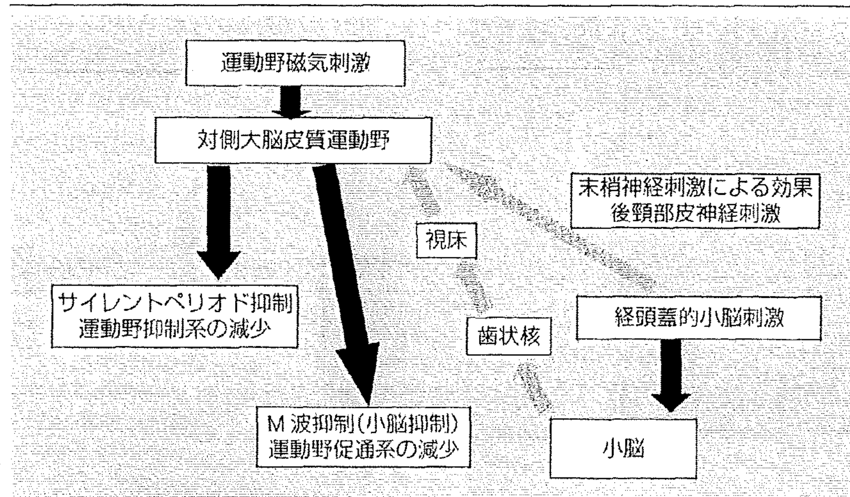
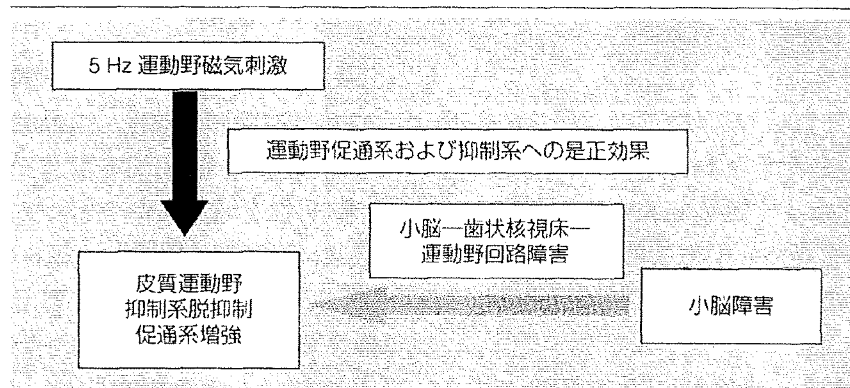
■図5　小脳刺激による大脳運動野—錐体路への効果



■図6　5 Hz高頻度経頭蓋的運動野磁気刺激による大脳運動野への効果



経頭蓋的磁気刺激の中枢神経への効果の機序

(1)低頻度小脳磁気刺激による運動野への効果

経頭蓋的に小脳刺激を行うと運動野刺激によるM波が抑制される．この抑制効果は，小脳実質，小脳遠心路などが刺激された結果，大脳皮質運動野-錐体路が抑制されたと考えられる．また，同様に，経頭蓋的小脳刺激は，抑制的反応であるサイレントペリオドの潜時を短縮するため，大脳皮質運動野の抑制系自体も抑制すると考えられる．これらの結果より，経頭蓋的小脳刺激は，運動野促通系および抑制系回路の両者に関与し，運動野-錐体路系への効果を表すと考えられる(図5)．こうした機序により，SCA 3や多系統萎縮症などの錐体路症状を呈する群への効果あるのかもしれない．

(2) SCA 6に対する高頻度経頭蓋的磁気運動野刺激による効果

正常対象では，小脳磁気刺激によって，運動野刺激によるM波は抑制される[7]が，純粋小脳型であるSCA 6においては，この小脳抑制は消失している．また，サイレントピリオドはSCA 6で延長している．従来の経頭蓋的磁気刺激による研究の結果から，小脳-歯状核-視床-運動野回路の機能は低下しており，またその結果，サイレントピリオド延長より運動野抑制系の増強あるいは抑制系への脱抑制が疑われる．

正常対象での検討では，5 Hz高頻度経頭蓋的磁気運動野刺激によって，MEP振幅は変化せず，運動野促通系および抑制系には効果を有しなかったが，一方SCA 6では，5 Hz高頻度経頭蓋的磁気運動野刺激により，安静時，筋収縮時ともに

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MEP振幅は増大することより，運動野促通系および抑制系への効果を有すると考えられた．SCA6において，小脳-歯状核-視床-運動野回路の機能は低下による運動野抑制系の増強あるいは抑制系への脱抑制は，5Hz高頻度経頭蓋的磁気運動野刺激により是正される可能性が考えられた（図6）．

脊髄小脳変性症の磁気刺激治療とリハビリテーション

脊髄小脳変性症への経頭蓋的磁気治療自体の有効性が確立されていないため，リハビリテーション（以下リハ）との併用療法についての確立されたものはない．経頭蓋的磁気刺激治療の効果は，臨床的にも効果自体が確立されていないが，有効であった症例でも効果の持続は，約1カ月程度であり，その効果を持続させることも課題である．SCDへのリハ効果は，症状の進行を遅延させ日常生活動作を維持させるために重要である．今後，経頭蓋的磁気治療法の発展とともに，リハとの併用療法の開発が必要である．

