Motor Cortex Plasticity Predicts Recovery in Acute Stroke

Repetitive transcranial magnetic stimulation of the brain given as intermittent theta burst stimulation (iTBS) can induce long-term potentiation (LTP)-like changes in the stimulated hemisphere and long-term depression (LTD)-like changes in the opposite hemisphere. We evaluated whether LTP- and LTD-like changes produced by iTBS in acute stroke correlate with outcome at 6 months. We evaluated the excitability of affected hemisphere (AH) and unaffected hemisphere (UH) by measuring motor threshold and motor-evoked potential (MEP) amplitude under baseline conditions and after iTBS of AH in 17 patients with acute ischemic stroke. Baseline amplitude of MEPs elicited from AH was significantly smaller than that of MEPs elicited from UH, and baseline motor threshold was higher for the AH. Higher baseline MEP values in UH correlated with poor prognosis. iTBS produced a significant increase in MEP amplitude for AH that was significantly correlated with recovery. A nonsignificant decrease in MEP amplitude was observed for the UH. When the decrease in the amplitude of UH MEPs was added to the regression model, the correlation was even higher. Functional recovery is directly correlated with LTP-like changes in AH and LTD-like changes in UH and inversely correlated with the baseline excitability of UH.

Keywords: LTD, LTP, transcranial magnetic stimulation

Introduction

The phenomenon of activity-dependent strengthening of synaptic transmission, known as long-term potentiation (LTP), is involved in neuroplasticity and is believed to play a central role in the adaptive changes and recovery observed after a brain lesion (Hagemann et al. 1998; Centonze et al. 2007; Di Filippo et al. 2008). Recently, protocols of repetitive transcranial magnetic stimulation (rTMS) of the brain that resemble experimental LTP models have been introduced. The rTMS paradigm known as intermittent theta burst stimulation (iTBS) produces a prolonged increase in cortical excitability (Huang et al. 2007), mediated by N-methyl-D-aspartic acid receptor (Huang et al. 2007), supporting the hypothesis that the aftereffects of iTBS involve LTP-like changes. iTBS may also induce changes in excitability in contralateral hemisphere, producing a long-term depression (LTD)-like phenomenon in this hemisphere (Di Lazzaro et al. 2008; Suppa et al. 2008).

The aim of the present study was to investigate the correlation between LTP- and LTD-like phenomena induced by iTBS in patients with acute stroke and the functional outcomes at 6 months. According to the currently influential concept that considers perilesional LTP as an essential mechanism of lesion-induced plasticity and a prerequisite for functional recovery (Hagemann et al. 1998) and the experimental demonstration of the role of synaptic plasticity in the process of recovery after stroke (Centonze et al. 2007), we expected a correlation between iTBS-induced changes and outcome. Our hypothesis was that higher levels of LTP in the affected hemisphere (AH) would be associated with a better outcome.

Methods and Patients

Patients

Seventeen patients (mean age, 68.2 ± 11.7 years) with first-ever stroke were recruited. Inclusion criteria were 1) ischemic stroke (both cortical and subcortical) involving the middle cerebral artery territory, 2) <10 days after stroke, 3) hand weakness, and 4) recordable motor-evoked potential (MEP) after TMS of the lesioned hemisphere. Exclusion criteria were 1) seizure history, 2) hemorrhagic stroke, 3) concomitant neurological or other severe medical problems, 4) complete paralysis of the hand, 5) inability to give informed consent, 6) treatment with drugs acting on central nervous system, and 7) contraindications for TMS and magnetic resonance imaging (MRI) studies. In order to identify patients at risk for poststroke epilepsy, all patients underwent an electroencephalography before entering the study (Rossini and Johnston 2005), and none of them showed any epileptic abnormality. The main clinical, neuroradiological, and demographic characteristics of the patients are reported in Table 1. The evaluation of neurological impairment in the acute phase was based on the National Institutes of Health Stroke Scale (NIHSS). Outcome at 6 months was clinically assessed using the modified Rankin Score (mRS). Though the mRS has important limitations, we have chosen this scale because no other outcome measure has been shown to be superior (Adams et al. 2004). The outcome was defined in agreement with previous studies in which favorable outcome depends on the initial severity of symptoms (Adams et al. 2004). Because in our patients, the baseline NIHSS score was >8, a favorable outcome was defined as mRS = 0 (no symptoms) (Adams et al. 2004). All patients underwent a standardized protocol of rehabilitation based on physical therapy for 2 months.

