

Recovery of upper-limb function due to enhanced use-dependent plasticity in chronic stroke patients

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Patients with chronic stroke often show increased flexor hypertonia in their affected upper limbs. Although an intervention strategy targeting the extensors of the affected upper limb might thus be expected to have benefits for functional recovery, conventional repetitive motor training has limited clinical utility. Recent studies have shown that repetitive transcranial magnetic stimulation could induce motor recovery. The present study tested whether 5 Hz repetitive transcranial magnetic stimulation of the upper-limb area of the primary motor cortex, combined with extensor motor training, had a greater effect on motor recovery than either intervention alone in stroke hemiparesis. Nine patients with chronic subcortical stroke and nine age-matched healthy subjects completed the crossover study. In separate sessions, we examined the single intervention effect of repetitive wrist and finger extension exercises aided by neuromuscular stimulation, the single intervention effect of 5 Hz repetitive transcranial magnetic stimulation and the combined effect of the two interventions. The motor functions were evaluated behaviourally in patients (Experiment 1) and electrophysiologically in healthy subjects (Experiment 2), both before and after the intervention. In addition, we tested the long-term effect by repeating the combined interventions 12 times in patients (Experiment 3). The motor functions were measured again 2 weeks after the end of the repetitive intervention period. In Experiment 1, the combined intervention, but neither of the single interventions, resulted in an improvement of extensor movement ($P < 0.0001$) and grip power ($P < 0.05$), along with a reduction of flexor hypertonia ($P < 0.01$), in their paretic upper limbs. In Experiment 2, only the combined intervention resulted in selective plastic changes of cortico-spinal excitability ($P < 0.01$), motor threshold ($P < 0.001$) and silent period ($P < 0.01$) for the extensors. In Experiment 3, we also confirmed long-term beneficial effects of the combined intervention in patients. These findings indicate that combining motor training with

repetitive transcranial magnetic stimulation can facilitate use-dependent plasticity and achieve functional recovery of motor impairments that cannot be attained by either intervention alone. This method could be a powerful rehabilitative approach for patients with hemiparetic stroke.

Keywords: stroke; repetitive transcranial magnetic stimulation; hemiparesis; use-dependent plasticity; rehabilitation

Abbreviations: EDC = extensor digiti communis; EEx = exercises for the extensors; FCR = flexor carpi radialis; M1 = primary motor cortex; MCP = metacarpophalangeal; MEP = motor evoked potentials; TMS = transcranial magnetic stimulation; UDP = use-dependent plasticity

Introduction

Stroke is the second most common cause of death and the leading cause of chronic disability in adults worldwide (Feigin *et al.*, 2003). After the onset of ischaemic insult, the brain starts to reorganize itself (Nudo *et al.*, 1996; Trompetto *et al.* 2000; Rossini *et al.*, 2003; Ward *et al.*, 2003; Di Filippo *et al.*, 2008). Later in the chronic phase, there is little probability of spontaneous neuronal plastic changes (Nakayama *et al.*, 1994; Verheyden *et al.*, 2008). However, recent reports suggested that functional recovery might occur even in chronic patients if plastic changes are induced in the primary motor cortex (M1) by intense motor training (Liepert *et al.*, 1998, 2000; Miltner *et al.*, 1999; Dong *et al.*, 2006; Wolf *et al.*, 2006) or non-invasive cortical stimulation (Hummel and Cohen, 2005; Khedr *et al.*, 2005; Mansur *et al.*, 2005; Takeuchi *et al.*, 2005; Fregni *et al.*, 2006; Kim *et al.*, 2006; Talelli *et al.*, 2007). However, the effectiveness of such strategies seems to have been limited to patients with mild hemiparesis (Hummel and Cohen, 2005).

Patients with chronic stroke, with moderate-to-severe hemiparesis, often suffer from motor deficits associated with flexor hypertonia, as well as motor weakness. A possible therapeutic strategy for these patients is selectively to induce long-term potentiation-like plasticity in the extensor function, to counteract the flexor hypertonia.

In healthy subjects, use-dependent plasticity (UDP) has been reported, in which motor training induced long-term potentiation-like changes in the cortico-spinal neurons representing only agonist, and not antagonist, muscles for a trained movement (Butefisch *et al.*, 2000, 2002). However, the beneficial effects of training in chronic-phase patients are relatively limited (Nakayama *et al.*, 1994; Verheyden *et al.*, 2008). Even in the subacute phase, additional extensor training of the affected hand did not change the clinical outcome (Trombly *et al.*, 1986).

To utilize UDP efficiently for stroke rehabilitation, one might combine excitatory repetitive transcranial magnetic stimulation (TMS) of the M1 area for the affected side with repetitive movements of the paretic limb. Since patients with moderate-to-severe hemiparesis have difficulty in executing the voluntary repetitive movements necessary for UDP, we used neuromuscular stimulation to aid extensor training.

We hypothesized that the upper-limb function of patients with chronic stroke with spastic hemiparesis would be improved by the repetitive wrist and finger-extension training combined with 5 Hz repetitive TMS, more than by either intervention alone. To test

this hypothesis, the effects of interventions on extensor motor functions were evaluated behaviourally in patients with subcortical stroke (Experiment 1) and electrophysiologically in healthy subjects (Experiment 2). Furthermore, we investigated the long-term effect in patients by 12 times of repeating the combined interventions (Experiment 3).

Subjects and methods

Subjects

The study protocol was approved by the Committee of Medical Ethics of the Graduate School of Medicine, Kyoto University, Japan, and written informed consent was obtained from all subjects.

Stroke patients

We investigated nine post-stroke patients (four male and five female) aged 33–62 years [mean $\pm$ standard deviation (SD), 51.6 ± 11.6 years], eight of whom were right-handed and one left-handed, according to the Edinburgh Handedness Inventory (Oldfield, 1971). All had experienced their first-ever subcortical stroke (seven with infarction and two with haemorrhage; eight in the internal capsule and one pontine) more than 5 months before the study (average $\pm$ SD, 24.0 ± 19.1). The lesions were documented by magnetic resonance imaging (MRI; T₁- and T₂-weighted images). None of the patients were on antispastic medications or other drugs that could interfere with cortical excitability. They were hemiparetic, three in the left hand and six in the right hand. Although they showed some voluntary contractions of extensors in their paretic upper-limbs in recording of electromyograms (EMGs), their voluntary movements of the wrist and fingers were limited to a greater or lesser extent (synchronously or separately): five could not extend the metacarpophalangeal (MCP) joints of the thumb, index finger and middle finger, and three could not extend one or two of the MCP joints by more than 0°. The paretic state of the affected upper limb was evaluated by the upper-extremity motor scores of the Stroke Impairment Assessment Set (Chino *et al.*, 1994; Sonoda *et al.*, 1997; Tsuji *et al.*, 2000). One patient had moderate hypesthesia in the paretic upper limb, whereas the others had no sensory disturbance (Table 1).

