 ± 0.09	0.49 ± 0.14
QPS-5 ms	59.8 ± 6.9%	42.8 ± 5.0%	60.8 ± 8.5%	92 ± 5% (85–97%)	0.43 ± 0.11	0.47 ± 0.28
QPS-10 ms	60.0 ± 9.7%	42.0 ± 4.9%	61.1 ± 10.0%	93 ± 4% (87–99%)	0.48 ± 0.18	0.54 ± 0.14
QPS-30 ms	58.3 ± 8.5%	41.7 ± 5.4%	60.8 ± 10.0%	95 ± 6% (86–100%)	0.51 ± 0.14	0.55 ± 0.18
QPS-50 ms	61.8 ± 7.6%	41.0 ± 5.1%	63.6 ± 8.1%	93 ± 5% (85–98%)	0.53 ± 0.21	0.53 ± 0.20
QPS-100 ms	60.8 ± 8.7%	40.3 ± 5.0%	61.5 ± 7.6%	92 ± 4% (86–96%)	0.53 ± 0.18	0.59 ± 0.21
Experiment 1c: With QPS-50 ms priming over SMA		Baseline (B1)			B0	B1
QPS-5 ms	61.6 ± 9.4%	41.6 ± 5.3%	62.6 ± 9.3%	94 ± 3% (91–98%)	0.50 ± 0.09	0.52 ± 0.08
QPS-10 ms	60.6 ± 9.1%	43.8 ± 5.9%	63.5 ± 9.6%	95 ± 2% (92–98%)	0.57 ± 0.11	0.53 ± 0.21
QPS-30 ms	58.1 ± 9.9%	42.1 ± 4.8%	62.2 ± 11.4%	95 ± 5% (88–100%)	0.53 ± 0.09	0.48 ± 0.04
QPS-100 ms	60.5 ± 10.1%	42.0 ± 6.4%	65.2 ± 11.7%	97 ± 3% (93–100%)	0.52 ± 0.12	0.49 ± 0.16
Experiment 2		Baseline (B0)			B0	B1
Sham with priming	61.8 ± 13.7%	40.8 ± 7.8%	61.5 ± 7.2%	93 ± 4% (88–100%)	0.46 ± 0.06	0.51 ± 0.14
QPS-10 ms with sham priming	58.3 ± 7.4%	40.5 ± 7.4%	–	–	0.47 ± 0.12	0.48 ± 0.18
Experiment 3		Baseline			Baseline	Post 1	Post 2
QPS-5 ms priming	60.6 ± 11.0%	42.0 ± 8.2%	66.0 ± 7.7%	95 ± 4% (88–100%)	0.52 ± 0.24	0.54 ± 0.27	0.49 ± 0.26
QPS-50 ms priming	59.7 ± 10.1%	41.5 ± 9.8%	66.4 ± 8.3%	95 ± 5% (88–100%)	0.48 ± 0.14	0.53 ± 0.19	0.55 ± 0.11
Experiment 4					ISI 3 ms	ISI 6 ms	
	57.9 ± 9.6%	43.3 ± 7.8%	62.6 ± 9.4%	–	0.59 ± 0.23	0.58 ± 0.27	

10, 30 and 100 ms (Hamada *et al.* 2008*a*). Since it would be of value to compare those results and SMA priming effects in the present study to understand the difference in priming stimulation site (i.e. M1 *versus* SMA), we selected the four ISIs used in the previous paper for Experiment 1c. The stimulus intensity of each pulse of QPS conditioning was set at 90% AMT_{FDI}. MEP measurements were exactly the same as those of Experiment 1b.

Experiment 2: Control experiments

In the first control experiment (Fig. 6*A*), priming stimulation using QPS-5 ms over SMA for 10 min 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. 6*B*), sham priming stimulation was followed by QPS-10 ms over M1 to confirm that sham priming did not affect the QPS-induced plastic changes (QPS-10 ms with sham priming). QPS-10 ms was chosen as conditioning because this protocol induced mild facilitatory after-effects (see Results) which we thought might make it more susceptible to any bidirectional effects of sham priming.

The sham stimulation procedure used for these control experiments was identical to those described in our previous reports (Okabe *et al.* 2003; Hamada *et al.* 2008*b*). In

brief, four electric pulses (each electric pulse was of 0.2 ms duration with intensity of twice the sensory threshold) were given to the scalp at 0.2 Hz at an ISI of 10 ms for sham conditioning (first control experiment) and at 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 $M1_{FDI}$ or SMA and the vertex. A coil, which was disconnected from the stimulator, was placed over the left $M1_{FDI}$ or SMA to mimic real TMS. Another coil, which was connected to a combining module with four 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.

Experiment 3: Effects of priming over SMA on intracortical circuits of M1

Nine subjects participated in this experiment. To explore the effects of SMA priming alone on either excitatory or inhibitory circuits of M1, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF) (Kujirai *et al.* 1993), short-interval intracortical facilitation (SICF) (Tokimura *et al.* 1996; Ziemann *et al.* 1998; Hanajima *et al.* 2002), and long-interval intracortical inhibition (LICI) (Valls-Sole *et al.* 1992; Wassermann *et al.* 1996) were measured using the paired-pulse technique before and after QPS-5 ms or QPS-50 ms priming over SMA (i.e. four pulses at an ISI of 5 ms or 50 ms with an ITI of 5 s for 10 min; the stimulus intensity of each pulse, 90% AMT_{TA}).

SICI was examined at an ISI of 3 ms using a conditioning stimulus (CS) intensity of 80% AMT_{FDI}. ICF was measured at an ISI of 10 ms with a CS intensity of 90% AMT_{FDI}. SICF was measured at an ISI of 1.5 ms. The intensity of the second stimulus (S2) was set at 10% below AMT_{FDI}. LICI was measured at an ISI of 100 ms with a CS intensity of 110% RMT_{FDI}. The intensity of the test stimulus (TS) (i.e. the first stimulus (S1) for SICF) was adjusted to elicit MEPs of 0.4–0.5 mV from relaxed FDI at baseline. Twelve trials were recorded for each condition and randomly intermixed with 18 trials of TS alone with an ITI of 5.5–6.5 s (about 6 min in total). The SICI, ICF, SICF and LICI were all studied simultaneously in one session using the four magnetic stimulators (i.e. three stimulators produced the different CS and the other one gave TS). Measurements of these values were performed in blocks immediately before (baseline) and just after QPS-5 ms priming (post 1) as well as 20 to 26 min (post 2). Conditioning intensities and test intensity were not changed after the priming because the test MEP sizes were not altered by SMA priming (see Results and Table 1).

Experiment 4: Supplementary experiments

The aim of these experiments was to test whether the conditioning stimulus over SMA spreads to M1 or PMd (Experiment 4a), or whether it exerts a direct effect on the spinal motor neurons (Experiment 4b). Eight subjects participated in this series of experiments.

Experiment 4a: Effects of a conditioning stimulus over SMA on MEP. We investigated whether the stimulus over SMA spreads to other cortical areas using a paired-pulse technique. The test response in the right relaxed FDI elicited by single pulse TMS over the left $M1_{FDI}$ was conditioned by single pulse TMS over SMA at an ISI of 3 ms or 6 ms. The intensity of the TS was adjusted to elicit MEPs of about 0.5 mV in the relaxed FDI when given alone. The stimulus intensities for conditioning over SMA were set at 70, 90 and 110% AMT_{TA}. At each ISI (i.e. 3 or 6 ms), 12 trials were recorded for each condition (70, 90 and 110% AMT_{TA}) and randomly intermixed with 18 trials of TS alone with an ITI of 5.5–6.5 s in a single block. Thus, two blocks of measurements at each ISI were performed. The order of blocks was randomized. As the inhibitory interneurons of the $M1_{FDI}$ might have a lower threshold than the intrinsic I-wave circuits (Reis *et al.* 2008), we argued that, if the current over SMA spread to the $M1_{FDI}$, then some inhibitory effects on the test response would be observed at an ISI of 3 ms in a conditioning intensity-dependent manner. Indeed, it is known that conditioning over the PMd at an ISI of 6 ms has either inhibitory or facilitatory effects on MEP sizes depending on the conditioning intensity (Civardi *et al.* 2001). Thus, we argued that, if the current over SMA spread to PMd, then some inhibitory or facilitatory effects on the test response would be observed in an intensity-dependent manner.

