Low-frequency (1 Hz) repetitive transcranial magnetic stimulation (rTMS) can depress the excitability of the cortex locally and has been proposed for the treatment of disorders such as schizophrenia and epilepsy. Some have speculated that the depressant effect is related to long-term depression (LTD) of cortical synapses. Because in vitro LTD can be enhanced by pretreatment of synapses with higher-frequency stimulation, we hypothesized that if rTMS depression had mechanisms in common with LTD, higher-frequency priming would increase it also. In 25 healthy volunteers in two experiments, we measured motor-evoked potentials (MEPs) from TMS of the motor cortex to define the baseline response. Subthreshold rTMS (6 Hz, fixed rate or frequency modulated) was used to prime the motor cortex, followed by suprathreshold 1 Hz stimulation for 10 min at just above the MEP threshold. Over the next 60 min, we recorded MEPS every 10 sec and found significant increases in the amount of cortical depression with both types of 6 Hz priming rTMS relative to sham. The MEP depression from 6 Hz-primed 1 Hz rTMS showed no evidence of decay after 60 min. Pretreatment with 6 Hz primes both 1 Hz rTMS depression and LTD. Although not conclusive evidence, this strengthens the case for overlapping mechanisms and suggests a potent new technique for enhancing low-frequency rTMS depression that may have experimental and clinical applications.

**Key words:** priming; transcranial magnetic stimulation; plasticity; motor cortex; long-term depression; treatment

### Materials and Methods

**Subjects**

Twenty-six healthy individuals gave written informed consent for the study, which was approved by the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-1430.

### Subjects

M.I. was supported in part by the San Antonio Area Foundation (San Antonio, TX) and the Georgia State University (Atlanta, GA).

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Twenty-six healthy individuals gave written informed consent for the study, which was approved by the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-1430 (Atlanta, GA).
Disorders and Stroke Institutional Review Board. Subjects were inter-
viewed and examined by a neurologist and found to be free of any signif-
icant medical or psychiatric illness or medications known to affect the
CNS. Additional demographic data are given below.

Experimental setup
Subjects were seated with the right forearm and hand supported. The surface
electromyogram (EMG) was recorded from the right abductor pollicis brevis
(APB) and extensor carpi radialis (ECR) muscles. We chose to record from a
physiological flexor and extensor in the hand and forearm to determine
whether the effects of priming were present in muscles with different physi-
ology (Lemon, 1993) and to make artifacts of limb positioning, etc. less likely.
Subjects were instructed to maintain muscle relaxation throughout the
study. The EMG was amplified, analog filtered (100 Hz and 1 kHz), digitized
(2 kHz) using a micro1401 unit and Signal software (Cambridge Electronic
Design, Cambridge, UK), and stored on a standard computer for off-line
analysis. In each trial, the EMG was acquired for a total of 400 msec with 100
msec of baseline before delivery of the stimulus. The absence of voluntary
contraction was verified on-line by visual monitoring of the EMG and off-
line inspection of the individual traces. Trials with EMG activity in the base-
line were discarded.

A Magstim Super Rapid stimulator (The Magstim Company New
York, NY), which produces a monophasic pulse, and an 8 shaped coil
with two 70 mm diameter windings were used for the first six subjects in
experiment 1. Thereafter, to avoid the problem of coil heating, a Neo-
Pulse stimulator, which emits a biphasic pulse, and a coil consisting of a
ferrous core wound with eight turns of wire (Neotonus, Atlanta, GA)
were used for the subsequent 10 subjects in experiment 1 and all subjects
in experiment 2.

Placement of the coil over the motor cortex was done by finding and
marking the scalp site that was optimal for producing MEPs in the right
APB on a tightly fitting cap with a chin strap. In all subjects, stimulation
at this position produced smaller MEPs in the ECR as well. A custom-
built apparatus held the coil on a rigid arm and provided a chin rest for
the subject. The position of the coil was monitored visually throughout the
stimulation sessions.