Magnetic Resonance Imaging

In the acute phase, all patients underwent contrast-enhanced brain MRI. MRI was performed with a 1.5-T scanner (GE Signa; General Electric, Milwaukee, WI), with $T_1$ - fluid attenuated inversion recovery, diffusion-weighted imaging (DWI), $T_2$ - and $T_2$ -weighted images acquired before and after the administration of paramagnetic contrast agent. The lesion margins, as seen on DWI-apparent diffusion coefficient (ADC) images, were freehand contoured in each single axial slice, and the areas of the obtained regions of interest were multiplied by the slice thickness and then summed to obtain a lesional volume measurement. The lesion...
volume has been assessed on DWI-ADC because in acute phase the lesion extent on DWI consistently corresponds to the final infarct volume (Schafer et al. 2002; Srinivasan et al. 2006). Lesion size and location were also estimated by using the Alberta Stroke Program Early CT Score (ASPECTS) (Barber et al. 2000). This study was performed according to the Declaration of Helsinki and approved by the ethics committee of the university’s medical faculty. Patients gave their informed consent before participation.

**Table 1**

<table>
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<th>Patient No.</th>
<th>Age (y) / sex</th>
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**Magnetic Stimulation**

**Motor Cortex Excitability to Single-Pulse TMS**

Magnetic stimulation was performed with a high-power Magstim 200 (Magstim Co, Whitland, Dyfed). A figure-of-eight coil with external loop diameters of 9 cm was held over the motor cortex at the optimum scalp position to elicit MEPs in the contralateral first dorsal interosseous (FDI) muscle. The induced current flowed in a posteroanterior direction.

We evaluated the threshold and amplitude of MEPs. The resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 50 μV) at rest (Rossini et al. 1994). The active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 200 μV in 50% of 10 trials) during isometric contraction of the tested muscle. The MEP amplitude was evaluated using a stimulus intensity of 120% RMT with the muscle at rest. Ten data sweeps were collected, and the mean peak-to-peak amplitude of the MEPs was calculated.

We evaluated the RMT, AMT, and MEP amplitude of the AH and unaffected hemisphere (UH).

In a subgroup of 10 patients (patient numbers 2, 4, 5, 7, 10, 11, 12, 14, 15, and 16—Table 1), we also evaluated short-interval intracortical inhibition (SICI) to paired TMS both for the AH and the UH. SICI was studied using a paired-pulse magnetic stimulation paradigm (Kujirai et al. 1993): Two magnetic stimuli were given through the same coil with a pulse width of about 280 μs and a maximum magnetic field strength of 1.5 T. The initial direction of the current induced in the brain was anterior to posterior. The stimulation intensity was defined in relation to the AMT evaluated using the MagPro stimulator. An intensity of 80% AMT was used. We used the iTBS protocol in which 10 bursts of high-frequency stimulation (3 pulses at 50 Hz) are applied at 5 Hz every 10 s for a total of 600 pulses.

The MEP amplitude was evaluated using a stimulus intensity of 120% RMT with the muscle at rest. Subjects were given audiovisual feedback of the electromyographic (EMG) signal at high gain to assist in maintaining complete relaxation; trials contaminated by EMG activity were discarded. After iTBS, amplitude of MEPs was measured using the same stimulus intensity used under baseline conditions. Ten data sweeps were collected, and the mean peak-to-peak amplitude of the MEPs was calculated. We evaluated the effects of iTBS on RMT, AMT, and MEP amplitude, both for AH (stimulated) and UH. Because we were interested in evaluating the effect of iTBS on the interhemispheric balance of excitability, we calculated an index of the bilateral change produced by iTBS. It should be noted that a decrease in UH MEP amplitude, together with an increase in AH MEP amplitude, decreases the imbalance in excitability. An index of the effects of iTBS on both hemispheres (indicated by RMTbal, AMTbal, MEPbal) was obtained with the following formulas:

\[
\text{RMT}\text{bal} = \Delta \text{RMT}_{AH} - \Delta \text{RMT}_{UH},
\]

\[
\text{AMT}\text{bal} = \Delta \text{AMT}_{AH} - \Delta \text{AMT}_{UH},
\]

\[
\text{MEP}\text{bal} = \Delta \text{MEP}_{AH} - \Delta \text{MEP}_{UH},
\]

where Δ = before iTBS - after iTBS, for RMT and AMT, and Δ = after iTBS - before iTBS, for MEP.