Experiment 4b: Effects of a stimulus over SMA on MEP in active condition. To test whether the current over SMA spreads to the $M1_{FDI}$ directly and whether direct stimulation of SMA activates some neurons in SMA projecting to the spinal motor neurons directly (Dum & Strick, 1991, 1996), single pulse TMS was applied over SMA during contraction of the right FDI muscle (20% maximum voluntary contraction). If the current over SMA spread to $M1_{FDI}$ directly or if the current stimulated some SMA neurons enough to produce any descending volley, then small MEPs would be elicited during voluntary contraction in an intensity-dependent manner. Ten stimuli were applied every 5 s at an intensity of 100% AMT_{TA}. The stimulus intensity was then increased by 10% of AMT_{TA} and another 10 stimuli were applied. This process was repeated until the intensity reached 190% AMT_{TA} or 100% MSO.

Data analyses

Experiment 1a. The after-effects of different conditioning types were analysed with absolute MEP amplitudes using two-way repeated-measures analysis of variance (ANOVA) (within-subject factors, CONDITION (QPS-1.5 ms, QPS-5 ms, ···, QPS-100ms), and TIME (B1 and following six time points)). If the factors CONDITION and TIME showed a significant interaction, *post hoc* paired *t* tests (two-tailed) with Bonferroni's corrections for multiple comparisons were 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 1b and 1c. Absolute values of MEPs at B0 and B1 (Fig. 1) were compared using paired *t* tests in each experiment. To evaluate the priming effects on subsequent QPS-induced plasticity, the absolute amplitudes of MEPs collected in Experiment 1a (i.e. without priming) and Experiment 1b (i.e. with QPS-5 ms priming over the SMA) or Experiment 1c (i.e. with QPS-50 ms priming over the SMA) were entered in three-way repeated-measures ANOVA with PRIMING (with and without priming), CONDITION ((QPS-1.5 ms, QPS-5 ms, ···, and QPS-100ms) for Experiment 1b, and (QPS-5 ms, QPS-10 ms, QPS-30 ms and QPS-100ms) for Experiment 1c), and TIME (B1, and following six time points) as within-subject factors to match the measurement time points relative to QPS conditioning among experiments. 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 B0 and B1 were not significantly different (see Results). If the factors PRIMING, CONDITION and TIME showed a significant interaction, *post hoc* paired *t* tests (two-tailed) with Bonferroni's corrections for multiple comparisons were used.

Experiment 2. 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 (B1 and following six 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 (B1 and following six points)).

Experiment 3. 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 time course of after-effects was analysed using three-way repeated measures ANOVA (within-subject factors, PRIMING (QPS-5 ms and QPS-50ms), BISTIM (SICI, ICF, SICF and LICI) and TIME (baseline, post 1 and post 2)). If the factors PRIMING, BISTIM and TIME showed a significant interaction, Dunnett's *post hoc* test in each condition was used for further analyses.

Experiment 4a. 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 ratios were entered in one-way repeated measures ANOVA (within-subject factor, INTENSITY of conditioning stimulation). Paired *t* tests (two-tailed) were used for further analyses.

Data were analysed with the commercialized software (SPSS version 17.0 for Windows; SPSS Inc.). All figures depict the group data.

Results

None of the subjects reported any adverse effects during or after any of the experiments. Baseline physiological data did not differ significantly among different experiments (Table 1). The stimulus intensities for SMA priming were all below RMT_{FDI} (Table 1).

Experiment 1: Effects of SMA priming on subsequent QPS-induced plasticity

Experiment 1a: QPS-induced plasticity without priming over SMA. In line with our previous report (Hamada *et al.* 2008*a*), QPS at short ISIs (QPS-1.5 ms, QPS-5 ms and QPS-10ms) produced an increase in the MEP amplitude, whereas QPS at long ISIs (QPS-30 ms, QPS-50 ms and QPS-100ms) suppressed MEPs (Fig. 2*A*). Two-way repeated measures ANOVA revealed a significant CONDITION (QPS-1.5 ms, QPS-5 ms, ···, and QPS-100ms) × TIME interaction ($F_{(2.941,14.705)} = 4.384$, $P < 0.001$). Figure 2*B* presents the MEP amplitude normalized to the baseline MEP at 30 min after QPS as a function of the reciprocal of the ISI used in each QPS burst. There was a non-linear relation between MEP excitability changes and ISI indicating the presence of threshold for inducing LTP-like plasticity. *Post hoc* analysis revealed that QPS-1.5 ms, QPS-5 ms and QPS-50ms were significantly different from QPS-30 ms.

Experiment1b: QPS-induced plasticity with QPS-5 ms priming over SMA. Figure 3 shows the time courses

of MEP amplitude following QPS at various ISIs with and without QPS-5 ms priming over SMA. No difference was found in MEP amplitudes at B0 and B1 in any conditions (paired t test, $P > 0.5$). Although SMA priming with QPS-5 ms did not occlude MEP facilitation by QPS-1.5 ms, it produced lasting MEP suppression after QPS-5 ms, QPS-10 ms and QPS-30 ms (Fig. 3*B–D*). By contrast, MEP suppression induced by QPS-50 ms and QPS-100 ms was not enhanced, but its duration was shortened by SMA priming (Fig. 3*E* and *F*). Three-way repeated measures ANOVA revealed a significant PRIMING × CONDITION × TIME interaction ($F_{(3.464,17.319)} = 3.826$, $P = 0.025$). *Post hoc* paired t tests revealed a significant effect of SMA priming on the after-effects of QPS-5 ms, QPS-10 ms, QPS-30 ms, QPS-50 ms and QPS-100 ms (Fig. 3*A–F*).

Experiment 1c: QPS-induced plasticity with QPS-50 ms priming over SMA. Figure 4 shows the change in MEP amplitude following QPS at various ISIs with and without QPS-50 ms priming over SMA. No difference in MEP amplitude was found at B0 and B1 in any conditions (paired t test, $P > 0.5$). QPS-50 ms priming over SMA did not enhance MEP facilitation by QPS-5 ms. It produced slight enhancement of MEP facilitation by QPS-10 ms (Fig. 4*A* and *B*). In contrast, transient MEP suppression induced by QPS-30 ms turned to facilitation after SMA priming, but there was no change in the after-effects of QPS-100 ms (Fig. 4*C* and *D*). Three-way repeated measures ANOVA revealed a significant PRIMING × CONDITION interaction ($F_{(2.593,9.948)} = 5.612$, $P = 0.023$), but revealed no significant PRIMING × CONDITION × TIME interaction ($F_{(3.363,16.810)} = 3.079$, $P = 0.051$). 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 SMA priming with QPS-50 ms on the after-effects of QPS-5 ms and QPS-30 ms (Fig. 4*A* and *C*).

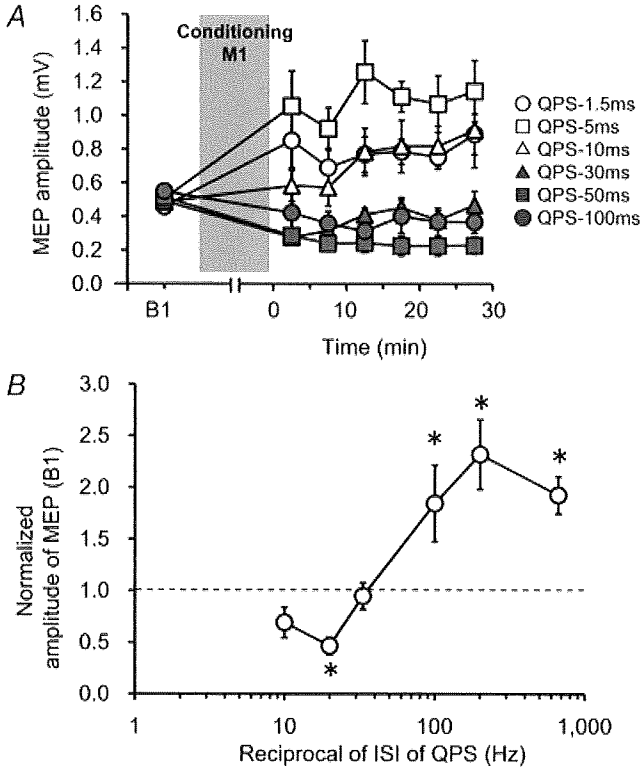

Figure 2. QPS-induced plasticity without priming over SMA
A, time courses of MEP amplitude following QPS at various ISIs without priming (mean ± S.E.M.). *B*, stimulus–response function of QPS-induced plasticity. Normalized amplitudes of MEP measured at 30 min after QPS as a function of the reciprocal of ISI of QPS: mean (± S.E.M.) of baseline. Note that the x-axis is logarithmic. Asterisks denote significant difference from QPS-30 ms (*$P < 0.05$).