Healthy subjects

We investigated nine healthy volunteers (four male and five female) aged 30–64 years (mean $\pm$ SD, 53.2 ± 13.8 years). None of the subjects had a history of neurological illness and all were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971).

Table 1 The proximal sites of paretic upper-extremities in Stroke Impairment Assessment Set (SIAS)

Patient	Age (years)	Gender	Months after stroke	SIAS (Shoulder, arm-fingers)	Haemorrhage (H)/Infarct (I)	Hemiparetic side	Lesioned site	Dominant hand before stroke	Sensory disturbance
1	60	F	51	3-1A	I	Right	Posterior limb of internal capsule and putamen	Right	None
2	53	F	15	2-1A	H	Right	Posterior limb of internal capsule and striatum	Right	None
3	62	F	41	4-1C	I	Left	Posterior limb of internal capsule and putamen	Right	None
4	59	F	5	2-1B	I	Right	Posterior limb of internal capsule and corona radiata	Right	None
5	33	M	11	5-1C	I	Right	Posterior limb of internal capsule and posterior putamen	Left	None
6	62	M	5	4-3	I	Right	Posterior limb of internal capsule and thalamus	Right	None
7	55	M	39	3-1B	I	Left	Pons	Right	None
8	33	F	44	5-4	I	Right	Posterior limb of internal capsule, corona radiata and basal area of temporal lobe	Right	None
9	47	M	5	3-1B	H	Left	Posterior limb of internal capsule, striatum and thalamus	Right	Moderate

In Stroke Impairment Assessment Set (SIAS), proximal sites of paretic upper-extremities, such as a shoulder and an arm, are evaluated by Knee-Mouth test (Score: 0, 1, 2, 3, 4, 5) and distal sites such as fingers are evaluated by the Finger-function test [Score: 0, 1 (1A, 1B, 1C), 2, 3, 4, 5]. The lower the value, the more severe the impairment. For example, a score of 0 = total paralysis, score of 1–2 = incompleteness of a task, score of 3–4 = completion of a task with clumsiness and the maximum score of 5 = normal status. In the Finger-function test, a score of 1A means they can do synkinetic flexion of fingers, 1B synkinetic extension of fingers and 1C partial separative flexion or extension of fingers. SIAS = Stroke Impairment Assessment Set.

None of the subjects had any contraindications to TMS (Wassermann, 1998).

Recording procedures

During the experiments, subjects were seated comfortably in an arm-chair with their forearms pronated (palm down) on an armrest and with the shoulder joint in the resting position and the elbow joint flexed at a right angle, in order to prevent the synchronized movement of extensors of the paretic upper limb. For the patients, EMGs were recorded from the flexor carpi radialis (FCR) muscle and the right biceps muscle of the paretic side, and the right and left extensor digiti communis (EDC), using a pair of silver electrodes. For the healthy subjects, the EMGs were recorded from the right and left EDC muscles, the right FCR and bicep muscles. The EMG was amplified, filtered (bandpass, 5–2000 Hz) and digitized at a sampling rate of 10 kHz using the Neuroscan system (Neuroscan Co., Herndon, VA, USA). Subjects were monitored by a video camera to check their behaviours.

Intervention protocols

Extensor exercises and repetitive TMS

The patients performed training of the paretic upper limb, whereas the healthy subjects performed training of the right hand combined with high-frequency repetitive TMS ('EEEx-TMS'; Fig. 1). All of the participants performed 15 cycles of exercises for the extensors of the wrist and fingers. Each cycle consisted of exercises for the extensors for 50 s followed by a train of 5 Hz repetitive TMS for 8 s, which was both preceded and followed by a resting period of 1 s (total time = 1 min). The exercises for the extensors comprised 50 repeats of 1 Hz rhythmic voluntary extension of the wrist and MCP joints of the five digits triggered by electrical neuromuscular stimulation across the EDC muscle, followed by brief relaxation (rather than voluntary flexion). The participants were asked to voluntarily make a movement of

two-thirds of the active range of extension at the same time as the electrical stimulation. For neuromuscular stimulation, the surface electrode pads (5 × 5 cm) were applied to the EDC muscle belly and distal tendon. The stimulation comprised a 40 Hz train of 250 μ s² constant current pulses, lasting for 500 ms (20 pulses). For the patients, the intensity was fixed at the level that produced 10° of extension of the paretic wrist in the relaxed state, so that the neuromuscular stimulation could assist the exercises for the extensors. For the healthy subjects, the stimulus intensity was fixed at 110% of the motor threshold, which was used as a trigger cue for the exercises for the extensors. In all subjects, the sensory threshold was assessed before the intervention.

High-frequency repetitive TMS was performed using a Magstim Super Rapid Magnetic Stimulator (Magstim, Whitland, Dyfed, UK) with an air-cooled figure-of-eight coil composed of two loops (outer diameter, 9 cm). The coil was placed in the optimal position in the M1 to elicit the best motor response in the target EDC muscles. In patients, if motor-evoked potentials (MEPs) were not elicited with the maximal intensity of the stimulator output, the optimal positions were determined as those symmetrically opposite to the optimal positions of the EDC muscles in the healthy side. The active motor threshold was defined as the lowest stimulus intensity required to elicit MEPs with a peak-to-peak amplitude of >150 μ V in the tonically contracting muscles in 5 out of 10 trials (Rossini *et al.*, 1994) for each individual subject. The intensity of the repetitive TMS was fixed at 100% of the active motor threshold and was kept constant throughout the intervention. If the active motor threshold could not be determined in patients, we used the maximum intensity of the stimulator output for the stimulus intensity.

Exercises for the extensors and sham TMS

The patients and healthy subjects performed motor training combined with sham TMS ('EEEx'; Fig. 1). They were instructed to perform 15 cycles of focal exercises for the extensors of the wrist and fingers.

