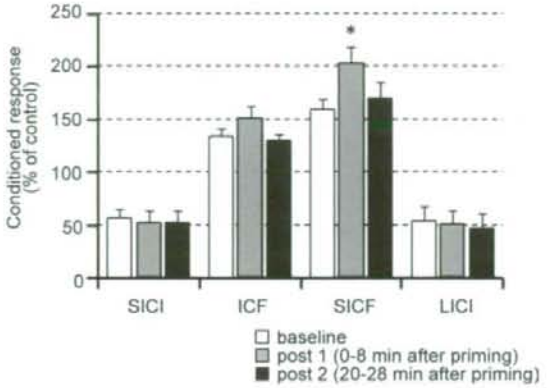

Figure 7. Effects of QPS-5 ms for 10 min on SICI, ICF SICF LICI
Neither SICI, ICF, nor LICI was altered by 10 min QPS-5 ms alone. SICF was enhanced after QPS-5 ms for only 10 min. Baseline (open bars); post 1 (grey bars), 0–8 min after QPS; post 2 (black bars), 20–28 min after QPS. $*P < 0.05$ by *post hoc* Dunnett's test.

(Bienenstock *et al.* 1982). A similar non-linear function has been demonstrated in the visual cortex (Kirkwood *et al.* 1996) and the hippocampus (Wang & Wagner, 1999; Zhang *et al.* 2005). These findings indicate that a BCM-like non-linear relation of synaptic plasticity to stimulation frequency is a fundamental characteristic of synaptic plasticity, although the critical factors for inducing synaptic plasticity are probably the integrated postsynaptic depolarization and Ca^{2+} entry, and not the stimulation frequency *per se* (Bear, 1996).

Possible mechanism of QPS-induced plasticity

Despite the general concordance of the non-linear property of the stimulus–response function of QPS with those of animal studies of synaptic plasticity, the lack of direct recording of synaptic response in conscious humans renders any hypothesis explaining precise neuronal mechanisms underlying rTMS-induced or QPS-induced plasticity speculative (Cooke & Bliss, 2006). Nevertheless, results obtained through the present study might provide some evidence that favours the long-term alteration of synaptic efficacy as the mechanism of QPS-induced plasticity.

First, motor thresholds which were not altered by QPS are considered to reflect the membrane excitability of postsynaptic neurons (Mavroudakis *et al.* 1994, 1997; Ziemann *et al.* 1996*b*; Chen *et al.* 1997). Consequently, general changes in membrane excitability, which play an important role in motor learning (Woody *et al.* 1991; Aou *et al.* 1992), might not be the main mechanism for QPS-induced plasticity, which is consistent with the results of our previous work (Hamada *et al.* 2007*b*). Second, QPS caused bidirectional modulation of the recruitment curves. The slope of the curve depends on the distribution of cortical neurons' excitability; its synaptic connectivity is a possible factor causing changes of this curve. Third, QPS-induced plasticity was topographically specific to the stimulation site, indicating one basic property of synaptic plasticity: *input specificity* (Bliss & Collingridge, 1993). Fourth, the plastic changes of QPS lasted for about 75 min. This persistence of plasticity might be comparable to that of LTP, rather than that of post-tetanic or short-term potentiation (Bliss & Collingridge, 1993).

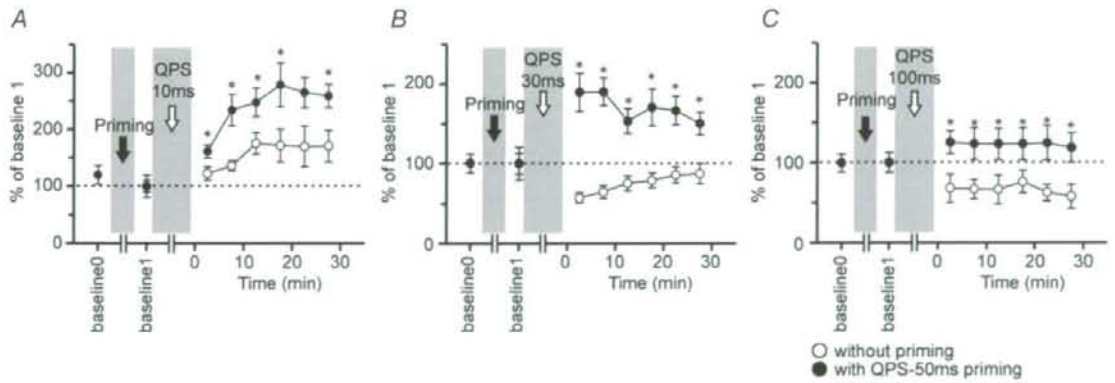

Figure 8. QPS-50 ms priming effects on QPS-induced plasticity ($n = 6$)
A–C, time courses of MEP amplitude following QPS at various intervals with (●) and without QPS-50 ms priming (○). Asterisks denote significant difference of MEP sizes with priming from those without priming ($P < 0.05$ by *post hoc* paired *t* tests). *A*, QPS-10 ms; *B*, QPS-30 ms; *C*, QPS-100 ms.

Fifth, the results of paired-pulse measurements to study intracortical excitability (Experiment 2c) revealed that QPS mainly modulated excitatory circuits within the primary motor cortex. Because SICI, which is considered to reflect γ-aminobutyric acid (GABA)-ergic inhibitory function of the human motor cortex (Kujirai *et al.* 1993; Ziemann *et al.* 1996*a*,*b*; Hanajima *et al.* 1998), remained unchanged (Fig. 3), modulation of the GABA-ergic system is probably not responsible for the MEP changes after QPS. Several reports have demonstrated that SICI is differentially modulated by various rTMS protocols which induce LTP or LTD-like plasticity. For example, SICI was altered by the theta burst stimulation (TBS) protocol (Huang *et al.* 2005), whereas SICI did not show any changes after the paired associative stimulation (PAS) (Stefan *et al.* 2002). These data provide corroborating evidence that lasting MEP modulation induced by several interventions in humans is not always conferred by some alteration of the GABA-ergic system contributing to SICI, even though GABA-ergic blockers are frequently required to induce LTP in neocortex in animal studies (Kirkwood & Bear, 1994; Hess *et al.* 1996).

By contrast, both ICF and SICF were altered by QPS protocols. Although ICF has been considered to be produced at the motor cortex (Kujirai *et al.* 1993; Ziemann *et al.* 1996*c*), the origin of ICF is still unclear since there were no changes in amplitude or number of descending volleys by ICF (Di Lazzaro *et al.* 2006). Therefore, we cannot draw any firm conclusion about the mechanism for ICF modulation by QPS. Firmer conclusions can perhaps be drawn from the studies of SICF.

SICF has been proposed to be caused by an interaction of I-wave inputs (Tokimura *et al.* 1996; Ziemann *et al.* 1998; Hanajima *et al.* 2002), probably by summation of excitatory postsynaptic potentials (EPSPs) elicited by the first suprathreshold TMS with subliminal depolarization of interneurons elicited by the second subthreshold TMS at cortical interneurons (Hanajima *et al.* 2002). Modulation of SICF after QPS would therefore be consistent with the idea that QPS alters the efficiency with which I-waves summate during paired-pulse TMS and would support the view that the effects of QPS involve changes at intracortical synapses. However, at some ISIs (e.g. QPS-5 ms), the trough of SICF was also substantially modulated as well as its peak (Fig. 4). In such cases, as at QPS-5 ms, it may still be possible that QPS did not specifically enhance intracortical excitatory circuits reflected by SICF, but changed the excitability of cortical interneurons and/or cortical output neurons undetectable by MT measurements and which in turn modulated both the peak and the trough of SICF. It should be noted that in these measurements of intracortical excitability, the intensity of the test stimulus after QPS was adjusted to match the amplitudes of test responses before QPS conditioning, so that the difference in test MEP sizes after QPS cannot contribute to the after-effects on ICF and SICF or the lack of changes in SICI.

Taken together, the points described above lead us to surmise that QPS mainly modulates the excitatory circuits of the primary motor cortex. We consider that the mechanism of QPS-induced plasticity involves long-term synaptic plasticity in those circuits with features of non-linear dependence on ISI of QPS, which is reminiscent of previous findings of synaptic plasticity in animal studies (Dudek & Bear, 1992). Further studies with pharmacological interventions are necessary to address synaptic mechanisms in more detail.