Stimulus–response function with priming over SMA. The normalized MEP amplitudes at 30 min post conditioning are plotted as a function of the reciprocal of the ISI used in each QPS with and without priming over SMA (Fig. 5). The crossover point from MEP suppression to facilitation appears to shift in either direction along the x-axis according to which priming stimulation was employed. *Post hoc* analysis revealed that QPS-5 ms priming over SMA significantly reduced MEP sizes after QPS-5 ms, QPS-10 ms and QPS-30 ms, but occluded MEP suppression by QPS-50 ms. QPS-50 ms priming over SMA inhibited MEP sizes after QPS-5 ms, whereas it facilitated MEP sizes after QPS-30 ms (Fig. 5).

Experiment 2: Control experiments

First, the after-effects of sham conditioning with real priming were monitored to examine whether priming alone (i.e. QPS-5 ms over SMA for 10 min) affects motor cortical excitability. Figure 6*A* shows the time course of the MEP amplitude following sham conditioning with real priming. No difference was found in MEP amplitudes at B0 and B1 (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, $F_{(6,30)} = 0.410$, $P = 0.866$). Second, the after-effect of real conditioning (QPS-10ms) with sham priming was compared to that of real conditioning without priming to confirm that sham priming did not affect motor cortical plasticity induced by real conditioning. Figure 6*B* shows the absolute amplitude of MEPs following real conditioning (QPS-10ms) without priming and with sham priming. No difference was found between MEPs at B0 and B1 (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), $F_{(1,5)} = 0.095$, $P = 0.770$; CONDITION × TIME interaction, $F_{(6,30)} = 0.410$, $P = 0.866$).

Experiment 3: Effects of priming over SMA on intracortical circuits of M1

Figure 7 shows the effects of priming over SMA on SICI, ICF, SICF and LICI. Three-way repeated measures ANOVA revealed significant PRIMING × BISTIM × TIME interaction ($F_{(6,48)} = 2.498$, $P = 0.035$). *Post hoc* tests revealed that only SICF was modulated after SMA priming; the effects on SICF were transient, being significant only at post 1 (Fig. 7*A* and *B*).

Experiment 4: Supplementary experiments

Experiment 4a: Effects of a conditioning stimulus over the SMA on MEP. Using the paired-pulse technique at an ISI of 3 ms, we investigated whether the stimulus over SMA spreads to $M1_{FDI}$. Figure 8*A* shows the changes of conditioned MEP relative to the unconditioned MEP in each block using three different conditioning intensities. One-way repeated measures ANOVA revealed a significant main effect of INTENSITY ($F_{(2,14)} = 3.904$, $P = 0.045$). *Post hoc* paired *t* tests revealed that the test MEPs were significantly inhibited by conditioning stimulus at 110% AMT_{TA} ($t = 2.39$, $P = 0.048$), whereas no significant inhibition was found at 70% or 90% AMT_{TA}.

Figure 8*B* shows the changes of conditioned MEP relative to the unconditioned MEP using the paired-pulse technique at an ISI of 6 ms. No significant effect of conditioning intensity over SMA at an ISI of 6 ms was found (one-way repeated measures ANOVA: effect of INTENSITY, $F_{(2,14)} = 0.679$, $P = 0.523$).

Experiment 4b: effects of a stimulus over the SMA on MEP in active condition. To test whether the current induced by TMS over the SMA directly spreads to the $M1_{FDI}$, single pulse TMS was applied over SMA during contraction of the right FDI muscle (20% maximum). Although the stimulus intensity reached 190% AMT_{TA} or 100% MSO, no MEPs were recorded in any condition.

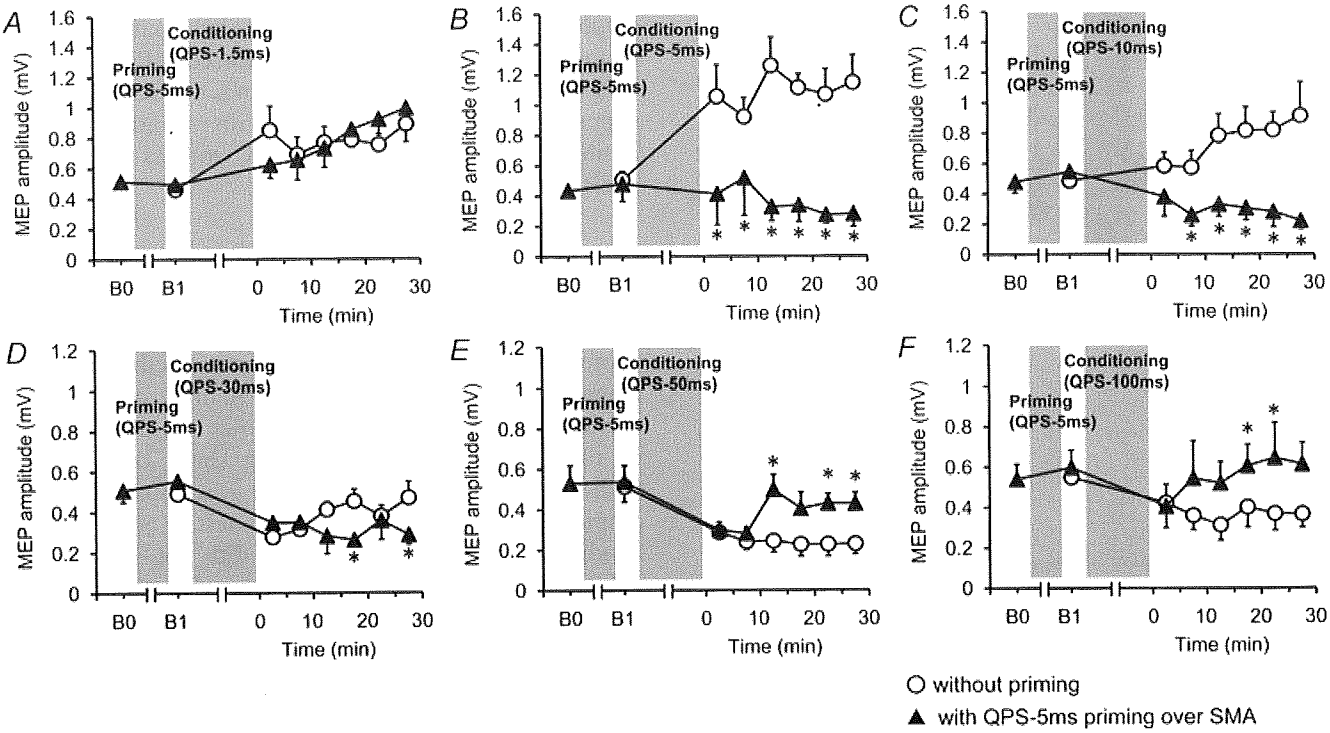

Figure 3. Effects of QPS-5 ms priming over SMA on QPS-induced plasticity
Time courses of MEP amplitude following QPS at various ISIs with (▲) and without (○) QPS-5 ms priming over SMA (mean ± S.E.M.). *A*, SMA priming did not change subsequent LTP-like plasticity induced by QPS-1.5 ms. *B* and *C*, priming reversed MEP sizes induced by QPS-5 ms (*B*) or QPS-10 ms (*C*). *D*, priming enhanced suppression of MEP by QPS-30 ms. *E* and *F*, MEP suppression induced by QPS-50 ms (*E*) and QPS-100 ms (*F*) were not enhanced, but shortened with SMA 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).

Discussion

We showed that LTD-like and LTP-like QPS-induced plasticity was altered by a preceding period of priming stimulation over SMA. QPS at short ISIs produced an increase in the MEP amplitude, whereas QPS at long ISIs suppressed MEPs (Experiment 1a, Fig. 2*A*). QPS-5 ms priming over SMA occluded MEP facilitation after QPS-5 ms and QPS-10 ms. Furthermore, it enhanced suppression of MEP after QPS-30 ms, but occluded MEP suppression by QPS-50 ms (Experiments 1b, Fig. 5). By contrast, QPS-50 ms priming over SMA inhibited MEP sizes after QPS-5 ms, whereas it facilitated MEP sizes after QPS-30 ms (Experiments 1c, Fig. 5). QPS-5 ms or QPS-50 ms priming over SMA did not change MEP sizes, SICI, ICF and LICI but altered SICF; QPS-5 ms priming enhanced SICF, whereas QPS-50 ms priming erased SICF (Experiment 3, Fig. 7). Finally, a single conditioning TMS over SMA at 110% AMT_{TA} with an ISI of 3 ms significantly inhibited test MEP sizes, whereas no effect was found at a conditioning intensity lower than 110% AMT_{TA}. In addition, no significant effects were found at any conditioning intensity using an ISI of 6 ms (Experiment 4, Fig. 8). We will argue that the present findings provide strong support for the hypothesis that priming over the SMA transiently altered the synaptic efficiencies of excitatory circuits within M1, and that such prior history of neuronal activity alters subsequent LTD-like and LTP-like QPS-induced plasticity through the metaplastic interplay between SMA and M1.