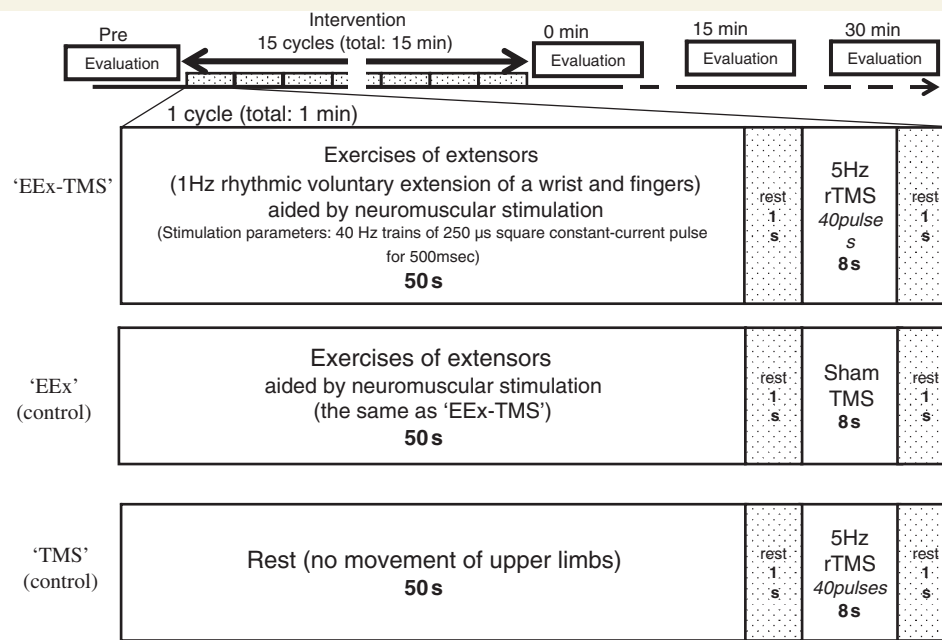


Figure 1 Experimental paradigm. In the main experiment ('EEEx-TMS'; upper), 15 cycles of focal extensor exercises ('EEEx'), consisting of 1 Hz rhythmic voluntary movements of the wrist and fingers aided or triggered by neuromuscular stimulation of the EDC muscles, were combined with high-frequency repetitive TMS ('TMS'). Each cycle consisted of exercises for the extensors for 50 s followed by a train of 5 Hz repetitive TMS for 8 s, which was both preceded and followed by a resting period of 1 s (total time = 1 min). In the control experiments, exercises for the extensors combined with sham TMS ('EEEx'; middle) or 5 Hz repetitive TMS alone ('TMS'; lower) were used. Motor functions (behavioural and electrophysiological parameters in each experiment) were evaluated before, immediately after, 15 min after and 30 min after the end of the intervention (the pre, post-0, post-15 and post-30 conditions, respectively). rTMS = repetitive TMS.

The exercises were the same as for 'EEEx-TMS'. However, the subjects received TMS using a sham coil that produced similar click sounds.

High-frequency repetitive TMS

The patients and healthy subjects received repetitive TMS alone without motor training ('TMS'; Fig. 1). During this intervention, repetitive TMS was given in 15 cycles as described for EEEx-TMS (at 5 Hz for 8 s with an interval of 52 s and 100% of the active motor threshold). They were seated with both of their upper extremities relaxed.

Motor assessment

The motor assessments (behaviour and TMS) were done before, immediately after, 15 min after and 30 min after the intervention (designated as the 'pre', 'post-0', 'post-15' and 'post-30' conditions, respectively). The order of measurements was randomized. The whole set of measurements took ~12 min to perform.

Behavioural assessment

Patients

To assess the effects of the interventions on behavioural parameters, and particularly to differentiate the effects of the training on agonist and antagonist muscles, clinical assessments were made of the active range of movement for the wrist joint and the MCP joints of the thumb, index finger and middle finger both in extension and flexion in the paretic side. We carefully checked that the shoulder joint remained in the resting position and that the elbow joint was flexed at a right angle, in order to prevent the synkinetic movement of the paretic upper limb. Pinch force was measured using a force transducer (range,

0–20 kg; diameter of contact surface area, 2 cm) with the thumb and index finger of the paretic hand, and grip power was also measured in the paretic hand using a custom handgrip dynamometer. Additionally, we assessed the changes of the passive range of movement in extension and flexion, and of the Modified Ashworth Scale scores (Ashworth, 1964; Bohannon and Smith, 1987) to evaluate the changes of hypertonia.

As a baseline, we measured the pinch force and grip power of the healthy hand before the intervention.

Healthy subjects

In the healthy subjects, we measured the active range of movement, pinch force and grip power of the right hand, as described for the patients.

As a baseline, we also measured the passive range of movement of the same joints on the right side. The pinch force and grip power of the left hand were measured before the intervention.

Electrophysiological assessment

Focal TMS was performed using a flat figure-of-eight coil (outer diameter of each wing, 9 cm) connected to a Magstim 200 magnetic stimulator. The coil was placed tangentially to the scalp with the handle pointing backwards and at 45° lateral to the midline.

The optimal scalp positions to induce the best motor response for the right EDC and FCR muscles were determined separately.

To assess the changes of cortico-spinal excitability and the recruitment of the cortico-spinal projection from the M1, we measured the resting motor threshold, the average of the 20 peak-to-peak

amplitudes of the MEPs with the fixed intensity of the TMS machine adjusted to produce a MEP of ~ 1 mV (stimulus intensity 1 mV) and the input–output functions from each of the right EDC and FCR muscles. The input–output functions were measured with the intensities of the single TMS individually adapted to the resting motor threshold. Eight MEPs were recorded from each of the right EDC and FCR muscles at intensities of 50, 80, 90, 100, 110, 120, 130 and 150% of the resting motor threshold, respectively. For each subject, the peak-to-peak amplitudes of the MEPs were measured in every single trial and averaged. Complete muscle relaxation was continuously monitored by visual feedback of the surface EMGs.

To investigate the motor inhibitory system, the silent period with a stimulation intensity of 140% of the resting motor threshold was assessed for the right EDC and FCR muscles, each of which was isometrically contracted at 40% of the maximum force. The individual 40% force level was maintained by visual feedback of the surface EMGs. The duration of the silent period was defined as the time from the onset of TMS until the return of voluntary EMG activity.

Additionally, to assess the effects of the intervention at the spinal level, the H-reflex in the right FCR muscle was measured. The right median nerve was stimulated at the elbow with an electric pulse of 1-ms duration during wrist flexion at 20% of the maximum force. The stimulus intensity was gradually increased from a level below the H-wave threshold to a level at which a stable maximum M-wave was elicited. Both H-waves and M-waves were recorded. The spinal excitability was assessed by the ratio of the maximum H-reflex amplitude ($H_{\max}$) to the maximum M-wave amplitude ($M_{\max}$).

Unfortunately, in seven out of the nine patients, we could not reach the stimulus intensity 1 mV due to the elevated motor threshold of the affected limb. Thus, TMS parameters could not be evaluated in detail for all the patients.

Experimental procedure

Experiment 1: comparison among interventions in patients

The nine stroke patients participated in three different interventions, 'EEEx–TMS', 'EEEx' and 'TMS' sessions, to test whether 'EEEx–TMS' had a greater effect on the extensor function than either intervention alone. The session was performed each on separate days, the order of which was randomized.

Experiment 2: comparison among interventions in healthy subjects

To investigate physiologic changes associated with the effects of interventions, we measured the electrophysiological parameters in nine age-matched healthy subjects, which we could not evaluate in the patients. As well as in Experiment 1, they participated in the three different interventions.

Experiment 3: long-lasting effects of repeated 'EEEx–TMS' in patients

Since we found that 'EEEx–TMS' could induce the functional improvements of extensor muscles in Experiments 1 and 2, we performed 12 times of 'EEEx–TMS' on the same nine patients, with one session per day, on two separate days per week, for 6 weeks in total, more than 7 days after the end of Experiment 1, to investigate the long-lasting effects of the repeated 'EEEx–TMS'. The same assessments were done before the 12 sessions (baseline), at the end of the 12 sessions (6 weeks) and 2 weeks after the 12 sessions (8 weeks). In five of the nine patients, the subjective feelings of the paretic limbs

were assessed by a visual analogue scale (ranging from 'very difficult or impossible to move' to 'very easy to move equally well as the healthy limb').

