Enhancing Encoding of a Motor Memory in the Primary Motor Cortex By Cortical Stimulation

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Enhancing use-dependent plasticity may result in improvements in the ability of the CNS to compensate for the loss of function (Bütefisch et al. 2002; Feeney et al. 1982). Transcranial magnetic stimulation (TMS) is a technique that allows noninvasive focal stimulation of the human brain (Hallett 2000) and that, in particular settings, can enhance cortical reorganization (Ziemann et al. 1998a) and information processing (Boroojerdi et al. 2001; Flitmann et al. 1998). Therefore one possible strategy to enhance use-dependent plasticity could be the synchronous application of TMS to the motor cortex engaged in performing the training motions, a paradigm reminiscent of in vitro experiments in which stimulation of cortical afferents was paired with depolarization of the synaptic target neuron in a specific temporal relationship (Baranyi and Szente 1987; Baranyi et al. 1991).

In the current study, we hypothesized that TMS applied to a thumb motor representation at a time it is engaged in driving a training motion would facilitate encoding of a motor memory for the trained movements.

METHODS

Subjects

Six of 11 healthy, right-handed volunteers (4 of them men; mean age: 34.2 yr; range: 23–42 yr) fulfilled the inclusion criteria (see following text) and gave written informed consent and participated in the study under a protocol approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke.

Experimental protocol

Subjects, unaware of the experimental purpose of the study, participated in different sessions testing the effects of four different interventions on the ability of training to form an elementary motor memory.

Encoding of a motor memory

Subjects were seated in a chair firmly connected to a frame that kept the head steady and the stimulating coil in a constant position with respect to the head. Head and coil stability were monitored with a three-dimensional laser system as previously described (Bütefisch et al. 2000). Each subject’s right forearm was immobilized in a molded armrest with the four long fingers supported and the thumb freely...
movable. Thumb movements were recorded with a two-dimensional accelerometer mounted on the proximal phalanx of the thumb (Classen et al. 1998). The direction of the thumb movements was calculated from the first-peak acceleration vector.

TMS was delivered from a custom-built magnetoelectric stimulator (Cadwell Laboratories, Kennewick, WA) through a figure-eight-shaped magnetic coil (wing diameter: 7.0 cm). The coil was placed tangentially to the scalp and rotated 45° away from the midline. The current induced in the brain was, therefore directed approximately perpendicular to the central sulcus, which is the optimal condition for activating the corticospinal tract transsynaptically (Kaneko et al. 1996; Werhahn et al. 1994). Stimuli were delivered to the optimal scalp position for eliciting mild isolated thumb movements. In addition to TMS-evoked movement directions, we recorded motor potentials (MEP) evoked by TMS from extensor pollicis brevis (EPB), a hand muscle mediating thumb movements in extension, and from flexor pollicis brevis (FPB), a hand muscle mediating thumb movements in flexion. MEP recorded from the muscle that acted as agonist to the training motions (see following text) is described as MEP agonist whereas that recorded from the muscle that acted as antagonist to the training movements is described as MEP antagonist. Motor threshold (MT) was defined as the minimum TMS intensity that evoked an MEP of ≥50 μV in ≥5 of 10 trials in the target muscle (Rossini et al. 1994). MT was determined to the nearest 1% of maximum stimulator output in the resting EPB and FPB muscles. MEP amplitudes after the training were expressed relative to MEP amplitudes at baseline.

Initially, we determined the direction of TMS-evoked thumb movements by stimulating the optimal scalp position overlying the contralateral motor cortex (total of 60 stimuli) at 0.1 Hz, a rate that does not affect cortical excitability (Chen et al. 1997). Subjects occasionally realized that the thumb had moved but could not determine its direction. In these trials, the baseline direction was defined as the mean angle of TMS-evoked movements that fell in the predominant direction (Fig. 1).