Effects of TMS over SMA

Given that there is now good evidence that TMS can stimulate SMA neurons (Civardi *et al.* 2001; Terao *et al.* 2001; Serrien *et al.* 2002; Verwey *et al.* 2002; Matsunaga *et al.* 2005; Hamada *et al.* 2008*b*), our results are compatible with the idea that rTMS can produce lasting changes in the excitability of these circuits. We argue that such mechanisms underpin the present findings, although we cannot be completely certain about the precise site of our SMA stimulus. According to previous studies, the optimal site of SMA stimulation has been shown to be between 2 and 4 cm anterior to Cz (Terao *et al.* 2001; Serrien *et al.* 2002; Verwey *et al.* 2002); neuroimaging methods also locate the hand area of the SMA proper some 2 to 3 cm anterior to Cz (Hikosaka *et al.* 1996; Lee *et al.* 1999). It is thereby conceivable that our TMS stimulus mainly activates SMA neurons.

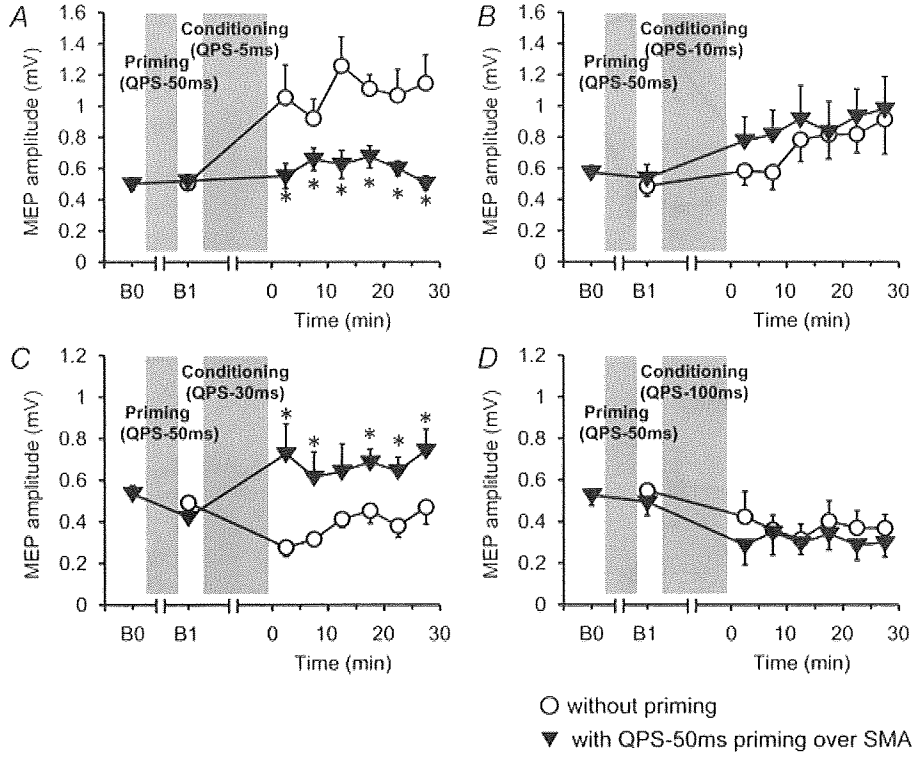

Figure 4. Effects of QPS-50 ms priming over SMA on QPS-induced plasticity
Time courses of MEP amplitude following QPS at various ISIs with (▼) and without (○) QPS-50 ms priming over SMA (mean ± S.E.M.). *A*, SMA priming occluded subsequent LTP-like plasticity induced by QPS-5 ms. *B*, priming did not change plasticity induced by QPS-10 ms. *C*, priming reversed suppression of MEP by QPS-30 ms. *D*, MEP suppression induced by QPS-100 ms was not altered with SMA 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).

A second question is whether currents induced by stimulation over SMA spread to other motor-related areas. The data suggest that this was unlikely with the intensities of stimulation that we used. Experiment 4a showed that a conditioning pulse over SMA at 110% AMT_{TA} with an ISI of 3 ms significantly inhibited test MEP sizes, which corresponds to the timing of SICI (Kujirai *et al.* 1993), whereas no effect was found at a conditioning intensity of either 70% AMT_{TA} or 90% AMT_{TA}. These findings suggest that the current induced by placing the coil over SMA spreads to $M1_{FDI}$ only when the stimulus intensity is higher than 110% AMT_{TA} in line with a preceding report (Matsunaga *et al.* 2005). In addition, no significant changes in the size of test responses were found at any conditioning intensity using an ISI of 6 ms, which corresponds to the optimal ISI to produce effects in the pathway from PMd to M1 (Civardi *et al.* 2001). Finally, Experiment 4b revealed that no MEPs were elicited during voluntary contraction with single pulse TMS over SMA at very high stimulus intensities up to 100% MSO, indicating no direct activation of excitatory interneurons or cortical output neurons within M1. We cannot completely exclude the possibility that various subliminally stimulated cortical areas including SMA, PMd, PMv, M1 and others regions were implicated in the effects of SMA priming. However, the present findings lead us to conjecture that the effects of priming over SMA can be mainly ascribed to stimulation of the cortex beneath the coil, namely SMA.

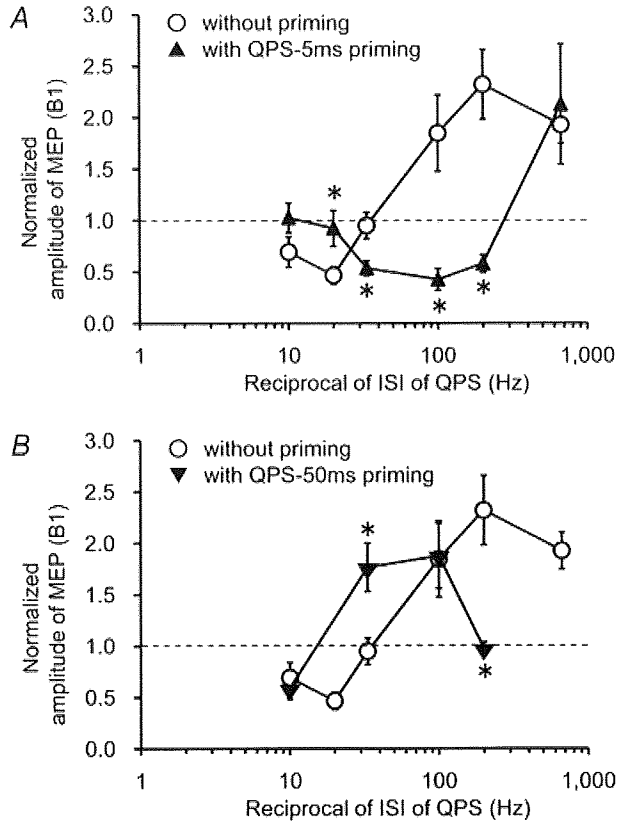

Figure 5. Priming-induced shifts in the stimulus–response function
The normalized amplitudes of MEP at 30 min post conditioning as a function of the reciprocal of ISI of QPS with and without priming over SMA (○). *A*, QPS-5 ms priming (▲). *B*, QPS-50 ms priming (▼). Note that the *x*-axis is logarithmic axis. $^*P < 0.05$ by *post hoc* paired *t* tests.

Effects of priming over SMA alone

We did not evaluate possible effects of sham priming on subsequent QPS-induced plasticity elicited by all kinds of QPS protocols used in the present paper. In Experiment 2, QPS-10 ms was chosen as a representative of all QPS protocols because it induced mild facilitatory after-effects rendering it more susceptible to possible effects of sham priming. In fact, two control experiments revealed that neither priming alone nor cutaneous sensation had any lasting effect on MEPs.