Statistical analysis

To assess the effects of the interventions, data on the MEP amplitudes, the intensity of the resting motor threshold and active motor threshold, the duration of the silent period measured for the EDC and FCR muscles, and the behavioural performance of the targeted hands were subjected to repeated-measures analysis of variance (ANOVA) with time (pre, post-0, post-15 and post-30) as a within-subject factor. For input–output functions, two-way repeated-measures ANOVA was performed using intensity (50, 70, 80, 90, 100, 110, 120, 130 and 150%) and time.

In Experiment 3, the behavioural performance measured in the paretic hands, and the visual analogue scale were subjected to repeated-measures ANOVA with time (baseline, 6 weeks and 8 weeks) as a within-subject factor.

If necessary, the Greenhouse–Geisser correction was used to adjust for the sphericity, changing the degrees of freedom using the correction coefficient epsilon. The Bonferroni correction for multiple comparisons was used for the *post hoc t*-test. Effects were considered significant at $P < 0.05$. All data are given as the mean $\pm$ SEM.

Results

No subjects experienced side effects during the experiments. By video-monitoring, we confirmed that similar amounts of upper-limb movements were achieved in each subject during the intervention across the 'EEEx–TMS', 'EEEx' and 'TMS' sessions in Experiments 1 and 2.

Experiment 1

The sensory threshold was 5.61 ± 0.65 mA and the actual stimulus intensity was 18.8 ± 2.14 mA for electrical neuromuscular stimulation in the patients. No significant difference was observed at baseline in behavioural parameters between three sessions.

Changes of motor behaviour

At baseline, the respective active range of movements in extension for the wrist joint, the thumb MCP joint, the index finger MCP joint and the middle finger MCP joint of the paretic upper limb were 34.2 ± 8.7 , -19.1 ± 6.0 , -17.9 ± 12.3 and $-23.4 \pm 11.7^\circ$, respectively, the active range of movement in flexion were 26.5 ± 12.1 , 56.2 ± 5.5 , 73.5 ± 6.4 and $74.7 \pm 6.7^\circ$, the passive range of movements in extension were 53.3 ± 6.3 , 5.7 ± 2.0 , 48.7 ± 5.9 and $43.8 \pm 5.3^\circ$ and the passive range of movements in flexion were 70.6 ± 4.9 , 70.6 ± 4.8 , 89.0 ± 4.6 and $91.2 \pm 3.6^\circ$.

The pinch forces in the paretic and healthy sides were 2.00 ± 0.05 and 5.20 ± 0.08 kg, respectively, and the grip powers were 8.2 ± 2.4 and 28.6 ± 3.7 kg.

After 'EEEx–TMS', repeated measures ANOVA followed by the *post hoc t*-test showed significant increases in the active range of movements in extension for the wrist joint, thumb, index and middle finger MCP joint (wrist: $F = 9.43$, $P < 0.0001$, thumb: $F = 14.08$, $P < 0.0001$, index finger: $F = 12.64$, $P < 0.0001$, middle

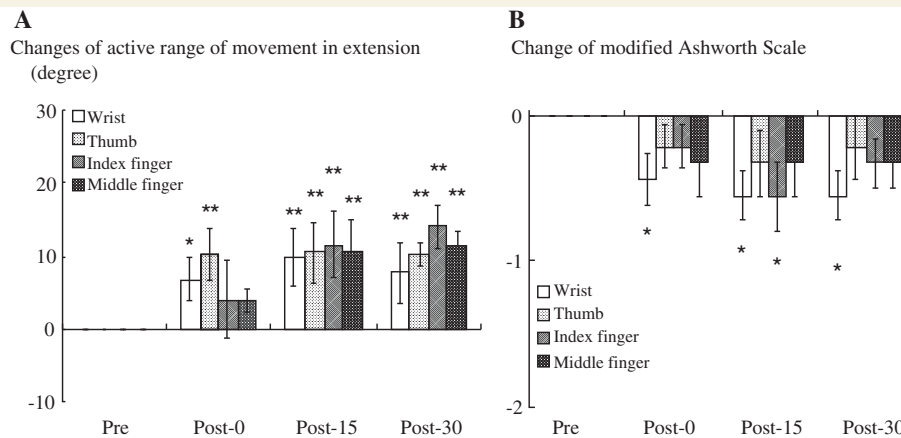


Figure 2 Behavioural evaluations before and after 'EEx-TMS' in stroke patients. After 'EEx-TMS', significant increases were found in the active range of movements in extension for the wrist joint and the MCP joint of the thumb in the paretic side in the post-0, post-15 and post-30 conditions, and in the MCP joints of the index and middle fingers in the post-15 and post-30 conditions (A). After 'EEx-TMS', the modified Ashworth Scale score of the wrist joint in the paretic side was significantly decreased in the post-0, post-15 and post-30 conditions, and that modified Ashworth Scale score of the MCP joint of index finger was decreased in the post-15 condition (B) (** $P < 0.001$, * $P < 0.05$).

finger: $F = 12.6$; Fig. 2A), while the active range of movements in flexion were not significantly changed. There were no significant changes in these parameters after 'EEx' or 'TMS' alone. The passive range of movements in both extension and flexion were not significantly changed after any of the three types of session.

In the post-30 condition, the grip power in the paretic side was significantly improved ($F = 3.23$, $P < 0.05$), whereas there was no change after 'EEx' or 'TMS' alone. The pinch force in the paretic side was not significantly changed after any of the three types of session.

Changes of hypertonia

At baseline, the modified Ashworth Scale scores were 1.9 ± 0.5 , 0.8 ± 0.3 , 0.7 ± 0.3 and 1.1 ± 0.4 for the wrist joint, and the MCP joints of the thumb, index and middle finger, respectively, in the paretic upper limb.

After 'EEx-TMS', repeated measures ANOVA followed by the *post hoc t*-test revealed that the modified Ashworth Scale scores of the wrist joint, thumb, index and middle finger MCP joint were significantly decreased (wrist: $F = 4.86$, $P < 0.01$, index finger: $F = 3.78$, $P < 0.05$; Fig. 2Bb). After the other two types of session ('EEx' and 'TMS'), the modified Ashworth Scale scores were not changed significantly.

Experiment 2

The sensory threshold was 4.14 ± 0.30 mA and the motor threshold was 8.01 ± 0.22 mA for electrical stimulation in the healthy subjects. No significant difference was observed at baseline in behavioural and electrophysiological parameters between three sessions.