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開催案内

◎バリアフリー2008
【テーマ】高齢者・障害者の生活を快適にする福祉機器・製品を含めた総合的な福祉情報を発信します．
【日　時】4月25日（金）～27日（日）10：00～17：00
【会　場】インテックス大阪2・3・4・5号館
【事務局】バリアフリー展事務局（担当：下河内）
〒540-0008 大阪市中央区大手前1-2-15
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FAX：06-6944-9912
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◎第8回兵庫医科大学呼吸リハビリテーションセミナー
【日　時】平成20年9月19日（金）～21日（日）
【会　場】兵庫医科大学
【事務局】兵庫医科大学病院リハビリテーション部
（山崎 允）
〒663-8501 西宮市武庫川町1-1
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◎転倒予防医学研究会「第5回研究集会」
【日　時】平成20年10月5日（日）9：00～17：00
【会　場】ニッショーホール（日本消防会館）
〒105-0001 東京都港区虎ノ門2-9-16
【事務局】転倒予防医学研究会事務局
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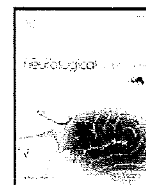

High-frequency rTMS over the supplementary motor area improves bradykinesia in Parkinson's disease: Subanalysis of double-blind sham-controlled study

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and The Effectiveness of rTMS on Parkinson's Disease Study Group, Japan

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ABSTRACT

A double-blind sham-controlled study demonstrated that high-frequency repetitive transcranial magnetic stimulation (rTMS) over the supplementary motor area (SMA) provided relief of motor symptoms in patients with Parkinson's disease (PD). However, it remains to be determined which parkinsonian symptoms were improved by this treatment. Subanalysis of Unified Parkinson Disease Rating Scale revealed that rTMS over SMA significantly improved bradykinesia in PD. Results support the hypothesis that neuronal activity of SMA was profoundly associated with hypokinetic symptoms in PD.

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1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive method used for human brain stimulation, offering potential for Parkinson's disease (PD) treatment [1]. High-frequency rTMS induces facilitation of some cortical neuronal excitability [1]. The supplementary motor area (SMA) executes complex function in motor regulation [2]; PD patients have shown SMA impairment [3–7]. In a double-blind sham-controlled study, the effect of high-frequency rTMS over SMA was compared with that of a realistic sham stimulation [8]. The SMA-stimulation group exhibited modest but significant improvements in motor symptoms: mean improvements in motor scores were 4.5 points in the SMA-stimulation group (i.e. 20% reduction from baseline) and −0.1 points in the sham-stimulation group (i.e. 0% reduction from baseline). The results implied to us that SMA is an appropriate stimulation site for PD treatment, but which symptoms were improved by SMA stimulation remains unknown. We therefore analyzed the subscores of UPDRS to clarify the nature of improvements provided by SMA stimulation.

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2. Patients and methods

2.1. Study design and patients

This study, performed at 15 centers throughout Japan, was a double-blind trial with a parallel design comparing SMA stimulation with sham stimulation. The study design — inclusion and exclusion criteria, clinical evaluations, evaluation time points, and procedures for interventions — has been described in detail [8].

In brief, all patients provided written informed consent before intervention. The protocol was approved by the ethics committee at each participating center. The inclusion criteria were idiopathic PD patients according to the British Parkinson's Disease Society Brain Bank criteria [9]. The exclusion criteria were dementia, major psychiatric illness, contraindications to TMS [10] and patients who had undergone TMS treatment prior to the study. Patients were assigned randomly to the SMA-stimulation group and sham-stimulation group at each center.

Clinical evaluations were conducted by another doctor who was completely blind to the type of intervention. All assessments were performed at the same time during the daily treatment cycle in each subject in all interventions to exclude some effects of time in daily life. The evaluation time points were selected when anti-parkinsonian drugs had some effect (neither the off state nor the best on state) to evaluate an add-on effect of rTMS to the usual treatment. Although a definite off and best on condition seem more appropriate for the treatment study, we were unable to set this level because our studied

patients were all outpatients. This possible heterogeneity might limit the validity of this study's results.

The Unified Parkinson's Disease Rating Scale (UPDRS) [11] was assessed before intervention (week 1) and immediately before the stimulation sessions at weeks 2, 4, 6, and 8. They were also assessed at weeks 10 and 12. The primary outcome measure was score changes in UPDRS part 3 (UPDRS-III). It was analyzed according to the intention-to-treat (ITT) principle using the last observation carried forward (LOCF) analysis.

One session of intervention was performed once a week for the first 8 weeks. For SMA stimulation, focal rTMS was applied using a hand-held figure-of-eight coil (9 cm external diameter at each wing) connected to a magnetic stimulator, which gives a biphasic pulse; 1000 magnetic stimuli were given in one session. One train consisted of 50 pulses at 5 Hz with inter-train interval of 50 s. The stimulus intensity was fixed at the 110% active motor threshold (AMT) for the right TA muscle. The coil was centered at points 3 cm anterior to the leg motor area in the sagittal midline. For sham stimulation, we employed a realistic sham-stimulation method [8,12].