The results of Experiment 3 show that QPS-5 ms or QPS-50 ms priming over SMA did not change MEP sizes, SICI, ICF and LICI. Its only effect was a transient modulation of SICF, which did not persist as long as the priming effects on QPS. We cannot exclude the possibility that subtle changes in inhibitory circuits were missed because paired-pulse measurements addressing

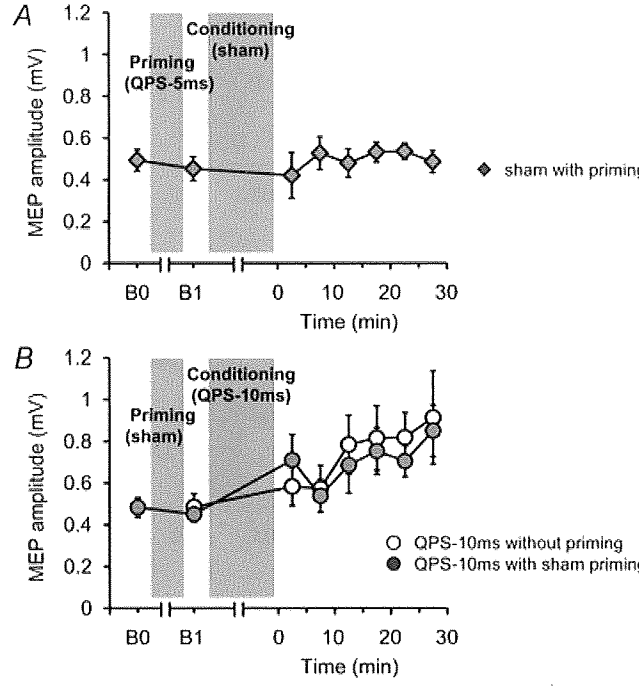

Figure 6. Control experiments
A, sham conditioning with real priming did not modify motor cortical excitability. *B*, the after-effects of QPS-10 ms without priming (open circles) were not different from those of QPS-10 ms with sham priming (grey circles).

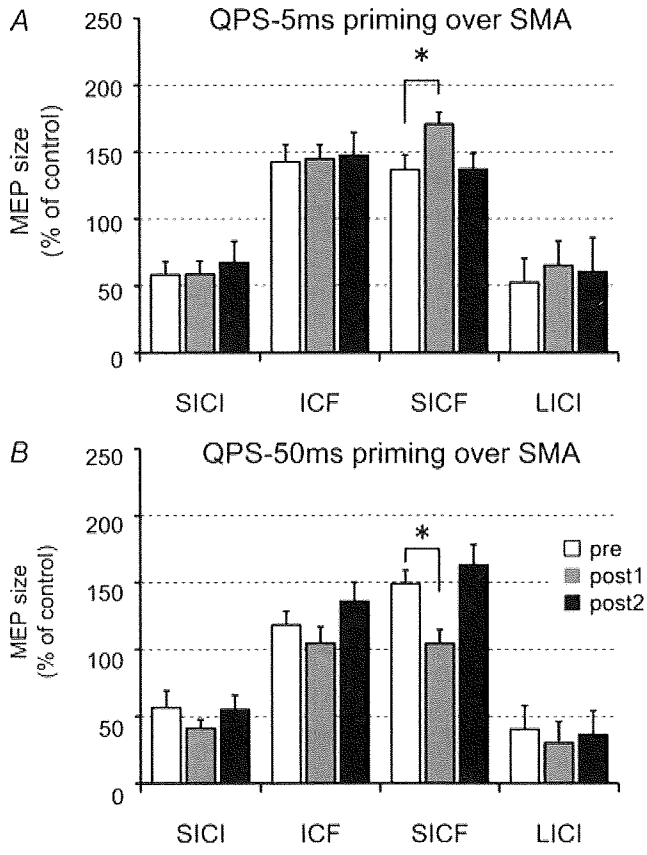

Figure 7. Effects of priming over SMA on intracortical circuits of M1

A, SICF was enhanced after QPS-5 ms. SICI, ICF nor LICI were altered by QPS-5 ms priming over SMA alone. *B*, SICF was suppressed after QPS-50 ms over SMA, whereas others were not altered. Baseline (white bars); post 1 (grey bars), 0–6 min after QPS; post 2 (black bars), 20–26 min after QPS over SMA. $^*P < 0.05$ by *post hoc* Dunnett's test.

intracortical excitability were assessed only at a single ISI with a single conditioning intensity. Nonetheless, the long period for measurements using various ISIs with multiple conditioning intensities might in turn miss the effects of priming owing to transient effects of priming on SICF.

The present results raise the question why such selective modulation of SICF was produced by SMA priming. Although this selectivity seems puzzling, it is not a unique observation since we have shown that QPS priming over M1 produced transient modulation of SICF without affecting MEP sizes, SICI, ICF and LICI (Hamada *et al.* 2008*a*). It has been also reported that selective modulation of SICI can be produced by low-intensity theta burst stimulation (TBS) without any changes in MEP (McAllister *et al.* 2009). Also, 5 Hz rTMS over PMd reduced paired-pulse excitability at an ISI of 7 ms without any changes in MEP amplitude (Rizzo *et al.* 2004). Thus, it is possible to modulate inhibitory or excitatory circuits selectively, which is not accompanied by lasting changes in MEP sizes under certain specified conditions.

What is the mechanism behind the modulation of SICF by SMA priming? At first, an interaction of I-wave inputs is thought to be the cause of SICF presumably by the summation of excitatory postsynaptic potentials (EPSPs) elicited by the first suprathreshold TMS stimulus with subliminal depolarization of interneurons that is produced by the second subthreshold TMS in cortical interneurons (Hanajima *et al.* 2002). Thus, modulation of SICF by SMA priming might therefore be consistent with the idea that SMA priming alters synaptic efficiency at which I-waves summate during paired-pulse TMS.

Then why are such changes in excitatory circuits of M1 produced by SMA priming? Matsunaga *et al.* (2005) proposed that there are at least two possible explanations for modulation of motor cortical excitability produced by rTMS over SMA. One possibility is that rTMS over SMA stimulates mainly cortico-cortical projections from SMA to M1 (Dum & Strick, 1991; Tokuno & Nambu, 2000; Nachev *et al.* 2008). The other is that rTMS over SMA alters local balance of excitability within SMA, leading to alteration of activity of cortico-cortical projections from

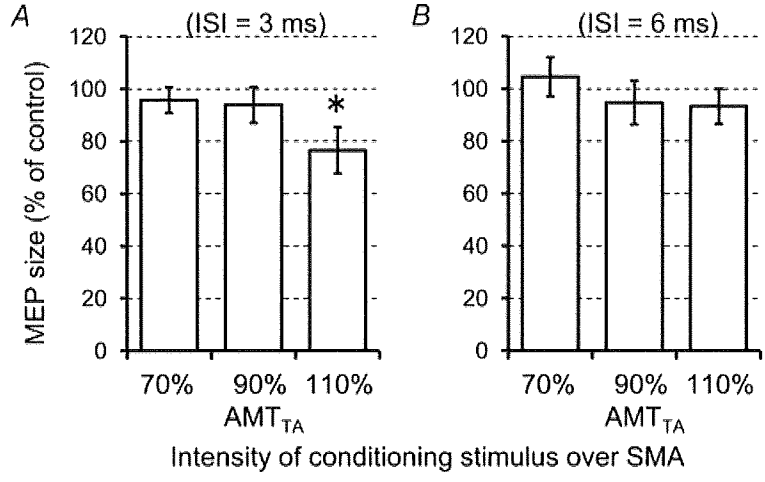

Figure 8. Effects of a conditioning stimulus over SMA on MEP

The test MEPs evoked by the left $M1_{FDI}$ was conditioned by stimulation of SMA at an ISI of 3 ms (*A*) or at an ISI of 6 ms (*B*). Asterisks denote a significant change relative to unconditioned response.

SMA to M1. They conclude that the latter might pertain to their findings since the intensity of stimulation was low (Matsunaga *et al.* 2005). The present study as with the previous study was not designed to test these hypotheses, thus we cannot comment on this issue further. Besides, it is also possible that functional modulation of basal ganglia via cortico-subcortical connections might contribute to the effects of priming over SMA (Inase *et al.* 1999; Kaneda *et al.* 2002; Akkal *et al.* 2007). Several studies also suggest that SMA proper sends direct projections to the spinal cord (Dum & Strick, 1991, 1996). Thus, SMA priming could produce some excitability changes in spinal motor neurons. However, it was impossible to evoke any MEPs during voluntary contraction of hand muscle using TMS over SMA at any intensities from 100% AMT_{TA} to 190% AMT_{TA} (i.e. 100% MSO), indicating that a higher intensity (above 100% MSO) is needed to stimulate neurons in SMA that project directly to the spinal cord. We cannot exclude the fact that repetitive stimulation of SMA with QPS, even at low intensities, might produce subthreshold temporal facilitation at spinal interneurons. In any case, we showed that QPS over SMA for 10 min produced bidirectional changes in SICF. Based on the fact that lasting motor cortical plasticity was induced by QPS over M1 for 30 min (Hamada *et al.* 2008*a*), the present result raises a new question as to whether QPS over SMA for 30 min produces plastic changes in bilateral M1. The issue should be addressed in future studies.