Testing TMS
The MEP threshold was measured at the start of each experiment. This
was defined as the minimum stimulus intensity required to elicit an MEP
of ≥50 μV in the relaxed APB on ≥5 of 10 consecutive trials at least 5 sec
apart. After measuring the MEP threshold, we applied TMS pulses at an
intensity of 115% of MEP threshold for 10 min to obtain a baseline
measure of MEP amplitude. The timing of the individual stimuli was
varied randomly by ≥20% around a mean frequency of 0.1 Hz to avoid
rhythmicty. After each treatment (see below), 0.1 Hz TMS was again
delivered at the same intensity for 60 min (Fig. 1A). It has been shown
(Chen et al., 1997a) that stimulation at 0.1 Hz for periods of up to 1 hr
does not cause detectable effects on motor cortex excitability.

Treatment rTMS
Priming. In each session, after the baseline MEP amplitude determina-
tion, one of three types of priming stimulation was delivered for 10 min.
The types of priming were as follows: (1) 6 Hz rTMS, with the pulses occurring at a fixed frequency; (2) frequency-modulated 6 Hz (6 Hz FM) rTMS, with the frequency modulated through a range of 4–8 Hz each second (Fig. 1B). [Burst-modulated stimulation has excitatory effects, and so-called “theta-patterned” stimulation has become a standard pro-
cedure in in vitro studies to produce long-term potentiation (LTP) and
prime LTD (Larson and Lynch, 1986; Larson et al., 1986);] and (3) sham
6 Hz rTMS, with the coil activated at 6 Hz but placed with the back of the
coil housing in contact with the head and the front tilted up at an angle.
Under this condition, there was an auditory artifact but no MEPs could
be produced with stimulation even at maximum intensity.

For priming, the TMS intensity was set at 90% of the MEP threshold.
Subjects received either 6 Hz, 6 Hz FM, or sham priming on different
days separated by at least 1 week. The order of priming types was ran-
domized and counterbalanced across subjects in each experiment. The
details of the regimens are given below in the descriptions of experiments
1 and 2.

1 Hz rTMS. After priming, we delivered 1 Hz rTMS at 115% of the
MEP threshold for 10 min to induce MEP depression.

Replication and the effect of priming dose
To look for an effect of the priming dose, all three priming types were
tested with two train lengths: In experiment 1, 16 individuals (10 men;
mean age, 37 ± 8 years; range, 23–49; two left-handed) were given 600
priming pulses (20 trains lasting 5 sec separated by 25 sec). In experiment
2, nine different individuals (six women; mean age, 26.6 ± 7.4 years;
range, 19–38; two left-handed) received 1200 priming pulses (20 trains
lasting 10 sec separated by 20 sec).

Reaction time testing
To screen for behavioral side effects of the combined treatment, we mea-
sured the auditory reaction time before and after each TMS session.
Subjects were instructed to press the space bar on a standard computer
keyboard by abducting the right thumb as quickly as possible in response
to a click delivered at a random interval after a warning cue.

Data analysis
The reaction time was measured from the go signal (click) to the re-
corded key press. Data from before and after each rTMS session were
compared within and across priming types and doses by repeated-
measures ANOVA.

The measurement of the MEPs and the data analysis were entirely
automated. The MEPs were measured peak-to-peak using Signal soft-
ware (Cambridge Electronic Design), and the data were exported to Mat-
lab (Mathworks, Natick, MA) or Statview (SAS Institute, Cary, NC) for
additional analysis. The post-treatment period of each experimental ses-
sion was divided into six 10 min epochs, and the mean MEP amplitude
was calculated for each epoch in each individual. Because of the high
variability in MEP amplitude from trial to trial and the skewness of the
means when there are many “zero amplitude” responses (Fig. 2), we
log-transformed the MEP amplitudes before analysis. This has been
shown to restore the lower tail of the distribution under similar condi-
tions (Wassermann, 2002).