Metaplasticity of QPS-induced plasticity

The second main finding of this study was that priming stimulation that itself had no effect on MEP amplitude led to a shift in the stimulus–response function of QPS. QPS-5 ms priming shifted the function to the right along the x-axis, such that suppressive plastic changes were promoted over a wider range of ISI than before priming. By contrast, QPS-50 ms priming shifted the curve to the left, with the effect that

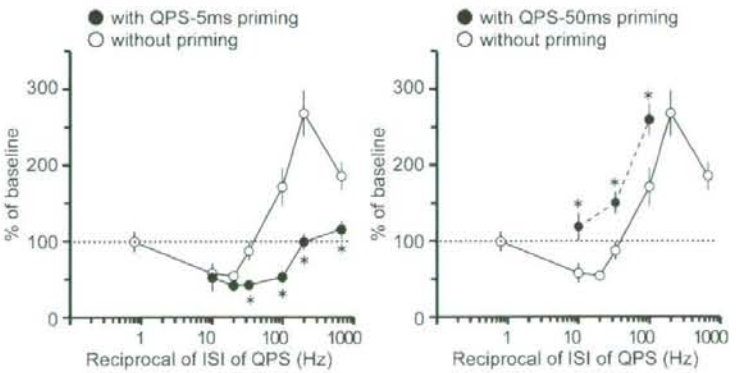

Figure 9. Priming-induced shifts in the stimulus–response function
Left, the normalized amplitudes of MEP at 30 min post conditioning as a function of the reciprocal of ISI of QPS with (●) and without QPS-5 ms priming (○) ($n = 6$). Right, the normalized amplitudes of MEP at 30 min post conditioning as a function of the reciprocal of ISI of QPS with (●) and without QPS-50 ms priming (○) ($n = 6$). *$P < 0.05$ by *post hoc* paired t tests.

facilitatory plastic changes were favoured over suppressive ones.

Effects of QPS-5 ms priming alone on excitatory and inhibitory circuits

Two control experiments revealed that neither priming alone nor cutaneous stimulation produced by the TMS pulses had any lasting effect on MEPs. Furthermore, QPS-5 ms priming did not change SICI, ICF or LICI. Its only effect was a transient increase in 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 intracortical excitability were only assessed at a single ISI with a single conditioning intensity. In addition, determination of SICI and LICI resulted in about 50% inhibition at baseline measurements (Fig. 6), which is submaximal for SICI and LICI according to previous paired-pulse studies (Valls-Sole *et al.* 1992; Kujirai *et al.* 1993; Wassermann *et al.* 1996), thus excluding a possible floor effect in the results. Another possibility to be ruled out is that the repeated application of suprathreshold TMS pulses during MEP measurements might have influenced priming and conditioning effects. However, such measurements do not alter SICF so that its effects, if present at all, should be negligible.

Priming-induced effects on subsequent QPS-induced plasticity

A homeostatic relationship between the prior history of neuronal activity and subsequent QPS-induced plasticity is consistent with the BCM theory, which states that θ_M, the point of crossover from LTD to LTP, is not fixed, but varies as a function of the activation history of postsynaptic neurons (Bienenstock *et al.* 1982). Compelling evidence now supports *sliding* θ_M, which is exemplified by the shift in crossover point of the frequency–response function of synaptic plasticity in conditioning frequency–response experiments (Kirkwood *et al.* 1996; Wang & Wagner, 1999; Zhang *et al.* 2005). According to the BCM model of sliding θ_M in animal experiments, we propose that priming stimulation transiently modulates neuronal activity of the human primary motor cortex and this prior history of cortical activity determines the sign of subsequent QPS-induced plasticity: QPS-5 ms priming transiently enhanced cortical activity, which led the following QPS conditioning to favour suppressive plastic changes. By contrast, QPS conditioning favoured facilitatory plastic changes after QPS-50 ms priming which might transiently reduce cortical activity.

Theoretically, the stimulus–response function should move only to the left or right along the horizontal axis as a function of postsynaptic activity (Bienenstock *et al.* 1982), but our stimulus–response function also seems to shift vertically (Fig. 9). Until further ISIs are tested we cannot comment in detail on this effect. However, a vertical shift in frequency–response function of synaptic plasticity has also been found in several animal studies and it has been proposed that its mechanism differs from that of the horizontal shift (Philpot *et al.* 2003; Zhang *et al.* 2005). For example, the priming-induced effects in the present study might be related to the substantial modulation of α-Ca^{2+}/calmodulin-dependent kinase II (αCaMKII) in activity-dependent form, which has been revealed to be a pivotal component for the vertical shifts in hippocampal slices (Zhang *et al.* 2005).

Duration of QPS conditioning: inverted-U relationship

We have additionally shown that QPS-5 ms for 20 min or 40 min did not produce any MEP size changes, whereas QPS-5 ms for 30 min induced LTP-like plasticity. The amount of facilitation and the duration of QPS conditioning showed an inverted U-shaped relationship (Fig. 6*D*), indicating that there is a threshold for inducing LTP-like plasticity, in line with our previous reports (Hamada *et al.* 2007*b*). These findings are consistent with previous animal studies. Christie *et al.* (1995) revealed that LTP induction depended on the number of TBS trains: 2 trains of TBS produced no plasticity and 8 TBS trains induced maximal LTP, while 16 TBS trains produced less LTP in the hippocampal slices. Another study showed that peak amounts of LTP occurred after 8–16 TBS trains, but 24 or 32 TBS trains produced no LTP (Abraham & Huggett, 1997). These lines of evidence suggest an inverted U-shaped relation between the amount of TBS and the degree of LTP (Christie *et al.* 1995). Such a time-dependent LTP reversal process (or the over-stimulation effect) was probably attributable to a depotentiation mechanism during the massed presentation of tetanic stimulation (Abraham & Huggett, 1997). Likewise, the after-effects of QPS-5 ms for 40 min might entail a similar mechanism.

The gap between priming and primary conditioning

The fact that the effects of QPS-5 ms for 40 min were comparable to those of QPS-5 ms for 30 min with 10 min QPS-5 ms priming suggests that the time gap between priming and subsequent primary conditioning is unnecessary. This finding also raises the intriguing possibility that the first part of the QPS conditioning might 'prime' subsequent conditioning. However, without data at different ISIs, it is difficult to comment further on this point.

Relationship to previous human studies of metaplasticity

The present findings with regard to the shifts in stimulus–response function of QPS-induced plasticity are in harmony with those of previous human studies which have revealed a similar effect of priming stimulation on subsequent rTMS-induced after-effects, suggesting metaplasticity of the human primary motor cortex. High-frequency rTMS priming enhanced the transient suppressive effect of 1 Hz rTMS (Iyer *et al.* 2003). The polarity of transcranial direct current stimulation (Nitsche & Paulus, 2001) determines the direction of subsequent after-effects of 1 or 5 Hz rTMS, which imparted no effect on motor cortical excitability when applied alone (Lang *et al.* 2004; Siebner *et al.* 2004). Reportedly, priming using PAS affected subsequent PAS-induced plasticity (Müller *et al.* 2007). Although they have explored only a limited number of rTMS protocols for primary conditioning (Iyer *et al.* 2003; Lang *et al.* 2004; Siebner *et al.* 2004; Müller *et al.* 2007), they have all used priming which itself induced LTP or LTD-like phenomena.

Based on the experimental evidence of metaplasticity, however, priming which does not itself change the basic synaptic efficacy can also alter the subsequent synaptic plasticity (Huang *et al.* 1992; Abraham & Tate, 1997; Wang & Wagner, 1999). It has also been suggested that the investigation of metaplasticity is facilitated when the priming stimulation does not alter the strength of synaptic transmission, because if LTP is produced by the priming stimulation, it is difficult to preclude the possibility that a lack of further LTP induction by tetanic stimulation is attributable to the ceiling effect of LTP (i.e. saturation of LTP) or a homeostatic mechanism that entails active inhibition of LTP (Abraham, 2008). Consistent with these results in animals, the critical new finding of the present investigation is that priming stimulation over the primary motor cortex that did not itself change MEP sizes (but altered SICF for a short period), has a large impact on subsequent QPS-induced plasticity. Thus, prior induction of LTP- or LTD-like phenomena is unnecessary to induce metaplastic changes in humans. Our findings are, at least partly, consistent with recent studies which have shown that voluntary muscle contraction (which may be a consequence of cortical activity enhancement of various motor-related areas) that is not enough to induce lasting effects on motor cortical excitability influenced the subsequent after-effects induced by TBS (Gentner *et al.* 2007; Huang *et al.* 2008). Although a potential weakness of the study is that the interpretation of the present data is speculative and not based upon experimental data on human motor cortical function, our new protocol, giving bidirectional after-effects with prolonged duration, enables us to test the effect of priming over a wide range. In fact, we showed that priming stimulation induced bidirectional shifts of the non-linear stimulus–response function of motor cortical plasticity in agreement with those revealed in animal studies of metaplasticity.

Safety issues

No subject reported any adverse effects during or after any intervention. Moreover, the spread of excitation to proximal muscles was not observed. We have previously shown that QPS at 1.5 ms with higher intensity than in the present study (using $130 \pm 24\%$ AMT, that is $82 \pm 7\%$ RMT: 74–98% RMT) can safely induce motor cortical plasticity in normal subjects with regard to seizure induction, because (1) no spread of excitation was observed, and (2) the occurrence rate of post-TMS EMG activity, which was thought to be a possible correlate of after-discharge, was not different from those during sham stimulation (Hamada *et al.* 2007*b*). These findings provide evidence that QPS can safely induce motor cortical plasticity. Obviously, adequate EMG monitoring is absolutely imperative to recognize early signs of seizure during future rTMS studies. Recent advances in rTMS devices heighten the need for establishing new safety guidelines for complex rTMS protocols such as QPS (Hamada *et al.* 2007*b*), paired pulse stimulation (PPS) (Thickbroom *et al.* 2006; Hamada *et al.* 2007*a*) and TBS (Huang *et al.* 2005).