QPS-induced plasticity and synaptic plasticity

QPS is a newly developed protocol for inducing bidirectional (i.e. either facilitatory or inhibitory) motor cortical plasticity in humans (Hamada *et al.* 2007, 2008*a*). QPS at short intervals facilitated MEPs for more than 75 min, whereas QPS at long intervals suppressed MEPs for more than 75 min. The QPS-induced plasticity appears to be rather synapse-specific, as suggested by the following in the previous study (Hamada *et al.* 2008*a*); motor thresholds, which are dependent on ion channel conductivity and might reflect membrane excitability (Mavroudakis *et al.* 1994, 1997; Ziemann *et al.* 1996; Chen *et al.* 1997), were unchanged after QPS; SICI, which is considered to reflect γ-aminobutyric acid (GABA)-ergic inhibitory function of M1 (Kujirai *et al.* 1993), remained unchanged; SICF and ICF were enhanced by QPS-5 ms whereas reduced by QPS-50 ms (Hamada *et al.* 2008*a*). Based on the hypothesis of the mechanism of SICF (Hanajima *et al.* 2002), it is possible that QPS changes the quantity of EPSPs in excitatory circuits responsible for SICF (Hamada *et al.* 2008*a*). These results led us to surmise that the mechanism of QPS-induced plasticity involves synaptic plasticity in the excitatory circuits of M1 with features of non-linear dependence on the ISI of QPS, suggesting the presence of threshold for LTP- and LTD-like plasticity induction in line with previous findings of synaptic plasticity (Dudek & Bear, 1992).

Possible mechanisms of effects of priming over SMA on QPS-induced plasticity

The present results suggest that SMA priming influenced subsequent induction of synaptic plasticity in the excitatory circuits of M1. Priming over SMA elicited bidirectional shifts of the crossover point of the stimulus–response function of motor cortical plasticity (Fig. 5). The priming effects depended on the precise parameters of priming stimulation which specifically and transiently altered SICF without significant changes in MEP. We note that at least three possible mechanisms might account for the present findings: metaplasticity (Abraham & Bear, 1996), a gating mechanism (Ziemann & Siebner, 2008) and state-dependent effects (Fujiwara & Rothwell, 2004; Huang *et al.* 2008).

SMA priming-induced metaplasticity

First, it might be possible to interpret these findings within the framework of metaplasticity, or the plasticity of synaptic plasticity, in which neural activity at one point in time can change cells or synapses such that their ability to exhibit LTP or LTD after a later bout of activity is altered (Abraham, 2008). Metaplasticity is best documented by showing that an experimental manipulation which itself causes no persistent synaptic plasticity can nevertheless produce a shift in the crossover point of the frequency–response function of synaptic plasticity induced by a period of conditioning stimulation (Abraham, 2008). More specifically, if a manipulation reduces LTP then this should reflect elevation of the induction threshold for LTP; thus, the frequency of the conditioning stimulation required to produce LTP becomes higher, compatible with a shift in the crossover point of LTP and LTD in the conditioning frequency–response curve (Wang & Wagner, 1999; Zhang *et al.* 2005).

In this context, QPS-5 ms priming over SMA transiently enhances SICF, and this prior history of cortical activity elevated the threshold for inducing LTP-like plasticity. This is confirmed by the observation that QPS-5 ms priming over SMA did not occlude LTP-like plasticity by QPS-1.5 ms, whereas the same priming interfered with LTP-like plasticity induced by QPS-5 ms and QPS-10 ms, which produced LTD-like plasticity instead. These results indicate elevation of the threshold of LTP-like plasticity induction. In fact, QPS-5 ms priming over SMA shifted the crossover point of the stimulus–response function to the right along the x-axis (Fig. 5).

Theoretically, enhancement of prior neuronal activity should promote LTD (Bienenstock *et al.* 1982). This is confirmed by the fact that QPS-30 ms with QPS-5 ms priming produced a lasting decrease of MEP sizes as compared to QPS-30 ms without priming (Fig. 3*D*). However, LTD-like plasticity induced by QPS-50 ms and QPS-100 ms were not enhanced although the duration of suppression was shortened by QPS-5 ms priming over SMA. The disparity may be partly explained by previous studies of metaplasticity which revealed that the balance of NMDA receptor subunits (i.e. NR2A and NR2B) is one important factor in modulating the threshold for synaptic plasticity (Philpot *et al.* 2003, 2007). Some forms of metaplasticity are NMDA receptor dependent (Huang *et al.* 1992; Christie & Abraham, 1992; Abraham & Huggett, 1997; Zhang *et al.* 2005), and the ratio of NR2A/2B, which is increased by prior cortical activity, elevates the thresholds for both LTP and LTD induction (Yashiro & Philpot, 2008). Perhaps a similar mechanism is implicated in our present observation that QPS-5 ms priming over SMA elevates the thresholds for both LTP- and LTD-like plasticity.

In contrast, after QPS-50 ms priming over SMA, which transiently reduced SICF, LTP-like plasticity was induced by QPS-30 ms, which had produced transient MEP suppression when given alone. Indeed our results are compatible with the view that metaplastic changes can best be recognized by using a near-threshold stimulation regimen (i.e. near the crossover point) (Christie *et al.* 1995) since QPS-50 ms priming over SMA did not interfere with LTP- or LTD-like plasticity induced by QPS-10 ms or QPS-100 ms (Fig. 5). One result is apparently at odds with this explanation though; QPS-50 ms priming over SMA erased LTP-like plasticity usually seen after the QPS-5 ms protocol. According to the BCM rule, LTP-like plasticity should be enhanced after QPS-50 ms priming since it transiently reduced cortical activity. One possible explanation comes from animal studies of metaplasticity which suggest an inverted U-shaped relation between the amount of TBS and the degree of LTP (Christie *et al.* 1995; Abraham & Huggett, 1997). Such time-dependent LTP reversal process or overstimulation effect has been believed to be a normal feature of LTP induction, probably caused by a depotentiation mechanism during the massed presentation of tetanic stimulation (Christie *et al.* 1995; Abraham & Huggett, 1997). These authors found that only two trains of TBS were required to induce LTP but eight trains of TBS, which induce LTP on its own, were unable to induce LTP with low-frequency priming stimulation. This blocking of LTP has been suggested to reflect the overstimulation effect (Christie *et al.* 1995). We suggest that similarly, the present results might reflect comparable overstimulation effects with priming stimulation.

One conspicuous point of our results is that the SICF was modulated transiently by the priming stimulation alone. Thus, the effects of SMA priming on SICF could have returned to baseline by the time QPS was delivered following the collection of 20 MEPs at B1. This is consistent with our previous study showing transient modulation of SICF with priming over M1 (Hamada *et al.* 2008*a*). We favour the view that this transient modulation of SICF reflects prior history of cortical activity and that such prior activity substantially influences subsequent QPS-induced plasticity in a metaplastic manner (Hamada *et al.* 2008*a*). However, it is still possible that such transient modulation of SICF has a weak association with the priming effects because the time course of the influence on SICF did not parallel that of the priming effect.

Previous human studies demonstrated the effects of priming stimulation on subsequent rTMS-induced plasticity, indicating metaplasticity (Iyer *et al.* 2003; Lang *et al.* 2004; Siebner *et al.* 2004; Müller *et al.* 2007; Ragert *et al.* 2009). We have also shown metaplastic changes of QPS-induced plasticity by priming stimulation (Hamada *et al.* 2008*a*). In the previous reports, both priming and conditioning were applied over M1 using exactly the same stimulus intensity, suggesting that they modulated the same synaptic connections (Hamada *et al.* 2008*a*). According to animal experiments showing homosynaptic and heterosynaptic metaplasticity (Wang & Wagner, 1999; Abraham *et al.* 2001), our previous results indicate that the homeostatic changes were mediated via homosynaptic mechanism of metaplasticity. In contrast to the previous studies, in the present work priming was applied over SMA, leading to bidirectional modulation of SICF, whereas the subsequent conditioning was applied over M1. It is reasonable to believe that the synapses in M1 that are altered by the SMA priming are those involved in a SMA–M1 projection, and that these are likely to be partially different from those modulated by conditioning over M1. This raises the intriguing possibility that the effects of priming can be ascribed to a mixture of homosynaptic and heterosynaptic metaplasticity. Another implication from this paper is that SMA priming would have metaplastic effects on subsequent rTMS-induced plasticity of M1 which can be produced by any rTMS protocols that are capable of inducing LTP- and LTD-like changes.