In this way, a decrease in UH MEP is added to the change observed in the AH, whereas an increase in UH MEP is subtracted from the change observed in the AH. The changes induced by iTBS were computed in the opposite direction for RMT and AMT in order to give the “balancing” indexes the same meaning (the higher the values, the higher the balancing).

**Statistical Analysis**

The comparison of MT, MEP, and SICI between AH and UH was performed by means of paired t test (after logarithmic transformation on MEP amplitude data to improve Gaussianity and reduce the potential bias due to outliers). The effect of iTBS on UH and AH was assessed with analysis of variance (ANOVA) for repeated measures with hemisphere (AH vs. UH) and time (before vs. after stimulation) as within-subject factors.

The main analysis aimed to evaluate the correlation between modulation induced by iTBS and clinical outcome (mRS at 6 months). Our sample size did not allow us to identify and validate prognostic markers previously identified in the epidemiological literature. However, the experimental setup allowed us to obtain rigorous measurements of some neurophysiological features and to provide preliminary data regarding potential new prognostic indexes. Considering the relatively small sample size of our study, the prognostic value of the proposed neurophysiologic indexes was simply assessed in 2 steps. First, bivariate Spearman’s ρ correlations between mRS at 6 months and the measures obtained at the baseline were determined. The measures reaching statistical significance, as well as the measures that should always be taken into account (age, clinical status at baseline, and ASPECTS), were entered into an ordinal regression model with mRS at 6 months as the dependent variable. The output of this analysis provided a preliminary model, with adjustments made for the effects of all other factors considered. Because the frequency distribution of mRS at 6 months was skewed toward the lowest values of the scale (6 patients obtained a score of 0, 6 patients obtained a score of 1, and only 33% of the patients obtained a score above 1), the clinical outcome was
categorized using 2 classes full recovery (mRS = 0) and partial recovery (mRS higher than 0). A logistic regression model provided preliminary estimates of the probability of a full recovery according to the neurophysiologic markers.

Because SICI was measured in a subgroup of patients, the correlation between mRS at 6 months and AH and UH SICI was evaluated separately using bivariate Spearman’s $r$ correlations.

**Results**

iTBS was well tolerated; there were no adverse events, and the observation during the electrophysiological study and subsequent prolonged clinical observation in the stroke unit did not reveal any abnormal behavior that could have suggested seizures.

**Baseline Cortical Excitability**

The baseline amplitude of MEPs recorded in the AH (mean = 0.25 mV; 95% confidence interval [CI] = 0.16–0.40) was smaller ($t_{16} = 5.295$, $P < 0.001$) than the amplitude of MEPs elicited from the UH (mean = 0.98 mV; 95% CI = 0.64–1.50). As indicated by a paired $t$-test ($t_{16} = 2.823$, $P = 0.012$), the baseline RMT was higher for the AH (RMT: mean = 65.2% of maximum stimulator output [MSO], 95% CI = 53.8–72.7) than for the UH (RMT: mean = 54.5% of MSO, 95% CI = 48.0–61.1). Although with slighter evidence, similar findings were obtained for AMT ($t_{16} = 2.077$, $P = 0.054$), with a baseline value equal to 44.1% of MSO (95% CI = 37.2–50.9) in AH and 38.5% of MSO (95% CI = 34.3–42.7) in UH.

SICI was significantly reduced for the AH of the 10 studied patients ($t_9 = 2.425$, $P = 0.038$); inhibition was less pronounced in AH (mean responses reduced to 60.8% of the test size, 95% CI = 38.1–83.4) than in UH (mean responses reduced to 32.8% of the test size, 95% CI = 19.6–46.0).

**Effects of iTBS of the AH**

Table 2 reports the results of the repeated-measures ANOVA for the 3 considered parameters. Pre- versus post-iTBS comparisons (corrected for multiple comparisons by means of Sidak procedure) for each parameter in each hemisphere are graphically represented by the CIs of Figure 1, where clear changes were found for RMT and MEP. However, the main finding pertaining to the “time × hemisphere” interaction (RMT: $P = 0.001$, AMT: $P = 0.014$, MEP: $P = 0.002$), which indicated—for each of the 3 parameters—a balancing effect of iTBS: The changes induced in the AH were significantly different from the opposite changes in the UH. The differences between changes that occurred after iTBS will be hereafter designated as the RMTbal, AMTbal, and MEPbal. The RMTbal ranged between −1 and 9; the median was 2, and the mean was 2.5 (standard deviation [SD] = 2.6). The AMTbal ranged between −4 and 6; both median and mean were 2.0 (SD = 3.0). The MEPbal ranged between 0.06 and 2.02; the median was 0.25, and the mean was 0.51 (SD = 0.56). For each parameter, negative values indicate a further imbalance in excitability after iTBS, with increased excitability in the AH and/or decreased excitability in the UH, whereas the positive values are indicative of a reduction in the imbalance after iTBS with increased excitability in the AH and/or decreased excitability in the UH.