After identifying the baseline TMS-evoked thumb-movement direction, subjects practiced voluntary brisk thumb movements in a direction opposite to baseline in six blocks of 5 min for a total of 30 min at 1 Hz. After every single training movement, the thumb returned to the original position by relaxation as confirmed by electromyography (EMG). Acceleration and EMG signals of 20 training movements recorded at random in each training block were sampled at 1 kHz (120 training movements total). Quality of the motor training, as defined by the accuracy and consistency of training movements, was monitored on-line by one investigator. If necessary, the subject was encouraged to perform better. Additionally, quality and consistency of training movements was measured off-line by calculating the angular difference between the voluntary thumb-movement direction and the TMS-evoked thumb-movement direction at baseline, the dispersion of thumb movement training directions, and the magnitude of the first-peak acceleration of these movements. The measure for dispersion of thumb-movement direction is derived from the length of the individual vectors in the unit circle. In a unit circle, a mean vector of 1 means that the direction of the individual vectors was identical, while a mean vector close to 0 means maximal dispersion (Batschelet 1981). At the end of the 30-min training period, TMS-evoked thumb-movement directions were recorded for 30 min (post 1–post 3, see following text) in all conditions. Because testing post 3 in Train+TMS synchronous centre, revealed

![FIG. 1. Experimental set-up. A: the direction of transcranial magnetic stimulation (TMS)-evoked or voluntary movement was derived from the 1st-peak acceleration in the 2 major axes of the movement, measured by a 2-dimensional accelerometer. B: circular frequency histogram illustrating the main effect of training. Baseline TMS-induced movement directions are a combination of extension and abduction (open). Training movements were performed in a direction approximately opposite to baseline (arrow). The mean training direction is at the center of the training target zone (TTZ). The black bar scale shows the number of TMS-evoked movements (in this case, 20) that fall in each 10° bin (see METHODS). Posttraining TMS-induced movement directions fell largely in the TTZ (filled), close to a 180° change from the baseline direction. Circular frequency histograms in the following figures are constructed in the same way. C: schematic diagram of the experimental setup during training. TMS application was triggered by the electromyographic (EMG) activity of the muscle supporting the training movement of the thumb (training agonist). In the subject illustrated in this figure, the training direction was flexion; therefore the flexor pollicis brevis (FPB) operated as training agonist during training motions. EMG activity was recorded from the FPB. The potentiometer was adjusted in such a way that TMS was triggered when the EMG amplitude of the training agonist (FPB) was −10–20% of the maximal EMG amplitude during a ballistic movement. Accordingly, the TMS to the contralateral motor cortex occurred within the 1st half of the EMG burst that was generated by the training agonist. EPB, extensor pollicis brevis.

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asynchronous contra motor cortex asynchronous to the training movements (training combined with TMS at 0.1 Hz applied to the contralateral evoked by TMS in hand muscles antagonist to the thumb voluntary training motions. This study activates corticocortical connections targeting pyramidal tract neurons (Day et al. 1987). This session activates corticocortical connections targeting pyramidal tract neurons (Day et al. 1987). This intervention was identical to TRAIN + TMS synchronous contra except that TMS pulses were applied asynchronous in relation to the training movements. Specifically, TMS pulses were delivered regularly at 0.1 Hz at random in reference to the movements that occurred at 1 Hz.

Inclusion criteria

Participating subjects fulfilled the following inclusion criteria: ability of TMS to elicit isolated thumb movements in the absence of movements of any other digits, wrist, or arm; consistent (reproducible) direction of TMS-evoked thumb movements in the baseline condition; and postraining TMS-evoked thumb-movement directions matching the training direction.

Statistical analysis

The proportion of TMS-evoked movements in TTZ before (baseline) and after (post 1) training were compared using Wilcoxon signed-rank test. A distribution-free Friedman rank test compared changes in the proportion of TMS-evoked movements falling in the TTZ after the different interventions calculated for the entire 30-min postraining period (post 1–post 3). Wilcoxon signed-rank test was used to compare the magnitude of these changes for the entire 30-min postraining period of each intervention relative to Train alone and to compare the training kinematics of each intervention relative to Train alone.

RESULTS

Motor threshold, movement threshold, and the amplitudes of MEP, MEP training antagonist, and MEP training agonist prior to the training did not differ across conditions (Table 1). Similarly, training kinematics, including magnitude of the first-peak acceleration of training movements, dispersion of training movement directions, and angular difference between mean baseline and training angle, did not differ significantly across conditions (Wilcoxon signed-rank test: NS, Table 2).