Gating mechanism and state-dependent effect

Although we favour a metaplasticity theory to account for the present findings, other mechanisms might be involved. One possibility is a gating mechanism (Ziemann & Siebner, 2008); if QPS-5 ms priming had increased intracortical inhibition, then it might have rendered subsequent QPS conditioning less effective in exciting cortical output neurons trans-synaptically. In such a case, each burst of QPS would have produced a smaller amount of calcium

influx into the neurons, resulting in induction of LTD-like plasticity. However, neither SICI nor LICI was altered by priming over SMA, thus leading to the tentative conclusion that it is much less likely that the priming effects arise as a consequence of the alteration of intracortical inhibitory circuits.

Another possible explanation is related to several human studies in which voluntary contraction influences rTMS-induced plasticity (Fujiwara & Rothwell, 2004; Gentner *et al.* 2008; Huang *et al.* 2008). The after-effects of rTMS depend on the state of the cortex at the time the stimulation is applied. Thus, the effect of 5 Hz rTMS on SICI was reversed by muscle contraction during rTMS (Fujiwara & Rothwell, 2004). More recently, voluntary contraction during TBS abolished TBS-induced plasticity (Huang *et al.* 2008). Huang *et al.* (2008) proposed two possibilities to account for such state-dependent effects. First, the voluntary contraction perhaps changes the membrane potential or Ca^{2+} concentration of pyramidal neurons, directly affecting TBS-induced plasticity. 'Busy line' effects are another possible explanation; given that synapses stimulated by TBS are the same as those activated by voluntary contraction, extra activation of those synapses by TBS is negligible (Huang *et al.* 2008). Yet another study showed that voluntary contraction of sufficient duration changes the direction of TBS-induced plasticity; the authors contrastingly interpret their findings within the framework of metaplasticity theory (Gentner *et al.* 2008). Perhaps it is still an open question regarding the mechanism of effects of voluntary contraction on TBS-induced plasticity. In any case, SMA priming which produced changes in SICF might be a sign of some sort of baseline difference in the state of motor cortex independent of metaplasticity, resulting in alteration of subsequent QPS-induced plasticity.

SMA–M1 interplay and metaplasticity

Experimental observations of metaplasticity are considered to represent a major form of homeostatic mechanism of synaptic plasticity that prevents neuronal circuits from becoming destabilized and that maintains them within a dynamic range of modifiability (Abbott & Nelson, 2000; Abraham, 2008). Our findings might therefore highlight a homeostatic (or metaplastic) regulation of synaptic plasticity within excitatory circuits of M1 by input from SMA. Since SMA is implicated in higher motor control and the learning process (Luppino *et al.* 1993; Tanji & Shima, 1994; Tanji, 1996; Hikosaka *et al.* 1999; Nachev *et al.* 2008), the present results further raise the intriguing possibility that a preceding period of learning which entails a change in neuronal activity of SMA may regulate subsequent learning that is handled by neuronal circuits of M1 (Hikosaka *et al.* 1999; Sane & Donoghue, 2000). Since this study was not designed to test this hypothesis, future studies would be needed to shed light on possible metaplastic interplay between SMA and M1 during motor learning.

Finally, the shortcoming of the present study is that the lack of direct recording of synaptic responses in conscious humans renders any hypothesis about the precise neuronal mechanisms underlying QPS-induced plasticity or metaplasticity speculative (Cooke & Bliss, 2006). However, although the interpretation of the present data is inferential, the present study does suggest strongly that there may be important interactions between M1 and SMA in terms of metaplasticity.

Conclusions

Preceding stimulation over SMA elicited bidirectional shifts of the crossover point of the stimulus–response function of subsequent motor cortical plasticity. SMA priming transiently altered the synaptic efficiencies of excitatory circuits within M1. The data support the view that the homeostatic changes are mediated via mechanisms of metaplasticity. These findings highlight an important interplay between M1 and SMA regarding metaplasticity which might underpin learning and memory processes.

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Author contributions

M.H. and Y.U. contributed to the conception and design of the experiments. M.H. performed the experiments and analysed the data. All authors participated in data interpretation. M.H., Y.T. and Y.U. wrote the manuscript. All authors revised the manuscript and approved the final manuscript. The experiments were performed at Department of Neurology, the University of Tokyo, Japan.

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Postural Tremor in X-Linked Spinal and Bulbar Muscular Atrophy

Ritsuko Hanajima, MD, PhD,[1]* Yasuo Terao, MD, PhD,[1] Setsu Nakatani-Enomoto, MD,[1,2] Masashi Hamada, MD, PhD,[1] Akihiro Yugeta, MD, PhD,[1] Hideyuki Matsumoto, MD,[1] Tomotaka Yamamoto, MD, PhD,[1] Shoji Tsuji, MD, PhD,[1] and Yoshikazu Ugawa, MD, PhD[2]

[1]*Department of Neurology, The University of Tokyo Hospital, Tokyo, Japan*
[2]*Department of Neurology, Fukushima Medical University, Fukushima, Japan*

Abstract: Postural tremor is a common initial symptom in spinal and bulbar muscular atrophy (SBMA), but its pathophysiological mechanisms remain to be studied. This study was undertaken to examine the physiological mechanisms underlying postural tremor in SBMA. For eight patients (36–63 years old) with genetically confirmed SBMA, we recorded surface electromyograms (EMGs) from the forearm muscles and hand movements with an accelerometer (ACC) while maintaining a posture with and without a weight load. We then analyzed their power spectra and coherence. The peak tremor frequency was 6–9 Hz in seven patients and 2–3 Hz in one patient. Oscillatory movements were associated with EMG activity in five patients, but not in three patients. Weight loads and postural changes affected the tremor frequency in all patients. Tremor was classified as "reflex tremor" in five patients and "mechanical tremor" in three patients. These results suggest that peripheral factors play important roles in tremor genesis in SBMA, although its clinical features resemble essential tremor. Subclinical sensory disturbance or a decrease of motor unit numbers might be candidates for such peripheral factors contributing to tremor genesis in SBMA. © 2009 Movement Disorder Society

Key words: peripheral tremor; mechanical tremor; reflex tremor; coherence; SBMA

X-linked spinal and bulbar muscular atrophy (SBMA, Kennedy-Alter-Sung syndrome) is a slowly progressive, degenerative disorder of the lower motor neurons caused by expansion of cytosine, adenine, guanine (CAG) repeats in the first exon of the androgen receptor gene.[1–5] However, in some SBMA patients, nonmotoneuronal symptoms such as gynecomastia, muscle cramp, pain, and postural hand tremor precede muscular weakness. Among them, hand tremor is the most common initial symptom.[6–8] Patients with an initial symptom of tremor are often misdiagnosed as having essential tremor.[7] Despite the similarity of clinical features of tremor of these two types,[9] the tremor in SBMA is usually classified as "tremor syndromes in peripheral neuropathy" or as so-called "neuropathic tremor,"[10] whereas essential tremor is classified as central tremor: a tremor generated by central mechanisms. However, pathophysiological mechanisms for tremor of SBMA remain unclear.

In the present investigation, we performed frequency analyses of electromyograms (EMGs) and ACC recordings of tremor in patients with SBMA.

METHODS

Subjects

Eight patients (36–63 year old men) with SBMA were recruited. The diagnosis was confirmed genetically by expansion of CAG repeat sequences in the first exon of the androgen receptor gene.[3] Table 1 presents a summary of the clinical features of all patients, including the Medical Research Council (MRC) grading system for muscle power of the musculus extensor digitorum communis (EDC; Grade 5, full power; Grade 4, movement against gravity and

*Correspondence to: Ritsuko Hanajima, Department of Neurology, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan. E-mail: hanajima-tky@umin.ac.jp

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TABLE 1. *Clinical features of all patients*

Case	Age (yr)	Onset (yr)	CAG expansion	First symptom	Tremor onset (yr)	MRC scale of EDC	SCV (m/s) Median *N*; Ulnar *N*	SNAP (microV) Median *N*; Ulnar *N*
1	38	28	53	Tremor	28	5–	53; 52	**4.9; 3.1**
2	60	40	>40	Tremor	40	5–	57; 51	**2.5; 1.1**
3	42	20	45	Tremor	20	5–		
4	61	25	50	Tremor	25	5–	52; 51	16.3; 6.4
5	63	43	47	Muscle weakness	51	5–	59; 51	12.5; **1.9**
6	58	45	48	Muscle weakness	45	5–	52; **44**	12.5; **1.9**
7	49	20	46	Tremor	20	5–	52; 47	**6.3; 3.4**
8	47	25	43	Tremor	25	4	56; 59	**7.9; 4.6**

Normal range: SCV, 49–66 m/s and 47.0–69.0 m/s for the median and ulnar nerves; SNAP amplitude, 9.4–40.0 μV and 5.0–36.0 μV for the median and ulnar nerves.