2.2. Data analysis

We performed a subanalysis of subscores of UPDRS-III on the SMA and sham-stimulation groups. The tremor score was the sum of items 20 and 21. The rigidity score was the sum of item 22 for the neck and upper/lower limbs. Other scores were speech (item 18), facial expression (item 19), rising from chair (item 27), posture (item 28), gait (item 29), postural stability (item 30), and body bradykinesia (item 31). The "bradykinesia" score was the sum of items 23–26. Item 31 was not included because it might not reflect bradykinesia directly: it was rated by the examiner's global impression after observing spontaneous gestures while sitting, and the nature of rising and walking. The above scores at baseline (week 1) and those at week 12 were compared using two-way repeated measures analysis of variance (ANOVA) (between-subject factor, INTERVENTION (SMA/sham); within-subject factor, TIME (week)). The Greenhouse–Geisser correction was used if necessary to correct for nonsphericity. Post hoc paired t tests (2 tailed) were used for additional analyses: p values less than 0.05 were considered significant. These statistical analyses were conducted on actual values of the scores.

To evaluate possible effects of our SMA stimulus on the primary leg motor area adjacent to the SMA, we analyzed the bradykinesia score in SMA group in the following ways. First, the patients in SMA group were divided into two groups based on gait improvements (item 29). The improvement group comprised patients who showed −1 point or greater improvement in the gait score. The non-improvement group comprised patients who showed 0 points or worsening of item 29. Subsequently, we compared changes in bradykinesia scores (items 23–26) in these groups using Wilcoxon's rank sum test. We also performed Fisher's exact test to determine whether item 29 and the bradykinesia score are independent. Second, the patients in the SMA group were divided into two groups based on improvements of the lower extremity function (item 26). Here again, the improvement group comprised patients who showed −1 point or greater improvement, whereas the non-improvement group comprised patients who showed 0 points or worsening for item 26. We then compared the changes in upper extremity functions (items 23–25) in these groups using Wilcoxon's rank sum test. We also performed Fisher's exact test to determine whether score changes of items 23–25 and item 26 were independent. Finally, for general interest, we performed additional correlation analyses to explore a possible relation between the baseline UPDRS-III score and the degree of bradykinesia score response to SMA stimulus. Statistical analyses were performed using software (SPSS Statistical Package, ver. 13.0; SPSS Inc.).

3. Results

We have already shown that background clinical features such as gender, Hoehn and Yahr stage, age, age of onset, duration of illness, and initial values of UPDRS-III were not different between the two intervention groups (Table 1) [8]. The means (SD) of the modified Hoehn and Yahr stage were 2.8 (0.6) for the SMA-stimulation group, and 2.9 (0.7) for the sham-stimulation group. Of the 99 patients, one was excluded from analysis because the medical treatment was changed during intervention.

Among the subscores of UPDRS-III, a significant interaction between INTERVENTION and TIME was found only in the bradykinesia score (Table 2) (two-way repeated measures ANOVA: effect of INTERVENTION, $F_{1,96}=4.207$, $p=0.043$; effect of TIME, $F_{1,96}=9.012$, $p=0.003$; TIME × INTERVENTION interaction, $F_{1,96}=5.976$, $p=0.016$). Post hoc analysis revealed a significant improvement in the bradykinesia score at week 12. No significant interaction was found in the other subscores (Table 2).

Comparison of the bradykinesia scores (items 23–26) between gait improvement and non-improvement groups based on item 29 shows that the median of score changes in the improvement group was −3 (range, −11 to 1); that in the non-improvement group was −2 (range, −6 to 6). We found no significant difference between the two groups ($p=0.075$). Table 3 presents a 2×2 cross table of changes in item 29 and bradykinesia scores. Fisher's exact test also revealed these factors as independent ($p=0.304$). We next compared changes in upper extremity functions (items 23–25) between the lower limb function improvement and non-improvement groups. We argue that if the current over the SMA spreads to the primary motor cortex for leg muscles and if it might contribute to bradykinesia score improvement, then it would present some dissociation between the changes in these scores. The median of score changes in the improvement group was −2 (range, −9 to 2); that in the non-improvement group was −1 (range, −4 to 5). We found a significant difference between the two groups ($p=0.024$), indicating that the changes in items 23–25 were associated with those in item 26. Table 4 shows a 2×2 cross table of changes in items 23–25 (upper extremity functions) and item 26 (lower extremity function). Fisher's exact test revealed that these factors are dependent ($p=0.039$). Finally, no significant correlation was found between baseline UPDRS-III scores and changes in the sum of items 23–26 (bradykinesia score) (correlation coefficient, −0.222, $p=0.103$).

Table 1
Baseline Characteristics of Patients.

	SMA group ($N=55$)	Sham group ($N=43$)
Age (year)		
Mean (SD)	65.3 (8.9)	67.4 (8.5)
Median (range)	66 (39–82)	69 (43–82)
Interquartile range	59.0–71.5	63.5–72.5
Male sex – no. (%)	29 (53)	25 (58)
Age of onset (year)		
Mean (SD)	57.2 (9.9)	59.5 (10.2)
Median (range)	58 (28–78)	61 (34–79)
Interquartile range	50.0–65.0	56.0–66.5
Duration of illness (year)		
Mean (SD)	8.1 (4.2)	7.8 (6.7)
Median (range)	8.0 (1–16)	5.0 (1–32)
Interquartile range	5.0–11.0	3.0–10.5
Hoehn–Yahr stage – no. (%)		
1	0 (0)	0 (0)
2	19 (34.5)	13 (30.2)
3	33 (60.0)	23 (53.5)
4	3 (5.5)	7 (16.3)
5	0 (0)	0 (0)

No significant difference was found between two groups for any parameter.
SMA, Supplementary motor area.