Changes of motor behaviour

At baseline, the respective active range of movements in extension for the wrist joint, the thumb MCP joint, the index finger MCP joint

and the middle finger MCP joint in the right upper limb were 69.0 ± 3.5 , 5.9 ± 2.4 , 13.9 ± 2.4 and $10.9 \pm 1.9^\circ$, the active range of movements in flexion were 66.1 ± 3.2 , 64.6 ± 5.2 , 89.0 ± 1.9 and $89.5 \pm 2.0^\circ$, the passive range of movements in extension were 77.8 ± 2.3 , 6.4 ± 1.7 , 47.8 ± 3.6 and $46 \pm 3.3^\circ$, and the passive range of movements in flexion were 80.0 ± 2.1 , 67.5 ± 5.4 , 94.3 ± 3.1 and $95.7 \pm 2.9^\circ$. The respective pinch forces in the right and left hands were 4.06 ± 0.05 and 3.85 ± 0.09 kg, and the grip powers were 27.7 ± 3.2 and 23.1 ± 3.0 kg.

There were no significant changes in the active range of movements of the wrist joints and fingers, the pinch force and the grip power after all three types of session.

Changes of TMS parameters

Figure 3 illustrates the waveforms of the mean MEPs with stimulus intensity 1 mV (Fig. 3A) and the silent periods (Fig. 3B) for the right EDC muscles and the maximum H and M waves (Fig. 3C) for the right FCR muscles from one representative subject before, immediately after, 15 min after and 30 min after 'EEx-TMS'.

At baseline, the respective resting motor thresholds for the right EDC and FCR muscles were 49.4 ± 3.7 and $51.5 \pm 3.7\%$, and the active motor thresholds were 39.9 ± 2.5 and $38.9 \pm 2.8\%$ of the maximum stimulator output of the Magstim 200. Additionally, the active motor threshold for the right EDC muscle was $68.4 \pm 3.7\%$ of the maximum stimulator output of the Magstim Super Rapid. The test intensities for the stimulus intensity 1 mV for the right EDC and FCR muscles were 72.0 ± 5.5 and $74.1 \pm 5.8\%$, respectively ($n = 9$).

After 'EEx-TMS', the MEP amplitudes of the right EDC muscles with stimulus intensity 1 mV were significantly enhanced ($F = 3.59$, $P < 0.05$), while those of the right FCR muscles were not ($F = 1.45$, $P > 0.05$) (Fig. 4A). After the 'EEx', the MEP amplitudes of the EDC and FCR muscles were not significantly changed ($F = 1.49$, $P > 0.05$ and $F = 0.69$, $P > 0.05$, respectively). After the 'TMS', the MEP amplitudes of the FCR muscles were significantly changed

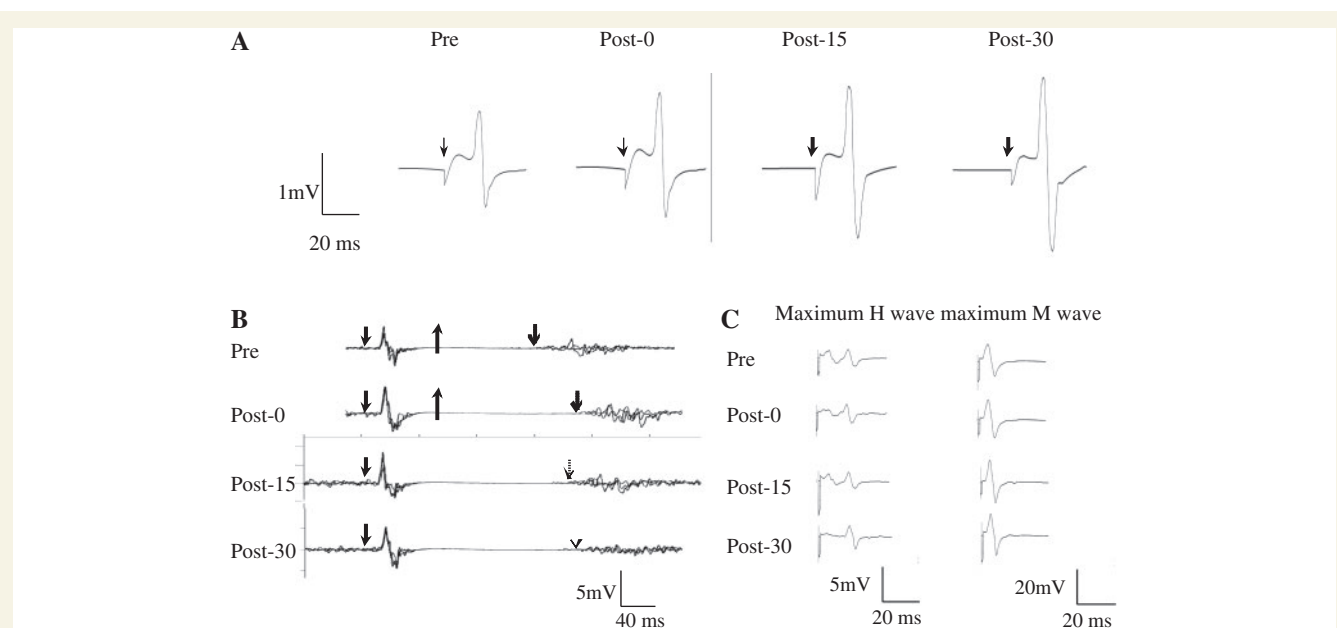


Figure 3 Waveforms of the mean MEPs with stimulus intensity 1 mV (A) and the silent periods (B) for the right EDC muscles and the maximum H and M waves (C) for the right FCR muscles from one representative subject in the post-0, post-15 and post-30 conditions. A solid arrow indicates the stimulus onset (A, B) and a dashed arrow indicates the silent period offset (B).

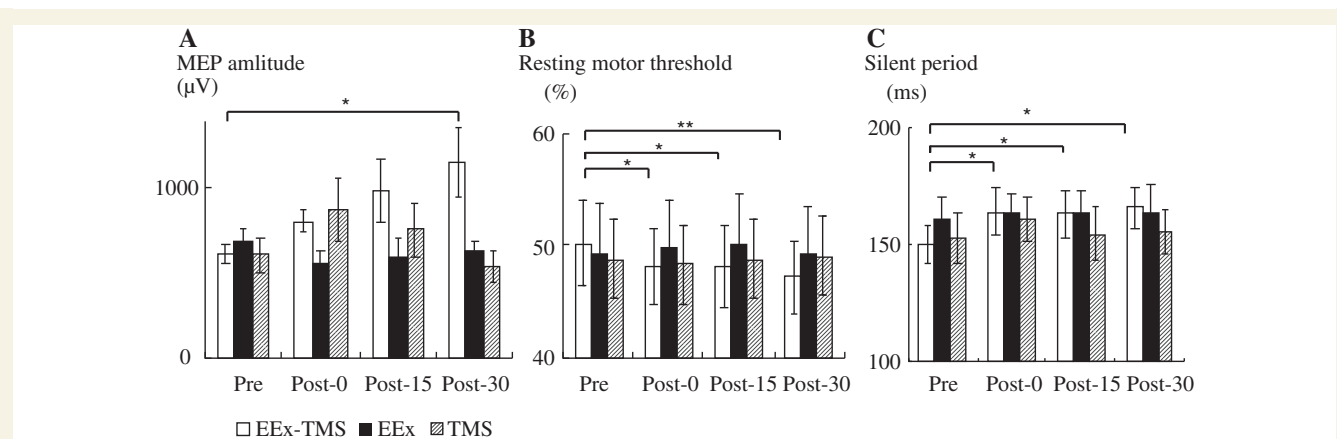


Figure 4 TMS evaluations for EDC muscle in healthy subjects. Only after 'EEEx-TMS', the MEP amplitudes of the right EDC muscles with stimulus intensity 1 mV were significantly enhanced (A), the resting motor threshold was significantly decreased (B), and the silent periods were significantly increased (C) (** $P < 0.001$, * $P < 0.05$).