Conclusions

The mechanism of QPS-induced plasticity favours long-term synaptic plasticity with features of non-linear dependence on stimulation frequency. Priming elicited bidirectional shifts in the stimulus–response function of motor cortical plasticity. The data support a BCM-like model of priming that shifts the crossover point at which the synaptic plasticity reverses from LTD to LTP. Such a broad range of after-effects produced by the new rTMS protocol opens up new possibilities for examining the details of metaplasticity theories in humans.

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ORIGINAL REPORT

INHIBITION OF THE UNAFFECTED MOTOR CORTEX BY 1 HZ REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ENHANCES MOTOR PERFORMANCE AND TRAINING EFFECT OF THE PARETIC HAND IN PATIENTS WITH CHRONIC STROKE

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Objective: **Recent reports demonstrated that low-frequency repetitive transcranial magnetic stimulation (rTMS) over the unaffected hemisphere improved the affected hand function in chronic stroke patients. We investigated whether 1 Hz rTMS improved the motor learning of the affected hand in patients after stroke.**

Design: **A double-blind study.**

Patients: **Twenty patients with chronic subcortical stroke.**

Methods: **The patients were randomly assigned to receive either a sub-threshold rTMS over the unaffected hemisphere (1 Hz, 25 minutes) or sham stimulation, and all patients performed a pinching task after stimulation. We evaluated the motor function of the affected hand and the excitatory and inhibitory function of the affected motor cortex by transcranial magnetic stimulation.**

Results: **Compared with sham stimulation, rTMS induced an increase in the excitability of the affected motor cortex ($p<0.001$) and an improvement in acceleration of the affected hand ($p=0.006$). Moreover, the effect of motor training on pinch force was enhanced by rTMS ($p<0.001$). These improvement in the motor function lasted for one week after rTMS and motor training ($p<0.001$).**

Conclusion: **rTMS improved the motor learning of the affected hand in patients after stroke; thus, it can apply as a new rehabilitation strategy for patients after stroke.**

Key words: repetitive transcranial magnetic stimulation, neuronal plasticity, motor learning, stroke, rehabilitation.

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INTRODUCTION

Functional recovery after stroke is related to various plastic processes leading to central nervous system reorganization (1–4). Various concepts are emerging that aim to enhance the beneficial plasticity and thus improve functional recovery (3, 5). However, it is necessary to develop strategies for improving the beneficial effects of neuro-rehabilitative treatments.

Recent reports demonstrated that low-frequency repetitive transcranial magnetic stimulation (rTMS) over the motor cortex of the unaffected hemisphere improved the motor function of the affected hand in patients after chronic stroke (6–8). Inhibition of the excitability of the unaffected hemisphere by rTMS at 1 Hz would decrease the transcallosal inhibition (TCI) from the unaffected to the affected hemisphere and increase the excitability of the affected hemisphere; this ultimately would translate into improved motor function of the affected hand (7). This study was based on the hypothesis that the unaffected hemisphere is disinhibited due to a reduction in the TCI from the affected hemisphere. Consequently, this disinhibited, unaffected hemisphere may increase the TCI to the affected hemisphere and impair the function of the affected hand (4, 9, 10).

It was thought that the application of rTMS at 1 Hz over the unaffected hemisphere may be useful as a new rehabilitation therapy for stroke patients (6–8). However, in a previous study, a continuous improvement in the motor function could not be induced by using only a single rTMS (7). Therefore, for rehabilitation of patients after stroke, it may be important to impart additional motor training or use neuropharmacological intervention while the changes are being generated by rTMS; this would improve the motor function. In particular, motor training after rTMS appears to be an attractive approach for enhancing motor recovery. Modulating the activity of a given neural network by rTMS may render the system more receptive to the motor learning process, thereby enhancing its efficacy (7). However, to our knowledge, no studies have investigated the effects of motor training combined with 1 Hz rTMS over the unaffected hemisphere in patients after stroke. Therefore, we studied whether the combination of rTMS at 1 Hz over the unaffected hemisphere and motor training could improve the function of the affected hand in patients after stroke.

METHODS

Subjects

The study population comprised 20 patients after stroke (mean age 62.3 (standard deviation (SD) 8.4) years (Table I)). The inclusion

Table I. *Clinical characteristics of patients after stroke*

Patient no.	Age (years)/sex	Duration after stroke (months)	Paretic side	FMS Total (%)	FMS Hand (%)	Lesion site
Real-rTMS group						
1	43/M	7	L	86	83	Corona radiata, internal capsule
2	56/M	8	R	79	96	Putamen
3	71/M	21	R	47	54	Thalamus
4	55/M	60	R	77	88	Putamen, corna radiata
5	61/M	10	R	33	29	Basal ganglia, internal capsule
6	70/M	41	R	86	96	Thalamus
7	72/F	18	R	77	83	Corona radiata
8	54/F	21	L	58	54	Corona radiata, internal capsule
9	59/M	60	R	67	58	Corona radiata
10	71/M	8	L	47	38	Internal capsule
Mean (SD)	61.2 (9.7)	25.4 (20.8)		65.7 (18.5)	67.9 (24.3)	
Sham-rTMS group						
11	64/M	8	R	91	96	Thalamus
12	72/M	24	R	58	63	Putamen, corna radiata
13	68/M	21	L	44	50	Internal capsule
14	55/F	9	R	67	63	Basal ganglia, internal capsule
15	60/M	34	L	92	96	Putamen, corna radiata
16	70/M	16	L	85	83	Internal capsule
17	52/M	15	R	89	88	Corona radiata, internal capsule
18	67/F	121	R	45	25	Internal capsule
19	55/M	8	L	82	96	Basal ganglia, internal capsule
20	71/M	88	R	39	46	Thalamus
Mean (SD)	63.4 (7.4)	34.4 (38.6)		69.2 (21.2)	70.6 (25.0)	

FMS: Fugl-Meyer scale (16) (percentages of maximum points in the upper limb (66 points) and in hand (24 points)); SD: standard deviation.

criteria were as follows: (*i*) first-time ischaemic stroke of more than 6 months duration; (*ii*) with subcortical infarction only, confirmed by magnetic resonance imaging (MRI); (*iii*) improved motor deficits of the unilateral upper limb to the extent that patients could perform a pinching task; and (*iv*) normal Mini-Mental State Examination score. The exclusion criteria were as follows: (*i*) severe internal carotid artery stenosis; (*ii*) seizure; and (*iii*) an intracranial metallic implant. The patients were randomly assigned to 2 groups: the rTMS group (10 patients) and the sham group (10 patients). The former received real rTMS, while the latter received sham stimulation.

All subjects gave their written informed consent, and the study protocol was approved by the local ethics committee of the Hokkaido University Graduate School of Medicine.

Experimental design

The measurements for assessing the motor function (acceleration and pinch force) were performed at pre-rTMS (Pre-rTMS) and post-rTMS (Post 1, immediately after the rTMS; Post 2, after motor training; and Post 3, 7 days after rTMS). The parameters of transcranial magnetic stimulation (TMS) (i.e. resting motor threshold (rMT), amplitude of motor evoked potentials (MEPs), and intracortical inhibition (ICI)) were evaluated at Pre-rTMS, Post 1 and Post 3. We did not evaluate the rMT, MEPs and ICI values immediately after motor training (Post 2) because the motor performance modulates the excitability of the motor cortex and ICI (11). It took 3 min to assess the motor function and 10 min to measure TMS parameters. Fig. 1 shows the time course of the experiment.

TMS parameters

Single pulse TMS was performed using a 70-mm figure-8 coil and Magstim 200 (Magstim Co., Dyfed, UK), and rTMS was applied using the same coil and a Magstim Rapid stimulator (Magstim Co.). The coil was placed tangentially over the motor cortex at an optimal site for the first dorsal interosseous (FDI) muscle. The optimal site was

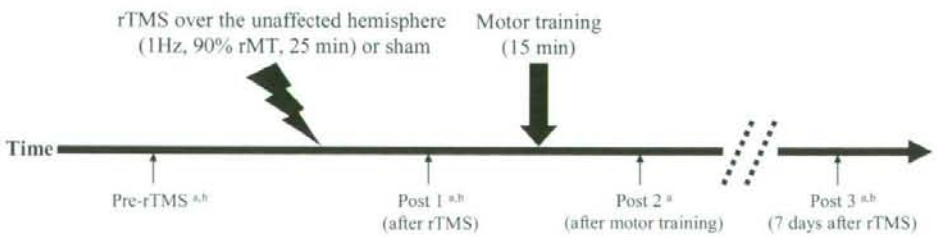

Fig. 1. Time course of the experiment. Repetitive transcranial magnetic stimulation (rTMS) was applied over the motor cortex of the unaffected hemisphere at a frequency of 1 Hz and a stimulus intensity of 90% resting motor threshold (rMT) for 25 min (or sham stimulation). After rTMS, the patients performed pinching task for 15 min as motor training. [a]The measurements for assessing the motor function (acceleration and pinch force) were performed at pre-rTMS, Post 1, Post 2 and Post 3. [b]The measurement of the transcranial magnetic stimulation parameters (rMT, amplitude of the motor evoked potentials (MEPs), and intracortical inhibition (ICI)) were performed at Pre-rTMS, Post 1 and Post 3.

defined as the location where stimulation at a slightly suprathreshold intensity elicited the largest MEPs in the FDI. This position was marked on the scalp and used throughout the experiment. Electromyographic (EMG) activity was recorded using silver–silver chloride (Ag-AgCl) electrodes positioned in a belly-tendon montage on the skin overlying the FDI. The signal was amplified, filtered (50–2000 Hz), and digitized at a sampling rate of 5000 Hz for off-line analysis (Neuropack; Nihon Koden, Tokyo, Japan). The rMT was determined separately for each stimulator and defined as the lowest stimulator output that could produce MEPs with a peak-to-peak amplitude greater than 50 μV in at least half of the 10 trials. The peak-to-peak amplitude of 10 averaged FDI responses obtained at 120% intensity of the rMT was also determined using Magstim 200.