Formation of a motor memory

Training alone or combined with TMS applied to the contralateral M1 either synchronous or asynchronous to the EMG.

### TABLE 1. Measurements of motor excitability prior to the interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>MT&lt;sub&gt;baseline&lt;/sub&gt;</th>
<th>MT&lt;sub&gt;training&lt;/sub&gt;</th>
<th>Percent Stimulation Output</th>
<th>Percentage MT&lt;sub&gt;baseline&lt;/sub&gt;</th>
<th>MEP&lt;sub&gt;agonist&lt;/sub&gt;, mV</th>
<th>MEP&lt;sub&gt;antagonist&lt;/sub&gt;, mV</th>
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<tbody>
<tr>
<td>Train alone</td>
<td>51.0 ± 2.6</td>
<td>52.8 ± 2.0</td>
<td>57.7 ± 1.9</td>
<td>113.8 ± 3.7</td>
<td>0.79 ± 0.17</td>
<td>0.68 ± 0.21</td>
</tr>
<tr>
<td>Train + TMS synchronous contra</td>
<td>50.2 ± 2.1</td>
<td>48.8 ± 1.6</td>
<td>54.8 ± 1.8</td>
<td>109.6 ± 2.0</td>
<td>1.59 ± 0.51</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>Train + TMS asynchronous contra</td>
<td>48.8 ± 1.8</td>
<td>48.8 ± 1.8</td>
<td>55.6 ± 1.9</td>
<td>114.1 ± 1.9</td>
<td>1.30 ± 0.33</td>
<td>0.62 ± 0.18</td>
</tr>
<tr>
<td>Train + TMS synchronous ipsi</td>
<td>50.0 ± 1.7</td>
<td>52.0 ± 1.7</td>
<td>56.5 ± 1.9</td>
<td>113.1 ± 1.5</td>
<td>1.22 ± 0.15</td>
<td>0.82 ± 0.18</td>
</tr>
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</table>

Motor threshold (MT) is given as a percentage of maximal stimulator output. Minimal stimulus intensity to elicit an isolated movement of the thumb (MovT). MovT is given as a percentage of MT of maximal stimulator output (Stim. output) and percent of MT of baseline muscle. Values are means ± SE. MEP<sub>agonist</sub>, motor potentials evoked by transcranial magnetic stimulation (TMS) in hand muscles supporting thumb voluntary training motions; MEP<sub>antagonist</sub>, motor potentials evoked by TMS in hand muscles antagonist to the thumb voluntary training motions.
TABLE 2. Training kinematics

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Peak Acceleration</th>
<th>Angular Dispersion</th>
<th>Angular Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Train alone</td>
<td>0.417 ± 0.032</td>
<td>0.994 ± 0.002</td>
<td>178.5 ± 2.1</td>
</tr>
<tr>
<td>Train +</td>
<td>0.586 ± 0.094</td>
<td>0.994 ± 0.002</td>
<td>182.2 ± 4.7</td>
</tr>
<tr>
<td>TMS synchronous&lt;sub&gt;contra&lt;/sub&gt;</td>
<td>0.484 ± 0.045</td>
<td>0.974 ± 0.012</td>
<td>175.9 ± 9.3</td>
</tr>
<tr>
<td>Train +</td>
<td>TMS asynchronous&lt;sub&gt;contra&lt;/sub&gt;</td>
<td>0.350 ± 0.035</td>
<td>0.992 ± 0.003</td>
</tr>
</tbody>
</table>

Peak acceleration is expressed in g. Angular dispersion is expressed as length of unit vector. Angular difference gives the difference between the mean training angle and the mean baseline angle and is expressed in degrees. The means ± SE are given for all data. There was no statistically different training parameters between conditions (Wilcoxon signed-rank test: NS).

The activity of the agonist muscle (Train+TMS synchronous<sub>contra</sub> and Train+TMS asynchronous<sub>contra</sub>) resulted in a significant increase in the proportion of TMS-evoked thumb movements in the TTZ 0–10 min after completion of the training (post1) in reference to baseline (Wilcoxon signed-rank test: Train alone, P = 0.05; Train+TMS synchronous<sub>contra</sub>, P = 0.03; Train+TMS asynchronous<sub>contra</sub>, P = 0.05; Fig. 2). TMS applied to the ipsilateral M1 (Train+TMS synchronous<sub>ipsi</sub>) blocked this effect (Wilcoxon signed-rank test: NS; Fig. 2).