($F = 3.08$, $P < 0.05$; pre- versus post-0, $P < 0.05$), whereas those of the EDC muscles showed a non-significant tendency to increase ($F = 1.66$, $P > 0.05$).

For the input–output functions, repeated-measures ANOVA showed significant main effects of time ($F = 12.40$, $P < 0.0001$), and the time $\times$ strength interaction ($F = 2.21$, $P < 0.005$) in the right EDC muscles after the 'EEEx-TMS' sessions while the FCR muscles did not. The *post hoc* *t*-test revealed significant increases of the MEP amplitude compared with the pre-condition at intensities of 120% of that of the post-0 condition, of 110, 120 and 150% of that of the post-15 condition, and of 120, 130 and 150% of that of the post-30 condition ($P < 0.01$, 0.01, 0.001, 0.001, 0.001, 0.01 and 0.001, respectively; Fig. 5A). The other two types of session did not cause any significant changes (Fig. 5B, C).

After 'EEEx-TMS', the resting motor threshold for the right EDC muscles was significantly decreased ($F = 10.03$, $P < 0.0005$; Fig. 4B), while that for the right FCR muscles was not ($F = 0.68$, $P > 0.05$). After the other two types of sessions, the resting motor thresholds for the right EDC and FCR muscles were not significantly changed ('EEEx': $F = 2.42$, $P > 0.05$ and $F = 0.92$, $P > 0.05$, 'TMS': $F = 2.65$, $P > 0.05$ and $F = 2.22$, $P > 0.05$, respectively).

After 'EEEx-TMS', the silent period for the right EDC muscles was significantly increased ($F = 5.08$, $P < 0.01$), unlike that for the right FCR muscles ($F = 0.52$, $P > 0.05$) (Fig. 4C). After 'EEEx' and 'TMS', the silent periods for the EDC muscles ($F = 2.26$, $P > 0.05$ and $F = 0.25$, $P > 0.05$, respectively) and the right FCR muscles ($F = 0.19$, $P > 0.05$ and $F = 1.26$, $P > 0.05$, respectively) were not significantly changed.

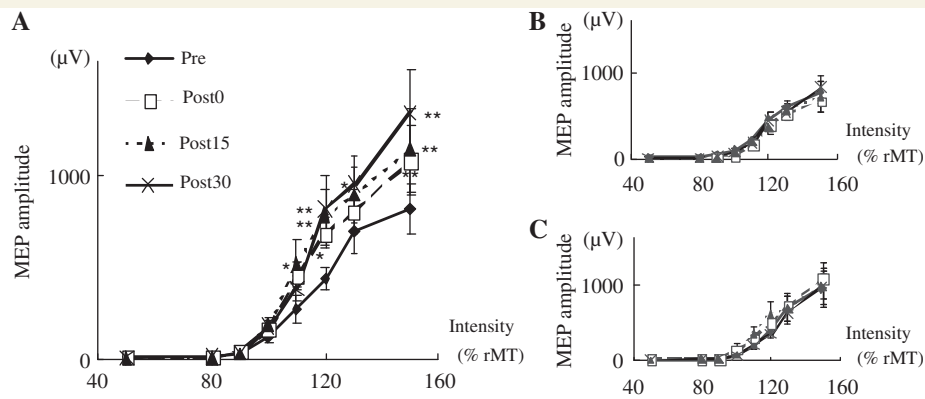


Figure 5 Input-output functions of MEP amplitudes for EDC muscles in healthy subjects. After 'EEEx-TMS', we observed significant increases of the MEP amplitude for the right EDC muscles compared with the precondition at intensities of 120% of that of the post-0 condition, of 110, 120 and 150% of that of the post-15 condition, and of 120, 130 and 150% of that of the post-30 condition (** $P < 0.001$, * $P < 0.05$) (A). 'EEEx' and 'TMS' did not cause any significant changes in the input-output function (B, C). rMT = resting motor threshold.

Changes of H-reflex

The H-latency was 16.14 ± 0.38 ms. The $H_{\max}/M_{\max}$ ratio was not significantly changed in the pre-, post-0, post-15 and post-30 conditions, respectively, after 'EEEx-TMS' ($F = 0.72$, $P > 0.05$) and 'EEEx' ($F = 1.13$, $P > 0.05$), but was significantly increased 30 min after 'TMS' ($F = 7.31$, $P < 0.005$, pre- versus post-30, $P < 0.001$).

Experiment 3

Changes of motor behaviour

Significant increases were found at the end of the repeated sessions (at 6 weeks) and 2 weeks later (at 8 weeks) in the active range of movements in extension for the wrist joint, thumb, index finger and middle finger MCP joint (wrist: $F = 9.55$, $P < 0.005$, thumb: $F = 9.79$, $P < 0.005$, index finger: $F = 14.05$, $P < 0.001$, middle finger: $F = 22.77$, $P < 0.0001$; Fig. 6A).

Significant increases were also found at 6 and 8 weeks in the active range of movements in flexion for the MCP joints of the thumb ($F = 11.66$, $P < 0.001$), index finger ($F = 13.71$, $P < 0.0005$) and middle finger ($F = 10.98$, $P < 0.001$; Fig. 6B).

We found significant increases at 6 and 8 weeks of the passive range of movements in extension for the wrist ($F = 9.51$, $P < 0.005$; baseline versus 6 weeks, $P < 0.05$ and baseline versus 8 weeks, respectively, $P < 0.001$) and the index finger MCP joint ($F = 4.52$, $P < 0.05$; baseline versus 6 weeks, $P < 0.05$ and baseline versus 8 weeks, $P < 0.05$), of the passive range of movements in flexion for the wrist joint ($F = 5.62$, $P < 0.01$; baseline versus 8 weeks, $P < 0.01$), and the MCP joints of the thumb ($F = 10.2$, $P < 0.001$; baseline versus 6 weeks, $P < 0.01$ and baseline versus 8 weeks, $P < 0.001$), index finger ($F = 7.56$, $P < 0.005$; baseline versus 6 weeks, $P < 0.01$ and baseline versus 8 weeks, $P < 0.01$) and middle finger ($F = 6.56$, $P < 0.01$; baseline versus 6 weeks, $P < 0.01$ and baseline versus 8 weeks, $P < 0.01$).