Paired-pulse stimulation was performed to investigate the ICI in the affected motor cortex (12). To apply paired pulses, a figure-8 coil was connected to a Bistim device (Magstim Co.) that triggered 2 magnetic stimulators. The stimulus intensity of the first conditioning shock was 80% of the rMT and that of the second pulse was 120% of the rMT. We performed the tests at inter-stimulus intervals (ISIs) of 2 and 3 ms. Ten trials were recorded for each ISI, and unconditioned trials (controls) were recorded during complete relaxation. The paired stimulation with each ISI was randomly mixed with the control stimulation. The MEP amplitudes obtained by paired-pulse stimulation were expressed as a percentage of the mean control MEP amplitude, and the ICI was then calculated by averaging these values. We obtained ipsilesional TMS data from 11 patients (6 patients, rTMS group; 5 patients, sham group). We excluded other patients who did not display an MEP of the affected hemisphere from the ipsilesional TMS study section, i.e. patients in whom an MEP was not induced even at 100% stimulator output (4 patients, rTMS group; 5 patients, sham group).

rTMS and motor training

rTMS was applied over the motor cortex of the unaffected hemisphere at a frequency of 1 Hz and a stimulus intensity of 90% rMT measured with Magstim Rapid for 25 minutes (1500 pulses). These rTMS protocols used in the present study were in accordance with the safety guidelines for rTMS application to the motor cortex (13). Sham stimulation was applied over the unaffected hemisphere by positioning the coil perpendicular to the scalp (14) and at the same frequency and intensity used for real rTMS. After rTMS, the patients performed a pinching task for 15 min as motor training, as described in a previous report (15). During the pinching task, the patients were asked to perform a metronome-paced pinch of their index finger and thumb of the affected hand as fast as possible (frequency individualized between 0.3 and 0.5 Hz).

Evaluation of motor function

For assessing the motor function, we determined the pinch force and acceleration. The maximum pinch force of the affected hand was determined using a pinch gauge (Pinch Meter SPR-641; Sakai Medical, Tokyo, Japan). The subjects were instructed to use only their thumb and index finger during the pinch force measurements. Ten pinch forces were averaged in each session. Movement acceleration was measured using an accelerometer (model MP110-10-101; Medisens, Sayama, Japan) that was firmly attached to the dorsal side of the proximal phalanx of the thumb. The signal was amplified with a power signal conditioner (model MP110-10-301; Medisens) and digitized at 2000 Hz with a personal computer using a CB-68LPR board (National Instruments, Austin, TX) and LabView software (National Instruments). Fifteen peak accelerations were averaged in each session. The patients were allowed to familiarize themselves with this motor evaluation method on the day before the rTMS experiment.

Data analyses

Data analysis was performed by an investigator blinded to the stimulation type. The data (age, duration after stroke, Fugl-Meyer scale (16), and rMT) were compared between the rTMS and sham groups by using Student's *t*-test. The effects of motor training or rTMS were evaluated using an analysis of variance (ANOVA) for repeated measures with TIME as a within-subjects factor and CONDITION (rTMS and sham) as a between-subjects factor. A *post-hoc* analysis was performed with Bonferroni correction. Any possible correlation between the changes in various parameters was determined by Pearson's correlation coefficient test as an exploratory analysis. All data were normalized by conversion to percentage change from the mean values of Pre-rTMS.

RESULTS

The subjects did not report any adverse effects during the course of the study. No difference was observed between the rTMS and sham groups with regard to the rMT (unaffected hemisphere: mean 46.9 (SD 9.0) vs 49.4 (SD 11.4) %; affected hemisphere: mean 62.2 (SD 12.3) vs 65.6 (SD 16.3) %), age, the duration after stroke, or Fugl-Meyer scale (Table I).

Fig. 2 shows the motor function after rTMS and motor training. A repeated measures ANOVA showed a significant interaction between TIME and CONDITION with respect to acceleration ($F_{3,54}=3.126$, $p=0.033$) and pinch force

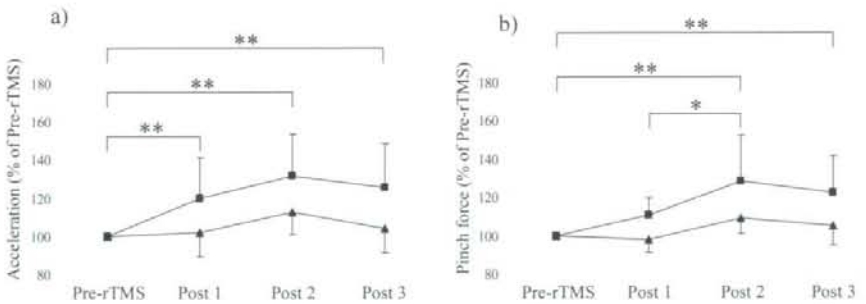

Fig. 2. The effects of rTMS and motor training. (a) Acceleration; (b) pinch force. Repetitive transcranial magnetic stimulation (rTMS) improved the acceleration of the affected hand (Pre-TMS vs Post 1; $p=0.006$) and this improvement in acceleration lasted for one week after rTMS and motor training (Pre-TMS vs Post 3; $p<0.001$). The motor training after rTMS improved the pinch force of the affected hand (Pre-rTMS vs Post 2; $p<0.001$; Post 1 vs Post 2; $p=0.020$). This improvement in pinch force also lasted for one week after rTMS and motor training (Pre-rTMS vs Post 3; $p<0.001$). *$p<0.05$; **$p<0.01$; Error bar, standard deviation; square, the rTMS group; triangle, the sham group.

($F_{3,54}=3.940$, $p=0.013$). It also showed a significant effect of TIME on both acceleration ($F_{3,54}=11.023$, $p<0.001$) and pinch force ($F_{3,54}=15.152$, $p<0.001$). The *post-hoc* test revealed an improvement in acceleration immediately after rTMS (Pre-rTMS vs Post 1: $p=0.006$). This improvement in acceleration lasted for one week after rTMS (Pre-TMS vs Post 3: $p<0.001$). The acceleration tended to increase after motor training; however, the effect of motor training on acceleration was not significant (Post 1 vs Post 2: $p=0.085$). The *post-hoc* test did not show any significant improvement in pinch force immediately after rTMS (Pre-rTMS vs Post 1: $p=0.061$). However, the motor training after rTMS improved the pinch force (Pre-rTMS vs Post 2: $p<0.001$; Post 1 vs Post 2: $p=0.020$). This improvement in pinch force also lasted for one week after rTMS (Pre-rTMS vs Post 3: $p<0.001$). In the sham group, the motor function increased after motor training; however, the effect was not significant (Pre-rTMS vs Post 2: acceleration, $p=0.067$; pinch force, $p=0.107$).

Fig. 3 shows the corticospinal excitability after rTMS. A repeated measures ANOVA for contralesional and ipsilesional MEPs showed a significant interaction between TIME and CONDITION (contralesional: $F_{2,36}=3.396$, $p=0.047$; ipsilesional: $F_{2,18}=5.867$, $p=0.011$) and a significant effect of TIME on both contralesional and ipsilesional MEPs (contralesional: $F_{2,36}=6.106$, $p=0.005$; ipsilesional: $F_{2,18}=3.946$, $p=0.038$). The *post-hoc* test revealed that a decreased contralesional MEP and an increased ipsilesional MEP were produced immediately by rTMS (Pre-rTMS vs Post 1; contralesional: $p=0.005$; ipsilesional: $p<0.001$) but not by sham stimulation (contralesional: $p=0.770$; ipsilesional: $p=0.629$). However, these changes induced by rTMS diminished at 7 days after rTMS (Pre-rTMS vs Post 2; contralesional: $p=0.652$; ipsilesional: $p=0.225$).