**Correlation between Electrophysiological Parameters and Clinical Outcome**

The bivariate associations between the clinical outcome (as indicated by mRS at 6 months) and the demographic, clinical, and neurophysiological parameters are reported in Table 3. Among baseline characteristics, only NIHSS at baseline and MEPs evoked by stimulation of the UH seemed to play a role in predicting the clinical status of patients 6 months after stroke ($P = 0.049$). Higher MEP values in the UH correlated with a poor prognosis ($P = 0.003$). Conversely, the increase in the MEP amplitude in the AH induced by iTBS was significantly associated with a better prognosis ($P = 0.012$).

When this increase in MEP was adjusted for the change in the MEP amplitude that occurred in the UH (indicated by MEPbal), the correlation between the MEP amplitude in the AH and prognosis reached the value of $-0.618$ ($P = 0.008$). When the UH MEP at baseline and the MEPbal were included in a multivariable ordinal regression model (also taking into account age, baseline NIHSS, and the extension of the lesion as evaluated by the ASPECTS), their prognostic effect was generally confirmed (Wald statistic = 4.967, $P = 0.026$, for UH MEP at baseline and Wald statistic = 3.451, $P = 0.063$, for MEPbal). Finally, a stepwise logistic regression analysis indicated that the strongest predictor of full recovery was MEPbal, although this analysis was underpowered (results were based on only 6 fully recovered patients) (Wald statistic = 3.352, $P = 0.067$) (Figure 2).

The log-transformed values of the MEP amplitudes of the 17 patients, followed by the changes produced by iTBS, MEPbal index, and clinical outcome at 6 months (mRS and dichotomous recovery) together with the regression equation used to model the correlation between the MEPbal and the probability of a full recovery, can be seen in Supplementary Table 1. In the subgroup of 10 patients who underwent SICI study, we did not find any correlation between AH and UH SICI values and mRS at 6 months (AH: $P = 0.532$, UH: $P = 0.619$, AH − UH: $P = 0.246$).

**Discussion**

We evaluated the characteristics and prognostic value of the LTP-like phenomena produced by iTBS in patients with acute stroke. We also evaluated the LTD-like effects produced by iTBS in the contralateral motor cortex (Suppa et al. 2008). iTBS resulted in a reduction in the AMT and RMT and an increase in the MEP amplitude for the AH. The increase in MEP amplitude was significantly correlated with recovery at 6 months. The correlation between the changes produced by iTBS and the functional recovery at 6 months after stroke shows for the first time in humans that the level of LTP-like phenomena in the AH
is correlated with long-term recovery. A reduction of the threshold of the AH has been previously reported by Fregni et al. (2006) after suppressive rTMS of the UH performed over a period of 2 weeks in a group of patients with chronic stroke, and, interestingly, the change in threshold of the AH was significantly correlated with motor function improvement. This finding supports the correlation between the ability of inducing excitability changes using rTMS and the process of motor recovery.

When the LTD-like changes produced in the UH are also taken into consideration, it appears that the strongest predictor of full recovery is represented by a measure that combines the LTP-like effects produced by iTBS in the stimulated motor cortex with the LTD-like effects produced in the contralateral motor cortex. This suggests that the recovery of function after stroke relies on reorganization activity in both hemispheres. The AH LTP and the UH LTD produce a balancing effect on the excitability in the 2 hemispheres, the more pronounced the effect of iTBS on these measures, the higher the probability of full recovery.

The LTD-like effect produced by iTBS in the contralateral hemisphere is conceivably correlated with the level of ongoing interhemispheric inhibition (Suppa et al. 2008). A role of the level of interhemispheric influences in the recovery process after a stroke has been hypothesized by Traversa et al. (1997). More recently, Murase et al. (2004) suggested that a stronger inhibition from the UH to the AH (conceivably together with a weakened inhibition from the AH to the UH) could contribute to motor disability in stroke patients (Murase et al. 2004). This is consistent with models considering hemispheric rivalry or competition as a key factor for recovery after a stroke. According to this model, the AH is doubly disabled both by its own damage and by interfering output from UH (Murase et al. 2004; Ward and Cohen 2004). However, the present data provide a more complex picture of the interhemispheric rivalry in that it suggests that, together with the baseline excitability of the 2 hemispheres, the response of both hemispheres to a protocol of stimulation producing an artificial form of plasticity is relevant to recovery.