SCV, sensory conduction velocity; SNAP, sensory nerve action potential amplitude, MRC scale, Medical Research Council grade for muscle power.

resistance; Grade 3, movement against gravity during no resistance; Grade 2, movement with gravity eliminated; Grade 1, flicker; Grade 0, total paralysis), sensory nerve conduction velocity (SCV), and sensory nerve action potential (SNAP) amplitude of the median and ulnar nerves. All patients had fine postural tremor in both hands. In all these patients, the ulnar and median sensory nerve action potentials were smaller than those of normal subjects, although they had no clinical sensory disturbance.

The experiments were performed according to the Declaration of Helsinki. The procedures were approved by the Ethics Committee of The University of Tokyo.

Recordings

We recorded surface EMGs from the upper limb muscles (e.g., EDC, musculus flexor digitorum superficialis [FDS], and musculus interossei dorsalis I [IOD1]) on the more affected side using 9-mm diameter Ag-AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the tendon. The EMG signals were amplified by an amplifier (Multi Amplifier MA 1116; Teac, Japan) through filters set at 5 and 1000 Hz. A mono-axial accelerometer (ACC, charge sensitivity 100 Hz, 1.85 pC/ms^{-2}, sense 100 PC/G, Piezoelectric accelerometer SV 1104, 9F04; NEC) was placed on the tip of the index finger or thumb to record hand movements. The ACC signal was amplified by a band pass filter between 0.2 and 30 Hz. These signals were collected through an interface (CED 1401; Cambridge Electronic Design, Cambridge, UK), digitized at a sampling rate of 2 kHz and fed to a computer for data storage and averaging using a software (Spike 2 ver. 5.05; Cambridge Electronic Design, Cambridge, UK).

Patients were seated comfortably on a reclining chair. For all patients, we recorded tremor with no load in two postures: with arms outstretched horizontally (Posture 1) and with wrist extension (Posture 2). In some patients, other postures were also examined, such as elbow flexion (Cases 3 and 7) or with the forearm-in supinated, in a palm-up posture (Cases 1 and 8). Movements were also recorded when a 1 kg weight load was imposed on the hand dorsum in all patients.

Fast Fourier transformation was performed using several epochs for 30 s. We calculated the power spectra of the rectified EMG and ACC, in addition to their coherence with FFT size 4096 using a software (Spike 2 version 5.05; Cambridge Electronic Design, Cambridge, UK). The tremor frequency was estimated by the peak of ACC power spectrum. Results obtained from different recording conditions were compared.

RESULTS

All patients showed postural tremor in the forearm and finger muscles. Although no patients had any clinical symptoms of sensory disturbance, the SNAP of the ulnar nerve was abnormally smaller in six patients; that of the median nerve was abnormally smaller in four patients.

The peak tremor frequency during Posture 1 ranged from 6 to 9 Hz in seven patients and 2–3 Hz in one patient. The EMG activities displayed coarse grouping discharges during Posture 1 (Fig. 1A). In five patients (Cases 1–5), the power of EDC EMGs had a peak frequency that was compatible with the ACC frequency peak; their coherence was significant at the same frequency (Fig. 1B, Table 2). In the other three patients (Cases 6–8), EMG spectra had no peaks at the same frequency as the ACC frequency peak (Fig. 2A).

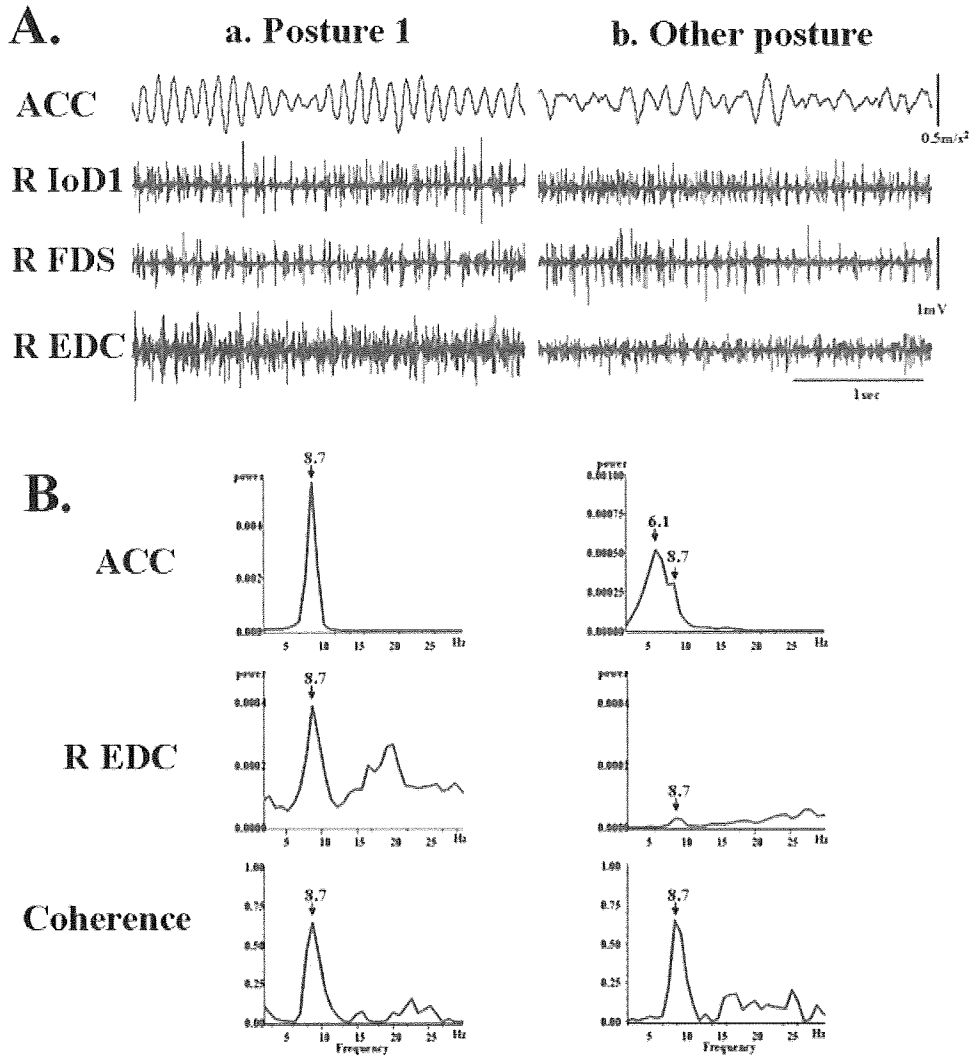

FIG. 1. (**A**) Waveforms of the ACC signal and EMGs from IOD1, FDS, and EDC for Case 4 while holding the hand in the elbow extension posture (Posture 1; **a**), and in the elbow flexion posture (other posture; **b**). The ACC signal showed oscillation of about 8.7 Hz in Posture 1. The frequency was lower in Posture 2. The EMGs tended to be grouped but were not synchronized completely with ACC oscillations. Those of EDC and FDS were neither reciprocal nor synchronizing. (**B**) Power spectra of ACC (top row), EDC EMG (second row), and coherence between ACC and EDC EMG (bottom row) in Posture 1 (left graphs) and another posture (right graphs) in Case 4. The peak tremor frequency was 8.7 Hz when holding out the arm in Posture 1. Both EDC EMG and ACC showed a power peak at the same frequency at 8.7 Hz. In Posture 2, the ACC spectrum showed a broader power increment with two peaks at 6.1 and 8.7 Hz. A small peak was observed at 8.7 Hz in the EDC EMG power spectrum. Significant coherence was observed at 8.7 Hz between EDC EMG and ACC.

The postural change from Postures 1 to 2 or other postures affected the tremor frequency in all patients. The magnitude of frequency changes was about 1 Hz for some of them (Cases 1, 2, 7, and 8), and more than 2 Hz in the others (Cases 3–5). In one patient, another peak (2.9 Hz) of tremor movement appeared in addition to the original peak in Posture 2 (Case 6; Fig. 2B). The EMG–ACC coherence was also affected by postural changes. In two patients (Cases 3 and 5), the significant coherence was not observed in Posture 2 or other postures. In contrast, significant coherence was observed only in Posture 2 in three patients (Cases 6–8). The other three patients (Cases 1, 2, and 4) showed a significant coherence in both postures. Figure 1A shows EMG and ACC activities in Case 4. When the patient kept the elbow, hand, and fingers outstretched horizontally (Posture 1), the ACC showed 8.7 Hz tremor (Fig. 1A and B). A peak was observed at 8.7 Hz in both the EMG power and coherence. When the patient flexed the elbow (the other posture), another