A repeated measures ANOVA for the rMT of the unaffected hemisphere (rTMS group: 102.8 (SD 9.0) % at Post 1 (% of Pre-rTMS) and 102.6 (SD 10.1) % at Post 3; sham group: 102.4 (SD 5.2) % at Post 1 and 102.0 (SD 6.8) % at Post 3) did not show a significant interaction between TIME and CONDITION ($F_{2,36}=0.019$, $p=0.981$); furthermore, there was no significant effect of CONDITION ($F_{1,18}=0.025$, $p=0.877$) or TIME ($F_{2,36}=1.550$, $p=0.226$) on the rMT of the unaffected hemisphere. A repeated measures ANOVA for the rMT of the affected hemisphere (rTMS group: 98.5 (SD 6.6) % at Post 1 (% of Pre-rTMS) and 99.7 (SD 5.4) % at Post 3; sham group: 99.8 (SD 4.6) % at Post 1 and 101.2 (SD 5.2) % at Post 3) did not show a significant interaction between TIME and CONDITION ($F_{2,18}=0.120$, $p=0.888$), and there was no significant effect of CONDITION ($F_{1,9}=0.234$, $p=0.640$) or TIME ($F_{2,18}=0.326$, $p=0.726$) on the rMT of the affected hemisphere. A repeated measures ANOVA for the ICI of the affected hemisphere (rTMS group: 96.4 (SD 29.9) % at Post 1 (% of Pre-rTMS) and 102.4 (SD 36.6) % at Post 3; sham group: 110.9 (SD 25.4) % at Post 1 and 104.3 (SD 30.1) % at Post 3) did not show a significant interaction between TIME and CONDITION ($F_{2,18}=0.333$, $p=0.721$), and there was no significant effect of CONDITION ($F_{1,9}=0.267$, $p=0.618$) or TIME ($F_{2,18}=0.088$, $p=0.917$) on the ICI of the affected hemisphere.

In the rTMS group, the improvement in the motor function after rTMS (Post 1) or motor training (Post 2) showed no significant correlation with the age of the subject, duration after stroke, the Fugl-Meyer scale, or the changes in ipsilesional MEPs and ICI.

DISCUSSION

This study reports that non-invasive cortical stimulation using rTMS over the unaffected hemisphere can improve the motor learning of the affected hand in patients after stroke. These results demonstrate that priming by rTMS enhances the motor training effect of improving the affected hand function in patients after stroke.

We found that rTMS at 1 Hz over the unaffected hemisphere reduced the corticospinal excitability of this region; this result is in agreement with a previous report (17). Moreover, rTMS increased the corticospinal excitability of the affected hemisphere. This result is also consistent with that of a recent study (8). A previous study demonstrated that rTMS at 1 Hz over the unaffected hemisphere induced a decrease in the TCI

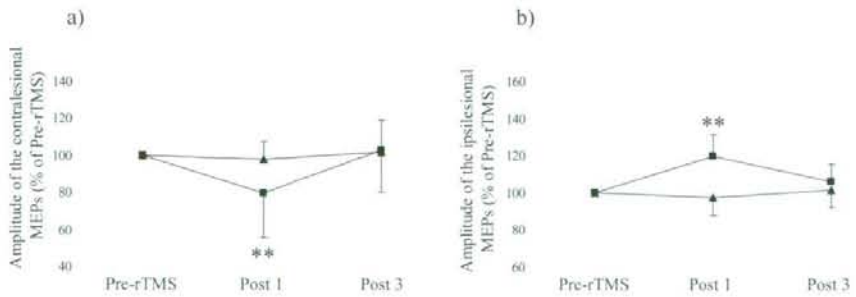

Fig. 3. The change in the corticospinal excitability after repititive transcranical magnetic stimulation (rTMS). (a) Amplitude of the contralesional motor evoked potentials (MEPs); (b) amplitude of the ipsilesional MEPs in the affected hemisphere. A decreased contralesional MEP and an increased ipsilesional MEP were produced immediately by rTMS (Pre-rTMS vs Post 1; contralesional: $p=0.005$; ipsilesional: $p<0.001$). However, these changes induced by rTMS had diminished at 7 days after rTMS. **$p<0.01$. Error bar, standard deviation; square, rTMS group; triangle, sham group.

from the unaffected hemisphere to the affected hemisphere (7). Therefore, rTMS at 1 Hz can facilitate the excitability of the affected hemisphere by reducing the TCI from the unaffected hemisphere. The enhancement of excitability in the motor cortex appears to be a necessity for motor learning (18). The motor cortex in humans is particularly engaged during the early stage of motor consolidation (19). Moreover, several studies have demonstrated that motor cortical plasticity depends on the motor cortex activation history (20–22). Based on these findings, the increased excitability of the affected motor cortex immediately after rTMS may contribute to a more suitable environment for the reorganization of the affected motor cortex by motor learning. Although the excitability of the affected hemisphere returned to baseline levels, the improvement in motor function continued for one week. Many studies have also reported that the acute effect of rTMS lasted for tens of minutes, as much as the stimulation period (23–25). Therefore, for improvement in motor function, it might be important that motor learning induced reorganization while the excitability of the affected motor cortex increased after rTMS. By another mechanism, rTMS over the unaffected hemisphere might reduce the disinhibition of the affected hemisphere, which was induced by the disruption of the TCI. A decrease in the inhibition unmasks the pre-existing, functionally latent neural networks around the lesion, thereby contributing to cortical reorganization (1). Kobayashi et al. (26) have reported that rTMS over the motor cortex induced disinhibition of the contralateral motor cortex. The disinhibition of the affected motor cortex may partly contribute to the functional improvement in the affected hand by unmasking the latent networks. However, we could not detect a change in the ICI of the affected motor cortex after rTMS. This hypothesis needs to be investigated using a larger number of stroke patients.

In patients after chronic stroke, the unaffected motor cortex might inhibit the motor performance of the affected hand via an abnormal TCI from the unaffected motor cortex to the affected motor cortex (7, 9). Several studies have suggested that the downregulation of the unaffected motor cortex results in an improvement in the motor function of the affected hand in patients after chronic stroke (6–8, 27). However, in patients after acute stroke, it is speculated that increased inhibitory input from the unaffected to the affected hemisphere might control the perilesional activity and reduce oxygen and glucose demands in the stroke penumbra in order to limit the extension of the lesion (2). Therefore, rTMS at 1 Hz over the unaffected hemisphere in acute stroke patients might lead to a poor prognosis due to the induction of an increase in neuronal death. Moreover, rTMS at 1 Hz over the unaffected hemisphere might induce the activation of the compensatory neural pathways, and ultimately, this activation might never result in a complete recovery. Further investigations are required to determine whether a low-frequency rTMS can promote recovery in acute stroke. Another concern of this study needs to be addressed. In this study, we selected patients with a better hand function who could perform pinching tasks and motor training. Moreover, the patients' lesion was subcortical infarction only. Therefore, this study might have a decreased external validity due to the homogeneous nature of the experimental population. This fact encourages future studies to investigate other stroke populations with different stroke types and clinical characteristics.

A previous study reported that rTMS without motor training improved acceleration for not more than 30 min and that it did not modulate the pinch force (7). However, the combination of a previously reported rTMS protocol and motor training prolonged the improvement in acceleration for 7 days. In addition, motor training after rTMS at 1 Hz induced an increase in the pinch force that was not improved by rTMS alone. Fregni et al. (8) demonstrated that the effects of rTMS in patients after stroke were cumulative and lasted for at least 2 weeks. Therefore, rTMS may be important in rehabilitation of patients after stroke – to impart additional motor training while the changes are being generated by rTMS at 1 Hz and to conduct rTMS cumulatively; this would sustain the effect of rTMS and improve the function of the affected hand.

In conclusion, our results demonstrated that the combination of rTMS over the unaffected hemisphere and motor training could lead to an improvement in the motor function of the affected hand of patients after chronic stroke. These findings will probably be pertinent to the design and optimization of neurorehabilitation strategies for patients after stroke.