Similar to a previous study (Delvaux et al. 2003), a negative correlation was found between the amplitude of motor responses evoked by single-pulse TMS in the UH and recovery. This finding provides further evidence for the idea that unbalanced excitability of the 2 hemispheres, with UH hyperexcitability, interferes with recovery after a stroke. The role of the interfering output from the UH in the genesis of
neurological disorders has also been recently demonstrated in nonmotor systems (Koch et al. 2008).

As previously reported (Liepert et al. 2000; Manganotti et al. 2002; Delvaux et al. 2003; Swayne et al. 2008), motor threshold was higher on the stroke side than on the healthy side, and the amplitude of MEPs elicited by stimulation of the AH was smaller than that of the MEPs evoked from UH. However, the baseline excitability of the AH seems to be less relevant: The amplitude of MEPs evoked by stimulation of the AH was not correlated with the recovery at 6 months. This finding is consistent with the results of the study by Swayne et al. (2008), showing a poor correlation between MEP amplitude and clinical deficit at 6 months after stroke. They hypothesized that the recovery of motor function is more dependent on the reorganization of alternative cortical networks than on the function of the original corticospinal pathways spared by the ischemic lesions. The LTP-like plasticity in the acute phase might be the expression of the potential of these alternative cortical networks.

In agreement with previous studies (Liepert et al. 2000; Cicinelli et al. 2003; Swayne et al. 2008), SICI, a test related to the excitability of inhibitory gamma-aminobutyric acid-A cortical circuits (Chen et al. 2008) was weaker in the AH. The amount of SICI was not correlated with long-term recovery.

Taken together, our results suggest that good functional recovery is directly correlated with a propensity for LTP-like activity in the AH and a propensity for LTD-like activity in the UH. As suggested by a previous study, these characteristics, which tend to favor the increase in excitability of the AH, may represent an adaptive form of plasticity, whereas an opposite pattern that would increase the imbalance may represent a maladaptive form of plasticity (Rossini et al. 2003).

In agreement with previous studies, DWI lesion volume did not predict outcome (Hand et al. 2006), whereas the NIHSS score at baseline confirmed to be a predictor of clinical status (Adams et al. 2004). However, in our study, the level of cortical plasticity seems to be the strongest predictor of the outcome. In a larger data set, other variables could be taken into account to ascertain the specific and independent role of the proposed measure of cortical plasticity. However, this first study indicated that its prognostic value held even after adjusting for baseline clinical status, age, lesion size, and neurophysiological baseline characteristics (MEP values in acute phase).

**Modulation of Motor Cortex Excitability: Perspectives for Rehabilitation**

An increasing interest is now being directed toward the development of rehabilitative, pharmacological, and neuro-modulatory approaches to promote functional recovery after stroke. Given the fundamental role of plasticity in poststroke brain reorganization, it would be extremely useful to develop a noninvasive test capable of evaluating the impact of therapeutic strategies on plastic phenomena in human brain. Knowledge of the mechanisms that promote recovery is fundamental for developing new strategies for the treatment of deficits related to stroke.

The present study shows that an imbalance in cortical excitability, hyperexcitability of the unaffected motor cortex, reduced propensity for the induction of LTP-like changes in the affected motor cortex, and reduced propensity for LTD-like changes in the unaffected motor cortex in the acute phase of brain ischemia are hallmarks of poor recovery. These findings provide further support for the use of neuromodulation techniques to promote recovery after stroke, enhancing adaptive plasticity (Mansur et al. 2005; Fregni et al. 2006; Talelli et al. 2007; Koch et al. 2008). TMS-related techniques may have a role both in driving cortical plasticity in the right direction and in assessing the effects of interventional protocols on cerebral cortex excitability. However, because it has been demonstrated that in the very early phases after stroke together with the physiological LTP that promote recovery there is a “pathologic” posts ischemic LTD, possibly involved in delayed neuronal death (Picconi et al. 2006), any procedure increasing cortical excitability should be performed with caution in the first hours after a stroke.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

**Notes**

Conflict of Interest: None declared.

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**References**