Table 2
ANOVA results.

Subscores of UPDRS-III	Item	SMA		Sham			df	F	p
		Baseline mean (SD)	Week 12 mean (SD)	Baseline mean (SD)	Week 12 mean (SD)				
Speech	18	0.87 (0.64)	0.92 (0.53)	1.05 (0.82)	1.16 (0.84)	TIME	1	2.907	0.091
						INTERVENTION	1	2.311	0.132
						TIME×INTERVENTION	1	0.380	0.539
Facial expression	19	1.12 (0.69)	1.10 (0.55)	1.40 (0.82)	1.35 (0.87)	TIME	1	0.470	0.495
						INTERVENTION	1	3.339	0.056
						TIME×INTERVENTION	1	0.007	0.933
Tremor	20,21	4.84 (3.65)	4.20 (3.01)	5.20 (4.10)	5.23 (4.38)	TIME	1	1.566	0.214
						INTERVENTION	1	2.099	0.151
						TIME×INTERVENTION	1	2.099	0.151
Rigidity	22	4.85 (3.18)	3.72 (2.77)	5.53 (3.63)	4.90 (3.72)	TIME	1	11.435	0.001
						INTERVENTION	1	2.239	0.138
						TIME×INTERVENTION	1	0.926	0.338
Bradykinesia	23–26	7.82 (3.68)	6.00 (4.07)	8.77 (4.90)	8.58 (5.59)	TIME	1	9.012	0.003
						INTERVENTION	1	4.207	0.043
						TIME×INTERVENTION	1	5.976	0.016
Arising from chair	27	0.60 (0.65)	0.53 (0.76)	0.86 (0.94)	0.98 (0.96)	TIME	1	0.104	0.747
						INTERVENTION	1	5.308	0.023
						TIME×INTERVENTION	1	1.965	0.164
Posture	28	1.20 (0.80)	0.98 (0.69)	1.51 (0.98)	1.44 (0.93)	TIME	1	6.818	0.010
						INTERVENTION	1	5.185	0.025
						TIME×INTERVENTION	1	1.811	0.182
Gait	29	1.15 (0.79)	1.04 (0.69)	1.30 (0.74)	1.30 (0.77)	TIME	1	0.996	0.321
						INTERVENTION	1	2.161	0.145
						TIME×INTERVENTION	1	0.996	0.321
Postural stability	30	1.12 (0.85)	0.93 (0.89)	1.21 (0.99)	1.21 (0.91)	TIME	1	1.816	0.181
						INTERVENTION	1	1.225	0.271
						TIME×INTERVENTION	1	1.816	0.181
Body bradykinesia	31	1.52 (0.79)	1.13 (0.77)	1.69 (0.99)	1.53 (0.93)	TIME	1	19.800	<0.001
						INTERVENTION	1	3.095	0.082
						TIME×INTERVENTION	1	3.517	0.064

4. Discussion

The present results showed that, in comparison to the sham stimulation, significant improvements in bradykinesia were induced by the SMA stimulation.

The pathophysiology of parkinsonian motor symptoms remains a matter of controversy [13]. Hypokinetic symptoms are apparently implicated in impaired activity of the SMA, presumably ascribed to decreased positive efferent feedback arising from the basal ganglia-thalamocortical motor loop [3–7]. The fact that only the bradykinesia scores were significantly decreased by the SMA stimulation concurs with the view that hypokinetic symptoms are associated with the SMA dysfunction in PD patients [2–7]. Furthermore, these improvements were observed two weeks after the end of the rTMS protocol. Although the mechanism of this delay remains to be determined, possible cumulative effects of rTMS and a long-lasting effect of rTMS, which lasted up to 8 days in the primate brain [14], might partly explain this delay.

We noted at least four limitations of this study. First, the SMA might not be stimulated or other parts might be affected, although the effects should be derived mainly from modulation of neuronal activity of SMA, according to several precedent reports [15]. Moreover, the evaluation of possible effects of our SMA stimulus on the primary leg motor area showed that our SMA stimulus produced substantial effects on motor functions of the upper extremities as well as on the lower extremities. Based on these observations, improvement in bradykinesia is ascribed to modulation of motor functions of upper and lower extremities. Second, more data related to UPDRS scores (in the off and best on) should be provided. Consequently, the improvement might well be attributable simply to the medication and not to rTMS of the SMA. However, it was impossible to assess an off state in our patients because all outpatients had difficulty in making hospital visits during off states. Furthermore, the baseline scores of assessments did not differ between SMA and sham groups. Importantly, we found no significant effect of the stage of the disease on the UPDRS score changes [8]. No significant correlation was found between the baseline state and the response of bradykinesia to our SMA stimulus. Third, the structure of the UPDRS motor part might be inappropriate to assess (possible) improvements of other motor symptoms (e.g. gait, postural stability,) which contain a single score item. The assessment might therefore lack sufficient sensitivity to detect small changes. Finally, recent studies of rTMS over SMA with small numbers of PD patients revealed worsening of complex movements [16]. That discrepancy might be ascribed to methodological differences such as the coil orientation (handle pointing laterally in this study versus no description in the previous study [16]), stimulus intensity (110% AMT for foot muscles in this study versus various stimulus intensities of 58–110% resting motor threshold for hand muscles in the earlier study [16]), stimulus frequency (5 Hz in

Table 3
2×2 cross table of changes in item 29 and bradykinesia score.