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Research Articles

High-Frequency rTMS over the Supplementary Motor Area for Treatment of Parkinson's Disease

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Abstract: Dysfunction of the basal ganglia-thalamocortical motor circuit is a fundamental model to account for motor symptoms in Parkinson's disease (PD). Using high-frequency repetitive transcranial magnetic stimulation (rTMS) over the supplementary motor area (SMA), we investigated whether modulation of SMA excitability engenders therapeutic effects on motor symptoms in PD. In this double-blind placebo-controlled study, 99 patients were enrolled and assigned randomly to SMA-stimulation and sham-stimulation groups. For SMA stimulation, 20 trains of 50 transcranial magnetic stimuli at 5 Hz were delivered at an intensity of 110% active motor threshold for leg muscles in one session. The sham stimulation was 20 trains of electric stimuli given through electrodes fixed on the head to mimic the cutaneous sensation during rTMS. Each session of intervention was carried out once a week for the first 8 weeks. The SMA stimulation, in contrast to the sham stimulation, engendered significant improvements in total scores and motor scores of the Unified Parkinson's Disease Rating Scale. Mean improvements in motor scores were 4.5 points in the SMA-stimulation group and −0.1 points in the sham-stimulation group. Results indicate that 5 Hz rTMS over SMA modestly improves motor symptoms in PD patients; SMA is a potential stimulation site for PD treatment. © 2008 Movement Disorder Society

Key words: Parkinson's disease; repetitive transcranial magnetic stimulation; supplementary motor area

Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive method to stimulate the human brain, offering potential for therapy in various neurological disorders including Parkinson's disease (PD).[1] Recent meta-analysis revealed a significant, but modest, effect of rTMS on motor function in PD,[2] although it remains controversial whether rTMS has therapeutic effects. Some authors have reported beneficial effects,[3–14] but others have not.[15–18]

It is difficult to explain these contradictory results definitively, but at least three possible reasons are implied. First, considerable methodological differences across studies in terms of the rTMS parameters and the evaluation methods appear to be simple explanations for the controversial results. They indicate a noteworthy lack of consensus on how these parameters should be specified for treatment.[18] Albeit circumstantially, repeated sessions of rTMS have been suggested as more efficacious than a single session because of the cumulative effects of rTMS.[11,13,14,18]

A second possibility is that the favorable outcomes[3–14] are confounded by the placebo effects, which improve the symptoms of PD[18,19] or induce dopamine release in basal ganglia.[20,21] Although meta-analysis in PD revealed that the placebo effect in clinical trials has a pooled effect size of only 0.1,[2] several issues related to procedures of sham stimulation remain to be resolved.[22] Accordingly, therapeutic effectiveness of rTMS cannot be estimated without further clinical study of rTMS using a pertinent sham stimulation.[18,22]

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Third, in terms of the stimulation site, arguments are mounting, which indicate the primary motor cortex as the potential site,[3–14] but a contrary view has also been given[15,16]; to put it the other way around, other brain regions might be more efficacious.

In light of evidence showing impaired activity of the supplementary motor area (SMA) in PD patients because of decreased positive efferent feedback arising from the basal ganglia-thalamocortical motor loop,[23–25] we hypothesized that the therapeutic effect on motor symptoms might be conferred by rTMS over SMA. It is reasonable to infer that high-frequency rTMS can facilitate underactivated SMA to some degree, engendering fundamental modification of the dysfunction of this motor circuit, which, in turn, provides symptomatic relief in PD patients. This study was conducted to compare the effect of high-frequency rTMS over SMA with that of realistic sham stimulation.

PATIENTS AND METHODS

Patients

Patients participating in this trial had idiopathic PD according to the British Parkinson's Disease Society Brain Bank criteria[26]; they had parkinsonian motor symptoms, neither dementia nor major psychiatric illness, and had no contraindications to TMS.[27] Patients who had undergone TMS treatment before this study were excluded, because they were probably able to differentiate the sham stimulation from real stimulation. All present patients were naive to TMS. Neurologists at the participating centers gave their assurance that each patient had received state-of-the-art antiparkinsonian medication before intervention and continued to take that medication throughout the study (12 weeks). We performed all the procedures in the outpatient clinic; no patients were admitted to the hospital for the treatment, because the aim of this study was to find out an rTMS treatment for outpatients.

Study Design

This study was a double-blind trial with a parallel design comparing SMA stimulation with sham stimulation at 16 centers in Japan (see Appendix 1). The protocol was approved by the ethics committee at each participating center. All patients provided written informed consent before intervention. Patients were assigned randomly to the SMA-stimulation group and sham-stimulation group at each participating center.

One session of intervention was performed once a week for the first 8 weeks. The rationale of performing weekly rTMS is as follows. First, it is a convenient and less demanding method for outpatients than daily rTMS. Second, we have already shown that a single series of 5 Hz rTMS had a long-lasting effect of up to 8 days in the primate brain on the local stimulated area and distant areas having anatomical or functional connections with the stimulated motor area.[28] If so, then, it is reasonable to infer that weekly rTMS for longer periods imparts cumulative effects. Considering the convenience and the potential effectiveness of weekly rTMS described earlier, we explored the effects of weekly rTMS for a longer period (8 weeks as in this study) using high-frequency rTMS over SMA to clarify the effect of weekly rTMS on motor symptoms in PD.

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 antiparkinsonian 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. On the basis of the self-assessment information from each patient, the doctors confirmed the timing of evaluation as neither *best on* nor *worst off* state. We did not evaluate the *worst off* state (which might be better to see the effect of rTMS[29]) because the subjects were outpatients. Although there might be some variability among subjects, all subjects, irrespective of group, were assessed similarly, and the baseline scores of assessments did not differ between SMA and sham groups (see Results). This approach may be a better, albeit not the best,[29] way to characterize an add-on effect of rTMS, which was the purpose of our study. However, this was a potential source of heterogeneity in this study and therefore a limitation of this design.

Three kinds of clinical evaluation were carried out by another doctor who was completely blind to the type of intervention. The Unified Parkinson's Disease Rating Scale (UPDRS)[30] was assessed before intervention (week 1) and immediately before sessions at weeks 2, 4, 6, and 8. The Hamilton Rating Scale for Depression (HAM-D) 17-item version[31] was used at weeks 1, 4, and 8. They were also assessed at the same time during the daily treatment cycle at weeks 10 and 12. Subjective improvement was assessed on every visit to the hospital. Subjects rated their clinical state using a visual analogue scale (VAS).[32] The scale was 0 to 100.

Interventions: 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 (Magstim Rapid; The Magstim Co., Whitland, Dyfed, UK, or SMN-1100; Nihon Kohden Corp., Tokyo, Japan, or MagLite; The Dantec Dynamics, Co., Bristol, UK). The SMA stimulation was given using a coil centered at points 3-cm anterior to the leg motor area in the sagittal midline. We cannot rule out the possibility that not SMA but other parts could be affected. However, several precedent reports combining TMS and neuronavigation system revealed that SMA is stimulated by TMS at 3-cm anterior to the hot spot for the tibialis anterior (TA) muscle,[33,34] so that at least some part of SMA is activated by this stimulation. Its effect might originate mainly from modulation of neuronal activity of SMA. The stimulus intensity was fixed at the 110% active motor threshold (AMT) for the right TA muscle. The AMT was defined as the minimum intensity that produced five motor-evoked potentials of 100 μV amplitude on 10 consecutive trials during voluntary contraction of the right TA, when the coil was centered over the leg motor area with the handle pointing leftward (the initial phase of induced current in the brain is rightward).

In one rTMS train, 50 stimuli were delivered at 5 Hz with an intertrain interval of 50 second; 1,000 stimuli were given in one session (20 rTMS trains). Currents in the coil at the initial phase of the biphasic stimulus flowed in the left or rightward direction for 500 stimuli and in the opposite direction for the other 500 stimuli. The stimulus intensity was determined at the first week and maintained as constant throughout the intervention in each subject. Surface cup electrodes were placed over the SMA and the leg motor area to mimic the cutaneous sensation of electrodes as that in the sham stimulation, but no current was given through these electrodes.

Interventions: Realistic Sham Stimulation[18] (see Fig. 1)

In all, 20 trains of electric stimuli (one train, 50 stimuli at 5 Hz; intertrain interval, 50 second) were given with a conventional electric nerve stimulator through the electrodes fixed on the head. The pulse duration was 0.2 millisecond, and the intensity was fixed at two times the sensory threshold. A figure-of-eight coil connected to an uncharged magnetic stimulator (coil A in Fig. 1) was placed over the SMA. Another coil (coil B in Fig. 1) was placed near the subject for sound stimulation. This magnetic stimulator was discharged, simultaneously with the electric stimuli to produce the same sound as that associated with real rTMS.

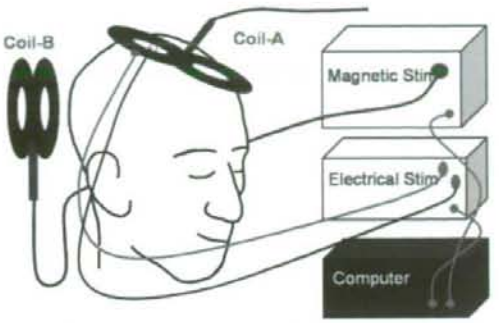

FIG. 1. A diagram depicting the realistic sham stimulation (see Patients and Methods).