At baseline, the amplitude of MEP training antagonist exceeded that of MEP training agonist (Table 1). Consistent with the kinematic data, Train alone and Train+TMS synchronous<sub>contra</sub> resulted in a differential modulation of MEP amplitudes, whereby the MEP training agonist increased and MEP training antagonist remained either unchanged or decreased (Fig. 3, A and B). Also consistent with the kinematic data, Train+TMS synchronous<sub>ipsi</sub> did not result in such differential modulation (Fig. 3D). In Train+TMS asynchronous<sub>contra</sub>, both MEP training antagonist and agonist increased although MEP training agonist still exceeds MEP training antagonist (Fig. 3C).

Longevity of the encoded motor memory

The longevity of changes in TMS-evoked movement directions differed according to the intervention [Friedman rank test (post 1–post 3 pooled): P < 0.001; Fig. 3]. The changes lasted longer in Train+TMS synchronous<sub>contra</sub> (Fig. 3B) than in Train alone [Fig. 3A; Wilcoxon signed-rank test (post 1–post 3 pooled data): P < 0.001], which was similar to Train+TMS asynchronous<sub>contra</sub> [Fig. 3C; Wilcoxon signed-rank test (post 1–post 3: NS)].

Changes in TMS-evoked movement directions lasted for ~20 min in Train alone (Fig. 3A) and Train+TMS asynchronous<sub>contra</sub> (Fig. 3C). Train+TMS synchronous<sub>contra</sub> led to a substantial enhancement of the effect to >60 min (Fig. 3B). Consistent with the kinematic data, the differential regulation of MEP training agonist and MEP training antagonist amplitudes in Train alone and Train+TMS synchronous<sub>contra</sub> was present for ≥30 min (Fig. 3, A and B). Data from a representative subject are shown in Fig. 4.

DISCUSSION

The main finding of this study was that TMS enhanced training-dependent encoding of an elementary motor memory when applied synchronously to the motor cortex engaged in generating the practice movements.

Motor training leads to encoding motor memories in the CNS (Shadmehr and Brashers-Krug 1997). Under our experimental conditions, training leaves a memory trace in the primary motor cortex that reflects the kinematic details of the practiced movements (Classen et al. 1998). Here, we investigated if it is possible to enhance this plastic process by synchronous stimulation to the motor cortex engaged in the training motions, a proposal consistent with the Hebbian principle that potentiation of synaptic efficacy (LTP) occurs when its pre- and postsynaptic elements are simultaneously active (Hebb 1949). When inputs converge onto a target neural structure in temporal synchrony, they can enhance cortical plasticity (Barany and Feher 1981; Barany and Szente 1987; Irikai et al. 1989). With these considerations in mind, we hypothesized that application of TMS, a technique that stimulates preferentially intracortical connections targeting pyramidal tract neurons (Rothwell 1997) at a time when the motor cortex is engaged in generating a training motion, should lead to an enhancement in this form of plasticity. Previous work demonstrated that TMS can increase the excitability of the primary motor cortex (Pascau-Leone et al. 1994b), enhance deafferentation-induced plasticity in humans (Ziemann et al. 1998), and induce LTP- and LTD-like changes in slice preparations of cortical tissue (Wang et al. 1996).