		Item 29 (gait)		Sum
		Improvement	Non-improvement	
Bradykinesia score (items 23–26)	Improvement	10	28	38
	Non-improvement	2	15	17
Sum		12	43	55

Table 4
2×2 cross table of changes in items 23–25 (upper extremity functions) and item 26 (lower extremity function).

		Item 26 (L/E)		Sum
		Improvement	Non-improvement	
Items 23–25 (U/E)	Improvement	24	5	29
	Non-improvement	14	12	26
Sum		38	17	55

this study versus 10 Hz [16]), session numbers (multiple sessions versus single session [16]), timing of evaluation (two weeks after rTMS versus immediately after rTMS session [16]), and the number of subjects (99 patients in this study, but only 10 subjects in the previous study [16]).

Although some shortcomings limit the scientific validity of this study, the SMA stimulation might exert modest improvement of hypokinetic symptoms in PD. These results support the hypothesis that neuronal activity of SMA is associated with hypokinetic symptoms in PD.

Acknowledgments

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Appendix A

The following doctors and institutions participated in the Group to Study Effectiveness of rTMS on Parkinson's Disease, Japan.

Principal investigator: Tsuji S

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Collaborators: Komori T, Chuma T, Kitagawa M, Matsunaga K, Saito Y, Sugiyama N, Miyagi Y, Tanaka T, Okabe S, Hamada M

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Primary motor cortical metaplasticity induced by priming over the supplementary motor area

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Motor cortical plasticity induced by repetitive transcranial magnetic stimulation (rTMS) sometimes depends on the prior history of neuronal activity. These effects of preceding stimulation on subsequent rTMS-induced plasticity have been suggested to share a similar mechanism to that of metaplasticity, a homeostatic regulation of synaptic plasticity. To explore metaplasticity in humans, many investigations have used designs in which both priming and conditioning are applied over the primary motor cortex (M1), but the effects of priming stimulation over other motor-related cortical areas have not been well documented. Since the supplementary motor area (SMA) has anatomical and functional cortico-cortical connections with M1, here we studied the homeostatic effects of priming stimulation over the SMA on subsequent rTMS-induced plasticity of M1. For priming and subsequent conditioning, we employed a new rTMS protocol, quadripulse stimulation (QPS), which produces a broad range of motor cortical plasticity depending on the interval of the pulses within a burst. The plastic changes induced by QPS at various intervals were altered by priming stimulation over the SMA, which did not change motor-evoked potential sizes on its own but specifically modulated the excitatory I-wave circuits. The data support the view that the homeostatic changes are mediated via mechanisms of metaplasticity and highlight an important interplay between M1 and SMA regarding homeostatic plasticity in humans.

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Abbreviations CS, conditioning stimulus; FDI, first dorsal interosseous muscle; ICF, intracortical facilitation; ISI, inter-stimulus intervals; ITT, inter-train interval; LICI, long-interval intracortical inhibition; LTD, long-term depression; LTP, long-term potentiation; M1, primary motor cortex; MEP, motor-evoked potential; PMd and PMv, dorsal and ventral premotor cortex; QPS, quadripulse stimulation; rTMS, repetitive transcranial magnetic stimulation; SICF, short-interval intracortical facilitation; SICI, short-interval intracortical inhibition; SMA, supplementary motor area; TA, ~~right~~ tibialis anterior muscle; TS, test stimulus.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a promising method to induce plastic changes in humans (Hallett, 2007). In some cases, the rTMS-induced plasticity is *N*-methyl-D-aspartate (NMDA) dependent, supporting the idea that changes in synaptic efficacy, such as long-term potentiation (LTP) and long-term depression (LTD), are implicated in rTMS-induced plasticity (Stefan *et al.* 2002; Huang *et al.* 2007). Several human studies have also shown effects of a prior history of neuronal activity on subsequent rTMS-induced plasticity (e.g. Siebner *et al.* 2004; Hamada *et al.* 2008*a*). These findings have been compared with metaplasticity, homeostatic regulation of synaptic plasticity in which the capacity of synapses to exhibit plasticity depends on prior levels of neuronal activity (Abraham & Bear, 1996; Ziemann & Siebner, 2008). This form of plasticity regulation might relate directly to the Bienenstock–Cooper–Munro (BCM) theory, a prevailing model for homeostatic mechanisms of synaptic plasticity (Bienenstock *et al.* 1982; Abraham, 2008).

The protocol for studying metaplasticity in an experimental context is to apply a period of priming

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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

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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

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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