The pinch force in the paretic side was significantly increased after 6 and 8 weeks ($F = 12.25$, $P < 0.0005$; baseline versus 6 weeks, $P < 0.0005$ and baseline versus 8 weeks, $P < 0.001$).

The grip power was significantly increased 2 weeks after the repeated sessions ($F = 6.75$, $P < 0.01$; baseline versus 8 weeks, $P < 0.005$).

Changes of hypertonia

After the repeated sessions, significant decreases were seen in the modified Ashworth Scale scores of the wrist joint ($F = 17.33$, $P < 0.0001$) and the MCP joints of the thumb ($F = 4.17$, $P < 0.05$) and middle finger ($F = 3.70$, $P < 0.05$) in the paretic side (Fig. 6C).

Subjective improvement of paresis

The visual analogue scale for the paretic upper limb was 2.4 ± 0.9 at baseline. It was significantly increased (4.0 ± 1.0) immediately after the intervention and remained high (3.6 ± 0.8) 2 weeks later ($F = 13.55$, $P < 0.005$; baseline versus 6 weeks, $P < 0.005$, baseline versus 8 weeks, $P < 0.05$). The patients reported that they felt their paretic hands to be better after the intervention and these feelings persisted 2 weeks later.

Discussion

The present study used a new rehabilitation approach for the hemiparetic upper-limb motor function in patients with chronic subcortical stroke of varying severity. The functional improvements in the range of movements, grip, pinch power and flexor hypertonia seemed to be brought about by the enhanced UDP in the affected M1 area controlling the agonist muscles involved in the exercises, which were combined with high-frequency repetitive TMS.

In Experiment 1, we found that the combined intervention, but neither of the single interventions of 'EEEx' or 'TMS', resulted in an improvement of extensor movement and grip power along with a reduction of flexor hypertonia in the paretic upper limbs of stroke patients. After 'EEEx-TMS', the improvement was sustained for at least 30 min, which could be attributed to a long-term

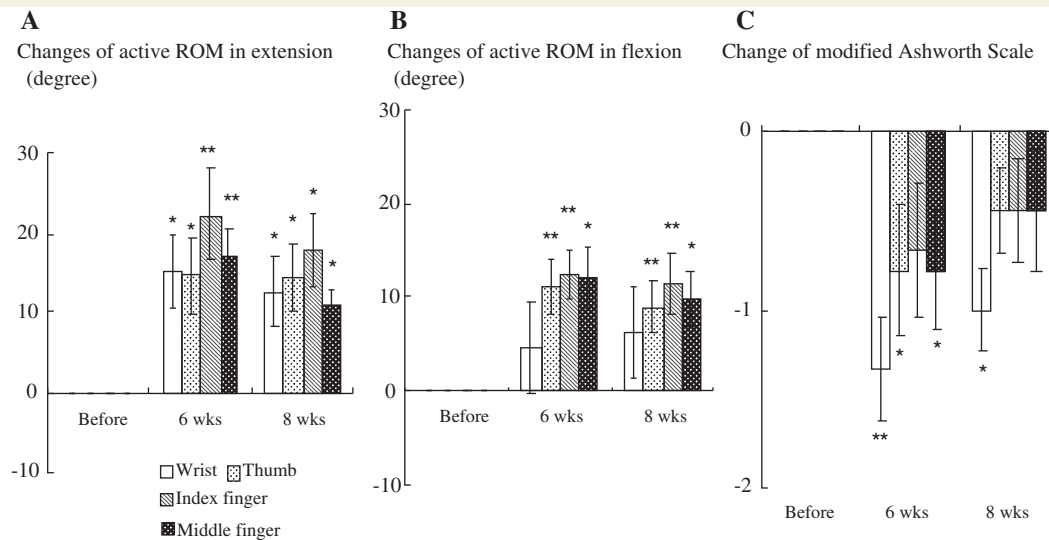


Figure 6 Long-term behavioural evaluations in stroke patients. Significant increases were found in active range of movements in extension for the wrist joint and the MCP joints of the thumb, index finger and middle finger (**A**), and in flexion for the MCP joints of the thumb, index finger and middle finger after the intervention (**B**); these increases remained 2 weeks later (**A**, **B**). After the repeated sessions, the modified Ashworth Scale scores of the wrist joint and of the MCP joints of the thumb and middle finger in the paretic side were significantly decreased (**C**) (** $P < 0.001$, * $P < 0.05$). ROM = range of movement.

potentiation-like mechanism, similar to those reported previously (Khedr *et al.*, 2005; Mansur *et al.*, 2005; Takeuchi *et al.*, 2005; Fregni *et al.*, 2006; Kim *et al.*, 2006; Talelli *et al.*, 2007). By contrast to the previous studies of the improved general motor function in paretic upper-limbs (Takeuchi *et al.*, 2005; Kim *et al.*, 2006), the aim of the current study was to improve the wrist and finger extension in order to overcome flexor hypertonia. After 'EEEx-TMS' sessions, we found significant changes of the active range of movement of extensors, but not flexors, in the paretic upper limbs. It was likely that plasticity for the specific movements—that is, UDP—had occurred. A significant improvement of the hypertonia measured by the modified Ashworth Scale score was also found. Hypertonia includes both muscle contracture and spasticity (O'Dwyer *et al.*, 1996); the former is brought about by alterations to the passive mechanical properties of muscle tissue (Tabary *et al.*, 1972; Dietz *et al.*, 1981; Williams and Goldspink, 1984), the latter is the result of hyperexcitability of spinal reflexes (Lance, 1980, 1990). It seems unlikely that the mechanical properties would have been changed by a 15 min intervention, so the effects of the 'EEEx-TMS' sessions could have been caused by the reduction of spasticity in addition to the enhancement of extensor function. In stroke, spinal circuitries released from the cortical control can cause abnormal spinal hyperexcitability and flexor hypertonia (Benecke *et al.*, 1983; Sheean, 2002) through compensatory sprouting in the segmental afferents to alpha-motoneurons. Improved output functions from the M1 to the extensors might have modified the activity of abnormal afferents through the reinforcement of the reciprocal inhibition over the flexors at the spinal level. In addition to the spinal mechanism, the functional reorganization of subcortical areas surrounding a lesion (Weiller *et al.*, 1993; Izumi *et al.*, 1998) might have contributed the effects of 'EEEx-TMS'. Further studies using neuroimaging techniques would be required to prove this point.

Repetitive TMS alone (the 'TMS' sessions) did not induce behavioural changes, although high-frequency repetitive TMS has been reported to cause long-term potentiation-like changes (Khedr *et al.*, 2005; Mansur *et al.*, 2005; Takeuchi *et al.*, 2005; Fregni *et al.*, 2006; Kim *et al.*, 2006; Talelli *et al.*, 2007). The affected M1 excitability might have been enhanced for both extensors and flexors, but failed to reach a sufficient level. After the training alone (the 'EEEx' sessions), we did not observe any significant behavioural changes.