Subjects participated in four different sessions testing the effects of motor training alone and motor training accompanied by TMS applied in synchrony and out of synchrony with the training motions. Preceding training, measures of corticomotor neuronal excitability, including motor thresholds (Mavroudakis et al. 1994), movement thresholds, and MEP amplitudes (Amassian et al. 1987; Ridding and Rothwell 1997) in two muscle groups controlling thumb movements, were similar across conditions. Additionally, monitoring of motor training kinematics revealed that the magnitude of the first-peak acceleration of training movements, the dispersion of training movement directions, and the angular difference between mean baseline and training angles did not differ significantly across conditions. The mean peak acceleration of training motions in
Train/H11001 TMS synchronous contra was slightly larger than in Train alone, similar to Train/H11001 TMS asynchronous contra (Table 2). It is possible that subthreshold TMS application in combination with training led to larger movement amplitudes. If present, this effect was very small because TMS was applied in only 1 of 10 trials, and in those trials, it did not evoke muscle twitches. More importantly, prolongation of learning occurred in the Train/H11001 TMS synchronous contra but not in Train/H11001 TMS asynchronous contra despite comparable acceleration levels. Overall, these results document comparable baseline motor cortical excitability levels preceding training and comparable motor training kinematics across the four interventions.

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First, we evaluated the effects of the different interventions on the formation of a motor memory. TMS applied to the motor cortex involved in the training task enhanced this form of plasticity, whereas TMS applied to the motor cortex ipsilateral to the training hand reduced it. This latter finding is consistent with interhemispheric competition models of cortical sensory processing (Mesulam 1999). Each hand motor representation exerts inhibitory influences on the homonymous representation in the opposite hemisphere (Di Lazzaro et al. 1999; Ferbert et al. 1992; Gerloff et al. 1998; Murase et al. 2004; Werhahn et al. 2002). It has been proposed that balanced interhemispheric interactions are required for the generation of proper voluntary movements (Ferbert et al. 1992). It is then possible that stimulation of the motor cortex ipsilateral to the training hand resulted in enhanced inhibitory drive exerted over the hand motor representation engaged in the training task, leading to the observed attenuation of the training effects (see Fig. 3D), a finding reminiscent of the reported reduction in deafferentation-induced plasticity in one hemisphere by application of TMS to the other hemisphere (Ziemann et al. 1998a).

The proposal is consistent with the reported modulation of corticomotor excitability (Schambra et al. 2003) and intracortical inhibitory circuits in one hemisphere by stimulation of homologous motor areas of the opposite hemisphere (Daskalakis et al. 2002; Schnitzler et al. 1996). Additionally, Train + TMS synchronous resulted in cancellation of training effects on encoding of this memory trace, consistent with a similar decrease in MEP amplitudes in agonist and antagonist muscles. It is of note that this form of plasticity relies on differential changes in excitability in agonist and antagonist muscles (Bütefisch et al. 2000) that were cancelled by application of ipsilateral TMS.

On the other hand, we found that stimulation of the motor cortex engaged in the training task enhanced the training effects if applied in synchrony with the training motions. Intracortical microstimulation (ICMS) alone results in cortical reorganization in the somatosensory and motor cortices in the rat (Nudo et al. 1990; Recanzone et al. 1992; Spengler and Dinse 1994). In humans, TMS increases motor cortical excitability (Pascual-Leone et al. 1994b) and can also facilitate functions mediated by the stimulated cortical areas (Boroojerdi et al. 2001; Flitmann et al. 1998). Interestingly, the facilitatory influence of TMS on training effects was observed in our study when stimulation was applied in synchrony with the training motions. The mechanisms underlying this effect remain to be determined. However, it is conceivable that LTP-like processes, known to operate in the motor cortex in vivo (Rioul-Pedotti et al. 1998) and thought to influence this particular form of plasticity (Bütefisch et al. 2000), could be enhanced by such Hebbian paradigm as also proposed when synchronized tactile-stimulation strategies were applied in the somatosensory system (Dinse et al. 2003). The duration of this elementary motor memory (>60 min when training was combined with synchronous stimulation but only ~20 min when TMS application was asynchronous to the training motions) is consistent with this hypothesis.

Therefore we demonstrate for the first time that it is possible to enhance the effects of motor training and the duration of this memory trace by noninvasive cortical stimulation. This form of memory encoding may underlie the beneficial effect of preperformance practice (for example, in athletics or musical performance), and it may be a requirement for purposeful skill acquisition in intact humans and in the rehabilitation of persons with brain damage (Bütefisch et al. 1995). The results raise the exciting possibility that cortical stimulation combined with rehabilitative treatment could lead to more prominent behavioral gains than rehabilitative treatment alone in patients with cortical lesions like stroke.

Acknowledgments

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References