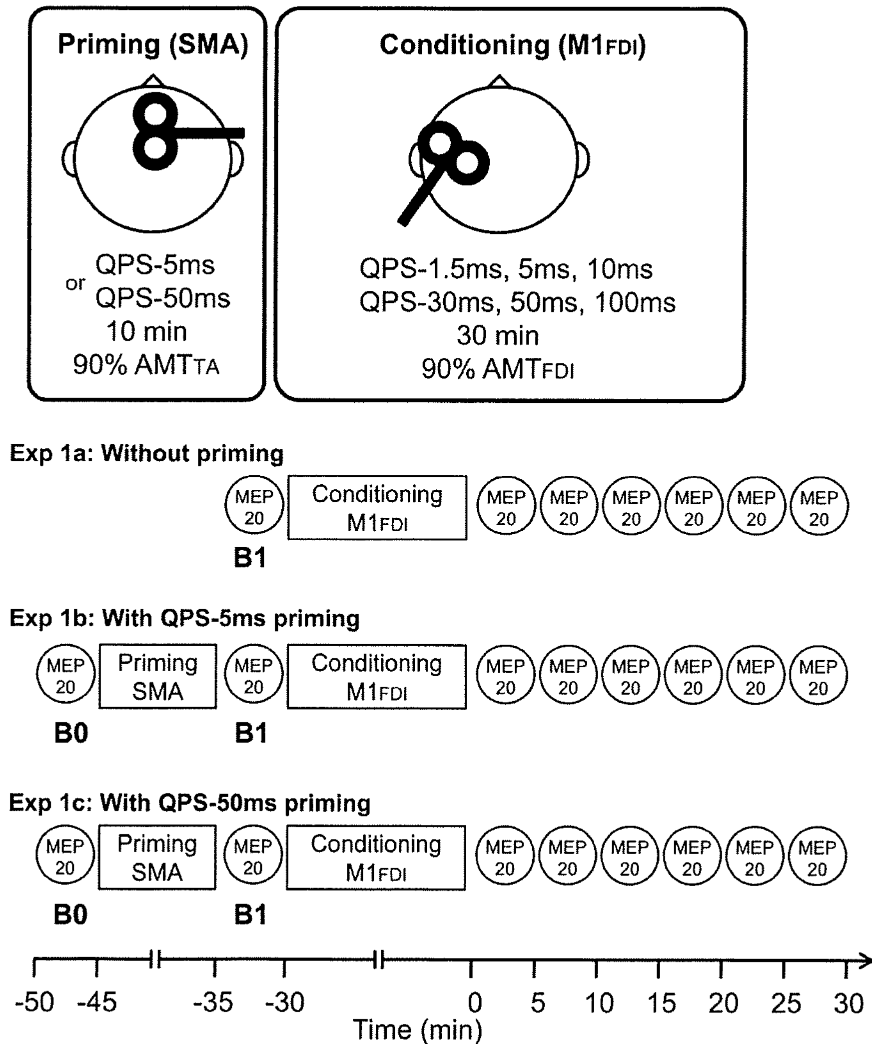

Figure 1. Timelines of experiments
(See Methods.)

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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

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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.

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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

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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*).

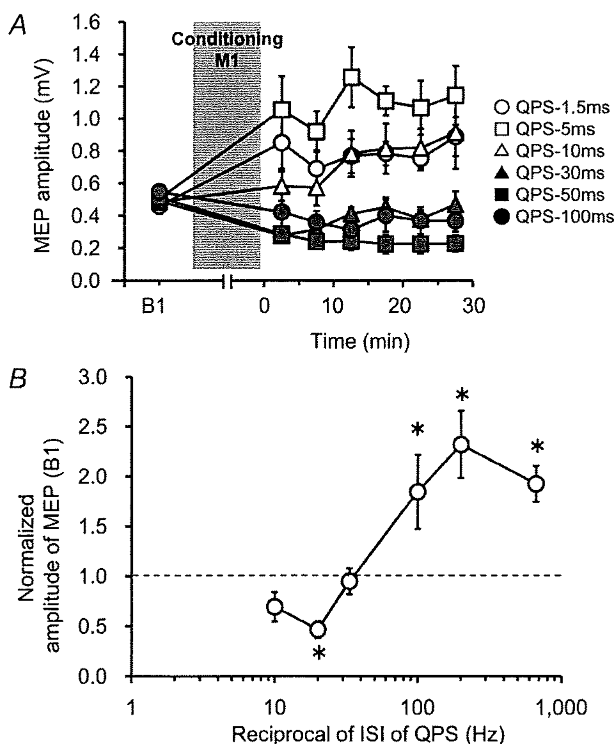

Figure 2. QPS-induced plasticity without priming over SMA
A, time courses of MEP amplitude following QPS at various ISIs without priming (mean ± S.E.M.). *B*, stimulus–response function of QPS-induced plasticity. Normalized amplitudes of MEP measured at 30 min after QPS as a function of the reciprocal 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

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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.