Although some previous studies suggested beneficial effects of neuromuscular stimulation on hypertonia of paretic upper limbs (Dewald *et al.*, 1996; Hummelsheim *et al.*, 1997; Fujiwara *et al.*, 2009), others found the opposite (Hines *et al.*, 1993). After 'EEEx' sessions, training aided by the neuromuscular stimulation did not induce any change of hypertonia, suggesting that neuromuscular stimulation of our protocol had little or no influence on hypertonia. A recent report showed that electrical stimulation of the spinal dorsal column activated dopaminergic neurons in an animal model of Parkinson disease (Fuentes *et al.*, 2009). Dopamine is considered to be a crucial factor for the induction of plastic changes as previous studies have indicated (Strafella *et al.*, 2003; Floel *et al.*, 2005; Ueki *et al.*, 2006), the peripheral afferent stimulation might facilitate dopamine release through the dorsal column resulting in reinforcement of the M1 plasticity during the 'EEEx-TMS' intervention.

In Experiment 2, we found that the 'EEEx-TMS' intervention could induce enhanced UDP that was specific for agonist, but not antagonist, muscles whereas the 'TMS' intervention could influence the muscles non-specifically, and 'EEEx' failed to induce any significant changes. Since the limb posture can influence the muscle representation and excitability (Melgari *et al.*, 2008), we carefully checked that the subjects kept their forearms in the pronated position during the assessments and interventions.

The 'EEx-TMS' intervention decreased the motor thresholds, which were reported to reflect the neuronal membrane-excitability level in the M1 (Mavrouidakis *et al.*, 1994; Ziemann *et al.*, 1996a; Hallett, 2000). The motor inhibitory system, as measured by the silent period, was increased only for the EDC muscles, and not for the FCR muscles, after 'EEx-TMS', suggesting the enhanced inhibitory function. However, since the silent period is a hybrid parameter including the earlier spinal and later cortical inhibitory circuits (Hallett, 1995; Chen *et al.*, 1999, 2000), its interpretation is not straightforward. To make more precise evaluations of the intracortical inhibitory networks in M1, the method of paired TMS would be necessary (Kujirai *et al.*, 1993; Ziemann *et al.*, 1996b).

In the M1 and the premotor area, neurons have been coded for specific movement (Georgopoulos *et al.*, 1982; Muir and Lemon, 1983; Rizzolatti *et al.*, 1996; Kakei *et al.*, 2001). The results suggested that the function of neurons for specific movement (in this case, extension of the wrist and fingers) could be improved by enhanced excitability or synaptic efficacy of the appropriate neuronal population. Contrary to a previous report by Butefisch *et al.* (2000), the extensor training alone did not induce any significant changes of the M1 excitability in our protocol. Butefisch *et al.* (2002) showed that specific training for >20 min was needed to induce UDP. The 15-min training periods used in our protocol might have been insufficient to induce UDP-like changes.

Previous studies showed that proprioceptive inputs reach the sensorimotor cortex and influence the cortico-spinal excitability (Marsden *et al.*, 1976; Porter and Rack *et al.*, 1976; Kasai and Komiyama, 1991). By pairing the peripheral sensory stimulation and the stimulation of M1 governing the same target muscle, the inhibitory or facilitatory effects could be induced in the sensorimotor cortex depending on their relative timing (Marioenzi *et al.*, 1991; Stefan *et al.*, 2000). Proprioceptive inputs from neuromuscular simulation might have enhanced the facilitatory effects of repetitive TMS.

The spinal excitability measured by the H-reflex was not changed, suggesting that these events might have occurred at the supraspinal level. However, it is possible that MEPs and H-reflexes might not reflect the same motor neuron pool (Meunier *et al.*, 2007), because H-reflex cannot evaluate all of motor neurons in the spinal cord. Thus, the effects of 'EEx-TMS' might be partly mediated by the spinal mechanism.

The electrophysiological assessment could not be done in stroke patients; however, similar plastic changes seemed to have occurred in both the healthy subjects and the stroke patients. It is possible that the physical effects produced by the neuromuscular stimulation during 'EEx' are different for patients and healthy subjects due to different intensities of electric pulses and also different viscoelastic properties of the normotonic and paretic/spastic muscles.

We did not use the non-affected hemisphere as a control condition in the patients but studied the age-matched healthy subjects, because the non-affected hemisphere of patients might not necessarily be normal because of the altered interhemispheric inhibition from the affected hemisphere (Murase *et al.*, 2004).

Regarding the long-term effects of our protocol, performing the 'EEx-TMS' sessions over 6 weeks (12 times in total) induced an improvement of active range of movement and passive range of movement not only for extension but also for flexion with the reduction of hypertonia. Continuing the intervention for a longer period than 6 weeks might have improved muscle contracture such as shortened muscle fibre and stiffened connective tissues produced by the persistent non-use condition (Edstrom, 1970; Tabary *et al.*, 1972; Akesson *et al.*, 1974; Goldspink *et al.*, 1974; Williams and Goldspink, 1984; Dietz *et al.*, 1986; Goldspink and Williams, 1990). The repeated 'EEx-TMS' resolved that condition, especially in extensors, resulting in flexors improvements.

In addition to the behavioural improvement, the patients felt that their paretic limbs moved better. The present approach might improve the quality of life of stroke patients. Although we did not quantitatively test the effects of intervention on daily living activities, it is likely that the improved subjective feelings and improvements in flexor hypertonia could lead to more frequent use of the upper limbs in daily life. It was reported that the Motor Activity Log can be improved after intensive motor training in patients with chronic stroke (Uswatte *et al.*, 2005, 2006; Wolf *et al.*, 2006).

It is possible that the spontaneous recovery might be contaminated in the present results, especially in the 5-month post-stroke patients. However, it is not likely to be the main cause of the functional improvement, since we could find the same results even if those patients were excluded.

In previous reports, therapeutic TMS protocols for stroke were able to induce long-lasting effects by repeating the stimulation for ~1 month (Khedr *et al.*, 2005; Fregni *et al.*, 2006), suggesting that transient long-term potentiation-like effects might have been consolidated through activated second messengers, novel gene expression and conformational changes of neuronal cells as reported in animal studies for learning and memory (Engert and Bonhoeffer, 1999; Bonhoeffer and Yuste, 2002; Matynia *et al.*, 2002; Caporale and Dan, 2008).

With regard to the cost-effectiveness, it is controversial as to which is more economical: community-based or day hospital rehabilitation (Brady *et al.*, 2005), while home-based physiotherapy costs less than hospital-based (Young and Foster, 1993). Our method could be embedded in day hospital rehabilitation. Since the duration of our intervention is only 15 min, its cost-effectiveness might be better than usual rehabilitation or intensive motor training (French *et al.*, 2008). Moreover, due to the long-term effects of our method, it might eventually reduce the cost of social service in the community. Our 'EEx-TMS' method could be a powerful rehabilitative approach for hemiparetic stroke patients.

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