Data Analysis

Background clinical features were compared between intervention groups. Gender difference and Hoehn and Yahr stage[35] were compared using the chi-square test. The patients' ages, ages of onset, and durations of illness were compared using the Wilcoxon rank-sum test. Initial values of total UPDRS score (UPDRS-total), sub-score of UPDRS, part III (UPDRS-III; motor score), HAM-D, and VAS were compared between intervention groups using Wilcoxon rank-sum test. The primary outcome measure was the score changes in UPDRS-III. The secondary measures were the UPDRS-total, HAM-D, and VAS. Primary and secondary outcome analyses were conducted according to the intention-to-treat (ITT) principle using the last observation carried forward (LOCF) analysis. In these scores, changes from the initial values were analyzed using two-way repeated measures analysis of variance (ANOVA) [between-subject factor, intervention (SMA/sham); within-subject factor, time (week)]. The time course of changes in UPDRS-III sorted by the Hoehn and Yahr stage was analyzed using three-way repeated measures ANOVA [between-subject factors, intervention (SMA/sham) and stage (Hoehn and Yahr stage 2, 3, and 4); within-subject factor, time (week)] to evaluate differences in the efficacy of the treatment among the stages of disease. The Greenhouse-Geisser correction was used if necessary to correct for nonsphericity. Bonferroni's post hoc method was used for further analyses: P values less than 0.05 were considered significant. These statistical analyses were carried out on actual values of the scores. To evaluate functionally relevant improvement, the effects of intervention were also graded by the score changes of UPDRS-III and UPDRS-total from the baseline at four levels; functionally relevant improvement (eight or greater decrease of the

TABLE 1. *Baseline characteristics of the patients*

	SMA group (N = 55)	Sham group (N = 43)	*P* value
Age (year)			
Mean (SD)	65.3 (8.9)	67.4 (8.5)	0.211
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)	0.593
Age of onset (year)			
Mean (SD)	57.2 (9.9)	59.5 (10.2)	0.133
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)	0.177
Median (range)	8.0 (1–16)	5.0 (1–32)	
Interquartile range	5.0–11.0	3.0–10.5	
Hoehn–Yahr stage, no. (%)			0.246
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 differences were found between two groups.
SMA, supplementary motor area.

score), slight improvement (zero to seven point decrease), slight worsening (one to seven point increase), and functionally relevant worsening (eight or greater increase). The quantities of patients at each level were compared between the SMA-stimulation and sham-stimulation groups using the chi-square test. Time courses of the scores were depicted as score differences from the initial values. Statistical analyses were performed using software (SPSS Statistical Package, ver. 13.0; SPSS).

RESULTS

Of the 99 patients who were enrolled between July 2005 and July 2007, one was excluded from analysis, because the medical treatment was changed during intervention. Tables 1 and 2 present clinical characteristics at the baseline and the initial values for evaluation. No significant differences were found between the two groups. The means (SD) of the Hoehn and Yahr stage were 2.8 (0.6) for the SMA-stimulation group and 2.9 (0.7) for the sham-stimulation group. Five patients dropped out of the study (Table 3). We concluded that these reasons for the decline were not attributable to the adverse effects of rTMS.

Primary and secondary outcome analyses were conducted according to the ITT principle using the LOCF analysis. All but one of the patients dropped out the study before week 4. Therefore, the data from these 4 patients were excluded from the analysis for HAM-D.

TABLE 2. *Initial values of UPDRS scores, HAM-D, and subjective scores (VAS)*

	SMA group (N = 55)	Sham group (N = 43)	*P* value
UPDRS			
Part III: Mean (SD)	23.0 (9.7)	25.8 (13.5)	0.387
Median (range)	22 (5–44)	24 (5–71)	
Interquartile range	16.5–30.0	17.5–33.0	
Total: Mean (SD)	37.6 (15.2)	44.0 (19.1)	0.133
Median (range)	37 (12–67)	44 (14–94)	
Interquartile range	26.0–49.0	29.0–55.0	
HAM-D: Mean (SD)	5.5 (4.8)	7.5 (5.6)	0.062
Median (range)	4 (0–19)	7 (0–23)	
Interquartile range	2.0–8.0	4.0–9.0	
VAS: Mean (SD)	51 (17)	49 (20)	0.662
Median (range)	50 (16–92)	50 (15–89)	
Interquartile range	39.5–60.5	32.5–61.0	

No significant differences were found between two groups.
SMA, supplementary motor area; UPDRS, Unified Parkinson's Disease Rating Scale; HAM-D, Hamilton Rating Scale for Depression, 17 item version; VAS, Visual Analogue Scale.

Unified Parkinson's Disease Rating Scale

In the 98 patients, the SMA stimulation significantly improved Unified Parkinson's Disease Rating Scale (UPDRS)-III scores, although they were unaffected by the sham stimulation (Fig. 2A) (two-way repeated measures ANOVA: effect of intervention, $F_{1,96} = 6.085$, $P = 0.015$; effect of time, $F_{6,576} = 4.182$, $P = 0.004$, and $\varepsilon = 0.586$; time × intervention interaction, $F_{6,576} = 3.258$, $P = 0.016$, and $\varepsilon = 0.586$). Post hoc analysis revealed a significant improvement of UPDRS-III in the SMA-stimulation group from weeks 4 to 12, although no changes were found in the

TABLE 3. *Dropouts*

Group	Age	Sex	Reasons for decline
SMA	58	M	He did not feel well because of the narrow room for stimulation; he withdrew after week 3.
	74	F	She felt lower back pain before enrollment of the study, and pain worsened after the second SMA-stimulation session. She accidentally fell down at the midnight and withdrew after week 2.
Sham	67	M	He had been diagnosed as having colon carcinoma at week 10 and could not be assessed at week 12 and thereafter.
	65	M	He thought his symptoms had deteriorated after the second sham session and withdrew from the study.
	72	F	She found it too difficult to visit our hospital every week and withdrew from the study at week 2.

M, male; F, female.

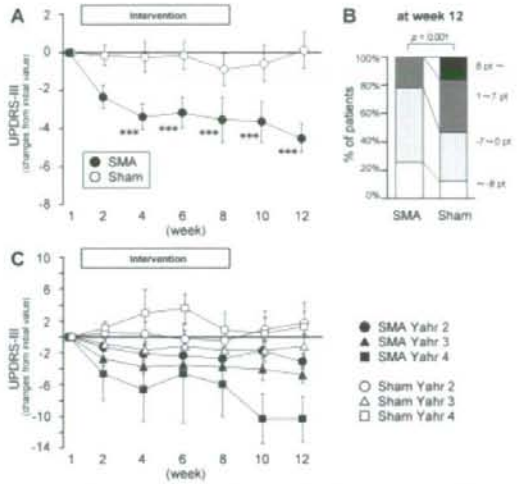

FIG. 2. Changes in UPDRS-III score. **A:** Time courses of changes in the UPDRS-III score. Significant improvement was found between weeks 4 and 12 in the SMA-stimulation group (●). Sham stimulation (○) did not improve UPDRS-III significantly. The times of evaluations are marked on the *X*-axis. Zero indicates no changes from baseline (week 1). Data are means ± SE. Asterisk, *** $P < 0.005$ using Bonferroni's method. **B:** Percentage of patients graded at four levels based on UPDRS-III score changes at week 12. Values on the right side of the graph denote the score changes of UPDRS-III from the baseline; functionally relevant improvement (eight or greater decrease), slight improvement (zero to seven point decrease), slight worsening (one to seven point increase), and functionally relevant worsening (eight or greater increase). **C:** Time courses of changes in UPDRS-III according to the initial Hoehn and Yahr stage. Data are means ± SE.

sham-stimulation group (Fig. 2A). An eight point or greater decrease of UPDRS-III score (functionally relevant improvement) was found in 25% of patients in the SMA-stimulation group at week 12 and 12% in the sham-stimulation group (Fig. 2B). Functionally, relevant worsening was found in 0% in the SMA-stimulation group and 16% in the sham-stimulation group at week 12. Significant differences were found between two groups ($P = 0.001$, chi-square test).

Figure 2C shows time courses of changes in UPDRS-III sorted by the Hoehn and Yahr stage. Three-way ANOVA revealed significant time × intervention interaction ($F_{6,552} = 4.015$, $P = 0.005$), but neither significant time × stage interaction ($F_{12,552} = 1.101$, $P = 0.362$, and $\varepsilon = 0.579$), nor time × intervention × stage interaction ($F_{12,552} = 0.573$, $P = 0.573$, and $\varepsilon = 0.579$), indicated that the effect of intervention did not depend on the stage of the disease.

Similar results were obtained for UPDRS-total scores (Fig. 3A,3B) (two-way repeated measures ANOVA: effect of intervention, $F_{1,96} = 7.660$ and $P = 0.007$; effect of time, $F_{6,576} = 6.139$, $P = 0.00041$, and $\varepsilon = 0.512$; time × intervention interaction, $F_{6,576} = 2.209$, $P = 0.086$, and $\varepsilon = 0.512$). The interaction revealed a trend for different effects of intervention, but this was not statistically significant. Therefore, post hoc analysis was done as an exploratory analysis to dissect substantial effects of intervention (irrespective of intervention types). A significant improvement of UPDRS-total scores in the SMA-stimulation group was found from weeks 4 to 12, although no changes from the baseline were found at any weeks in the sham-stimulation group (Fig. 3A). Functionally, relevant improvement at week 12 was found in 47% of patients in the SMA-stimulation group and 19% in the sham-stimulation group (Fig. 3B). By contrast, 4% in the SMA-stimulation group and 12% in the sham-stimulation group showed eight point or greater increase. A significant difference in the degree of improvement was also found between two groups ($P = 0.022$, chi-square test).