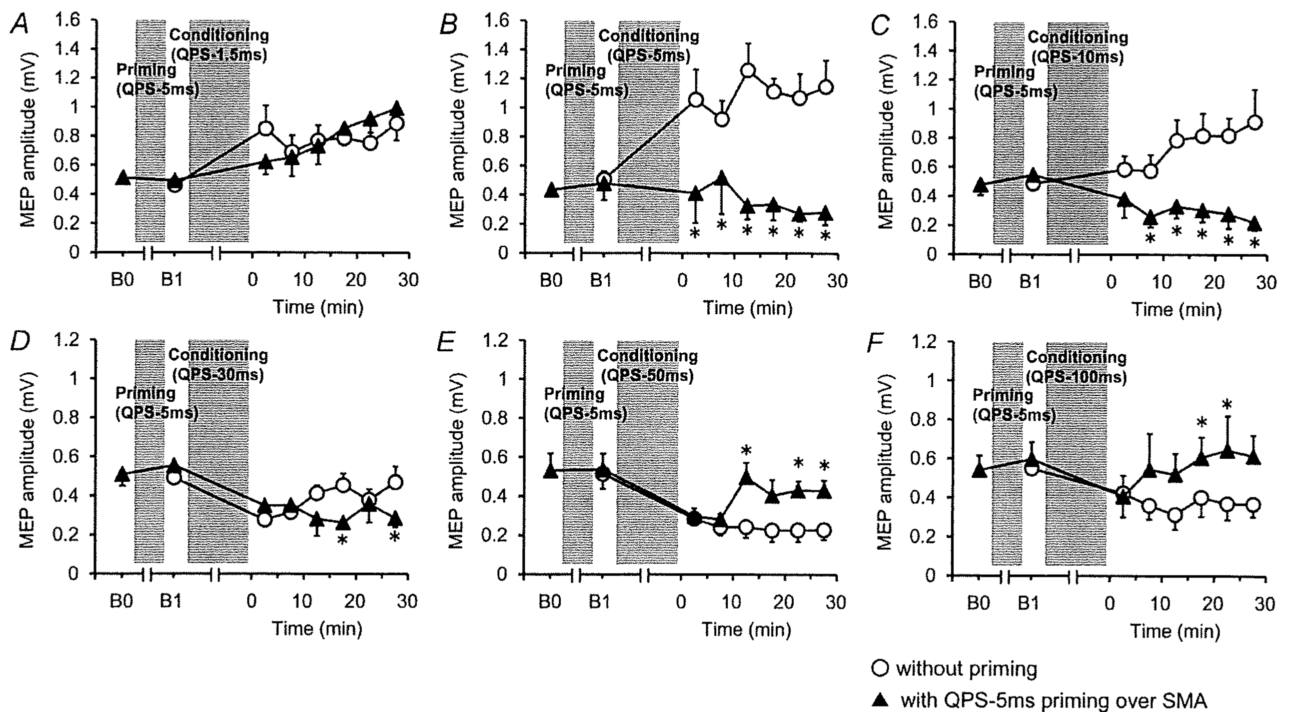

Figure 3. Effects of QPS-5 ms priming over SMA on QPS-induced plasticity
Time courses of MEP amplitude following QPS at various ISIs with (▲) and without (○) QPS-5 ms priming over SMA (mean ± S.E.M.). *A*, SMA priming did not change subsequent LTP-like plasticity induced by QPS-1.5 ms. *B* and *C*, priming 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).

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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.

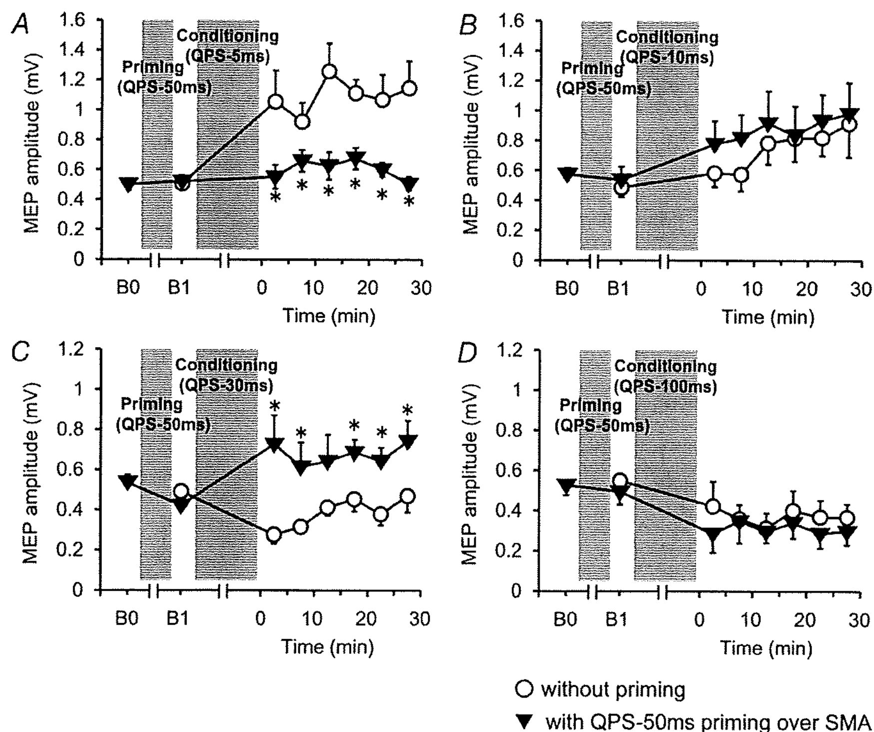

Figure 4. Effects of QPS-50 ms priming over SMA on QPS-induced plasticity
Time courses of MEP amplitude following QPS at various ISIs with (▼) and without (○) QPS-50 ms priming over SMA (mean ± S.E.M.). *A*, SMA priming occluded subsequent LTP-like plasticity induced by QPS-5 ms. *B*, priming did not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

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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.

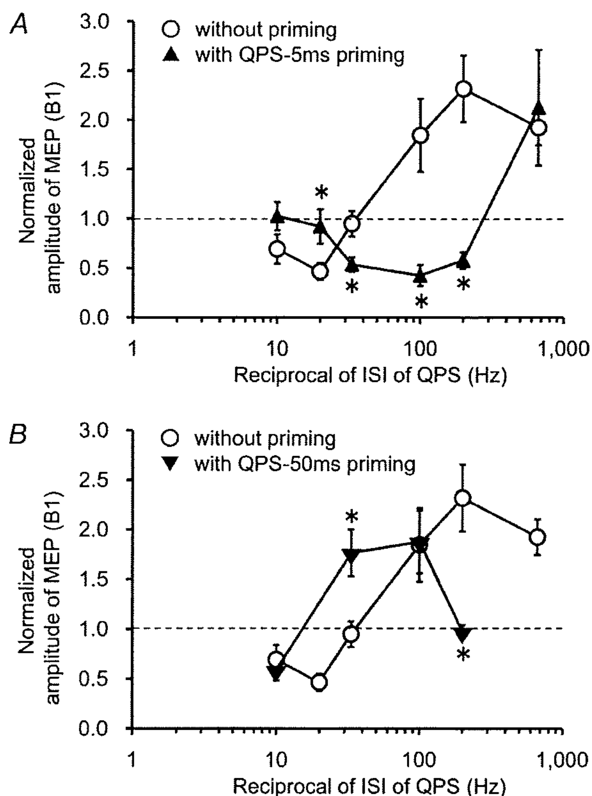

Figure 5. Priming-induced shifts in the stimulus–response function

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

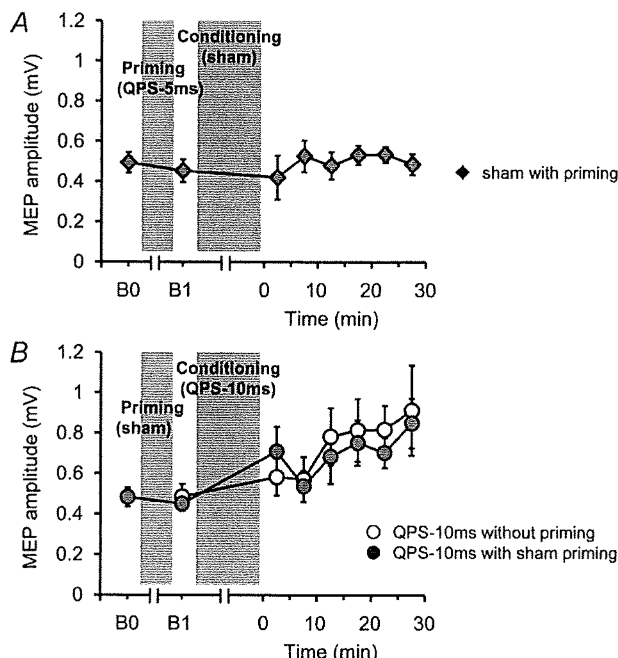

Figure 6. Control experiments

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).

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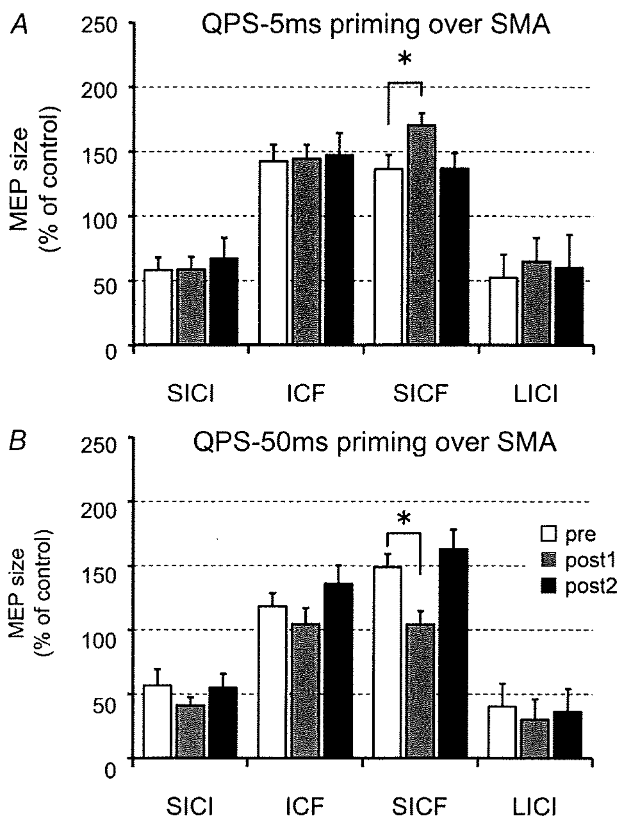

Figure 7. Effects of priming over SMA on intracortical circuits of M1

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

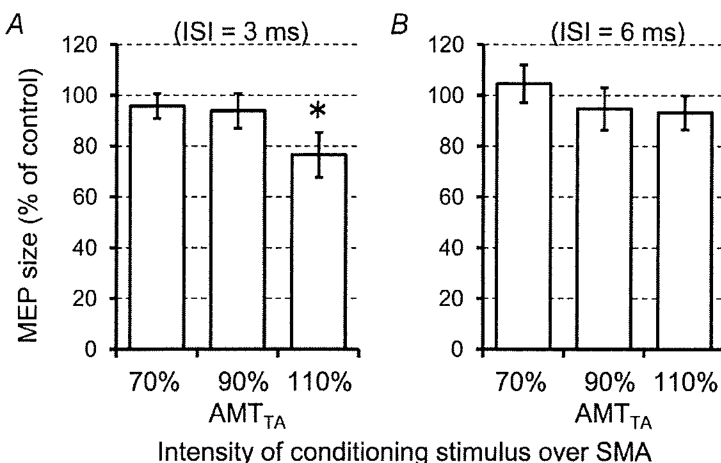

Figure 8. Effects of a conditioning stimulus over SMA on MEP

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.

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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).

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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

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