Hamilton Rating Scale for Depression

Figure 4 shows time courses of changes in Hamilton Rating Scale for Depression (HAM-D) in the 94 patients. No difference was found between two groups (effect of intervention, $F_{1,92} = 4.183$ and $P = 0.044$; effect of time, $F_{4,368} = 10.893$, $P = 0.0000023$, and $\varepsilon = 0.683$; time × intervention interaction, $F_{4,368} = 0.534$, $P = 0.711$, and $\varepsilon = 0.683$). Subsequent one-factor ANOVA performed as an exploratory analysis showed a significant improvement of HAM-D at weeks 4, 8, 10, and 12 in the SMA-stimulation group and at weeks 8, 10, and 12 in the sham-stimulation group (see Fig. 4).

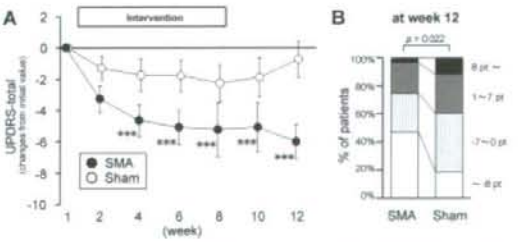

FIG. 3. Changes in UPDRS-total score. **A:** Time courses of changes in UPDRS-total score (means ± SE). Significant improvement was found between weeks 4 and 12 in the SMA-stimulation group (●). Sham stimulation (○) did not improve the total score significantly. asterisk, *** $P < 0.005$ using Bonferroni's method. **B:** Percentage of patients graded at four levels based on UPDRS-total score changes at week 12.

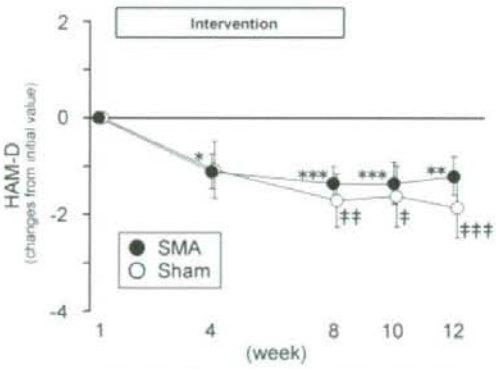

FIG. 4. Changes in HAM-D. No difference was found between two groups. Subsequent one-factor ANOVA in each group showed significant improvement of HAM-D at weeks 4, 8, 10, and 12 in the SMA-stimulation group (●) (asterisk, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ using Bonferroni's method), and at weeks 8, 10, and 12 in the sham-stimulation group (○) (asterisk, ‡ $P < 0.05$, ‡‡ $P < 0.01$, ‡‡‡ $P < 0.005$ using Bonferroni's method). Data are means ± SE.

Visual Analogue Scale

Figure 5 shows the time courses of changes in the VAS in the 98 patients. No difference was found between two groups, although the time had a significant effect on the scale (effect of intervention, $F_{1,96} = 1.602$ and $P = 0.209$; effect of time, $F_{9,864} = 5.520$, $P = 0.000037$, and $\varepsilon = 0.592$; time × intervention interaction, $F_{9,864} = 1.274$, $P = 0.272$, and $\varepsilon = 0.592$). Subsequent one-factor ANOVA was performed as an exploratory analysis. Significant improvement was found at weeks 8, 10, and 12 in the SMA-stimulation group, but no significant changes were found in the sham-stimulation group (see Fig. 5).

DISCUSSION

The main finding of this study was that, in comparison to the sham stimulation, modest, but significant improvements in motor symptoms were induced by the SMA stimulation. This result led us to presume that, in addition to the motor cortex,[3–14] SMA is a potent stimulation site for PD treatment.

Our findings are consistent with those of some previous studies, which have shown that only two sessions of weekly rTMS had no cumulative effects in healthy subjects[36] and in PD patients.[37] Consistent with these results, no significant differences were found in UPDRS-III between weeks 1 and 2 in our study (Fig. 2A). However, rTMS sessions for longer than 2 weeks engendered substantial improvements in UPDRS-III. In fact, significant improvement is apparent at week 4 in the SMA group. This finding indicates that the weekly sessions of rTMS for longer than 4 weeks might be necessary to have substantial effects in PD patients, probably because of some cumulative effects, which were not apparent from two sessions of weekly rTMS.

The impetus for this study is provided by the fact that the dysfunction of the basal ganglia-thalamocortical motor circuit, which plays a pivotal role in movement control,[38] accounts for the motor symptoms in PD.[39] In fact, impaired activity of SMA has been shown in PD patients because of decreased-positive efferent feedback arising from this motor circuit.[23–25] Given that rTMS induces neuronal excitability changes in the human brain,[1] the present result indicates that high-frequency rTMS facilitates underactivated neurons of SMA, resulting in a complement of dysfunction of these neurons and/or of this motor circuit. As we have already revealed in monkeys,[28,40] remote effects of rTMS might also have conferred improvement. Although these rTMS-related functional changes might contribute to the motor symptom relief that was imparted by SMA stimulation, recent studies of rTMS over SMA with small numbers of PD patients revealed no changes in time-perception tasks[41] or worsening of complex movements.[17] The discrepancy might be ascribed to the timing of evaluation. We therefore cannot comment on those earlier results. Additionally, we cannot rule out the possibility that SMA might not be stimulated, and other parts might be affected. However, according to several precedent reports,[32,33] the effects produced by our stimulation method might originate mainly from the modulation of neuronal activity of SMA.

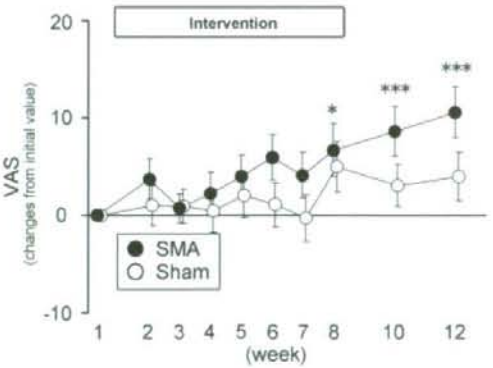

FIG. 5. Changes in VAS. No difference was found between two groups. Subsequent one-factor ANOVA in each group showed significant improvement (feel better) of VAS at weeks 8, 10, and 12 in the SMA-stimulation group (●) but no significant changes in the sham-stimulation group (○). Asterisk, * $P < 0.05$, *** $P < 0.005$ using Bonferroni's method. Data are means ± SE.

The precise neuronal mechanism underlying the present results remains speculative. However, our findings might provide a clue to suggest the optimal stimulation parameters in clinical use. Concurring results of several studies showing the cumulative long-term therapeutic effects of repeated rTMS sessions,[11,13,14,18] we have shown long-lasting effects of SMA stimulation. These data led us to surmise that repeated sessions of rTMS are preferred to achieve therapeutic benefits.

Notwithstanding, a potential weakness of the study is that only modest improvement of motor symptoms was found in the SMA-stimulation group. It is thereby invalid to conclude that 5 Hz rTMS over SMA produces clinically adequate benefits. The unoptimized rTMS parameters used in this study are probably attributable to the lack of clinical benefits such as the total number of sessions, the number of sessions per week, or the stimulus frequency. To determine the optimal stimulation parameters for treatment of PD, further studies are warranted to investigate the therapeutic effects of rTMS over SMA by varying these factors or using new rTMS protocols producing long-lasting after effects (e.g., theta burst stimulation[42] or quadripulse stimulation[43]).

Finally, the improvements in HAM-D and VAS did not differ between the SMA-stimulation and sham-stimulation groups. Interestingly, Fregni et al.[44] reported that placebo interventions in PD had an immediate subjective sensation of improvement but caused no significant objective motor changes. Consistent with their results, there were some improvements in VAS and HAM-D without significant improvement of UPDRS-III in the sham-stimulation group. However, the sham stimulation substantially improved motor symptoms in some patients. We cannot completely rule out the possibility that this sham procedure produced the improvement of HAM-D and VAS, because it stimulated some tissues outside the skull. It is very difficult, however, to speculate upon scientifically valid mechanism of this possibility. Because HAM-D and VAS might reflect some brain activity and because these tissues might be of little relevance to such brain activity, we suggest that the effect by sham treatment is a placebo effect. These considerable placebo effects are congruent with our previous findings that the realistic sham procedure had moderate placebo effects[18]; it should be used for control stimulation to show the definite efficacy of real rTMS.

In summary, results of this study showed that 5 Hz rTMS over SMA modestly improved motor symptoms in patients with PD. SMA is a potential stimulation site for treatment of PD.

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APPENDIX 1

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

Principal investigator: Tsuji S

Coordinators: Kaji R, Tobimatsu S, Nakajima K, Nakamura Y, Fukudome T, Yokochi F, Ugawa Y

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

Participating institutions:

University of Occupational and Environmental Health Hospital, Tokyo University Hospital, Fukushima Medical University Hospital, Tokushima University Hospital, Kyushu University Hospital, Tottori University Hospital, Kinki University Sakai Hospital, National Hospital Organization Nagasaki Medical Center of Neurology, Tokyo Metropolitan Neurological Hospital, Sapporo Azabu Neurosurgical Hospital, Saitama Medical University Hospital, Osaka University Hospital, Hamamatsu Medical University Hospital, Hamamatsu Seirei Hospital, Hokkaido University Hospital, Kumamoto Kinoh Hospital.

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