To exclude significant differences in baseline excitability between ses-
sions, the baseline MEP amplitudes were compared in each experiment
by repeated-measures ANOVA. The effect of priming on the depressant
effect of 1 Hz treatment was evaluated separately in the APB and ECR by
repeated-measures ANOVA comparing baseline with the first 10 min
ePOCH after treatment, with treatment (before vs after) and priming type as
independent variables. To look at the persistence of depression after 1 Hz
treatment, another set of ANOVAs was performed with time (10 min
epochs 1–6 after treatment) and priming type as independent variables.
The mean MEP amplitude data for each epoch were normalized across

A

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Figure 1. A, Flowchart showing the paradigm for experiments 1 and 2. B, Graphical repre-
sentation of the constant (6 Hz) and frequency-modulated (6 Hz FM) priming stimulation
patterns.
subjects by dividing each value by the same individual’s baseline mean MEP amplitude. Therefore, the baseline epoch was not included in the time course analysis. We also compared the data from the subjects in experiment 1 who were treated with each of the two stimulators by adding stimulator type as an independent variable to these analyses. To test for the effect of the dose of priming, the results of experiments 1 and 2 were compared for each muscle in separate ANOVAs. *Post hoc* comparisons were done with the Bonferroni–Dunn test. The threshold for statistical significance was set at $p < 0.05$.

**Results**

After her first session (sham), one subject who was anxious and apprehensive during the study experienced a “heavy” feeling in the right hand after treatment, which recurred episodically for a few days. Her reaction time did not change with treatment, and there were no abnormalities on neurological examination. She was excluded from further participation, and her demographic and physiological data were not included. No other participants experienced any subjective effects of stimulation that lasted beyond the study session. There were no significant effects of treatment on reaction time either within experiments or after pooling all data. Nor did the MEP threshold change significantly within subjects across sessions; the mean never varied by $>0.5\%$ of maximum stimulator output. There were no significant differences on any measure between the results from the subjects treated and tested with the Magstim and Neoto-nus stimulators.

**Experiment 1**

There were no significant differences between the MEP amplitudes in the pretreatment baseline between sessions in either muscle. The comparison of baseline with the first 10 min epoch showed significant depression of MEPs independent of priming type after the combined priming–1 Hz treatment (Fig. 3) (main effect of treatment: APB, $F_{(1,29)} = 32.7, p < 0.0001$; ECR, $F_{(1,29)} = 16.5, p = 0.001$). However, the effect was greater after 6 Hz and 6 Hz FM than after sham in both muscles (treatment × priming type interaction: APB, $F_{(2,28)} = 3.4, p = 0.046$; ECR, $F_{(2,28)} = 4.5, p = 0.02$).

*Post hoc* testing revealed significant differences when comparing 6 Hz and 6 Hz FM with sham in both muscles (all $p < 0.01$) but no significant differences between the two active priming types. There were no significant main effects of time or priming type × time interactions.

![Figure 2](image-url)
Experiment 2

There were no significant differences in the baseline MEP amplitudes between sessions in either muscle. Again, comparison of the baseline with the first 10 min epoch after treatment showed that priming–1 Hz treatment significantly decreased MEP amplitude (Fig. 3) (main effect of treatment: APB, $F_{(1,15)} = 18.4, p = 0.003$; ECR, $F_{(1,15)} = 11.1, p = 0.01$). Although active priming produced more MEP depression than sham in both muscles (Fig. 3), the priming type × treatment interaction did not reach significance in either muscle in this analysis. However, in the 60 min after treatment (Fig. 4), the main effect of priming type was significant in both muscles (APB, $F_{(2,78)} = 3.8, p = 0.04$; ECR, $F_{(2,78)} = 5.8, p = 0.01$). The only post hoc comparison to reach significance after adjustment for multiple comparisons was between sham and 6 Hz FM in the ECR ($p = 0.006$). As in experiment 1, there was no statistical evidence of wearing off (i.e., the main effect of time was nonsignificant) and there were no significant priming type × time interactions. Comparison of the two experiments showed no significant main effects of experiment or interactions of experiment with other factors.

Discussion

These results demonstrate that stronger depression of motor cortex excitability by 1 Hz treatment can be achieved if priming rTMS at 6 Hz precedes it. The difference from sham was present in a flexor and an extensor muscle at almost all points from 10 to 60 min after treatment in two experiments using two different groups of subjects. The reduction in average MEP amplitude of ∼30–40% was comparable with our own peak effects and those of other groups, but the lack of any sign of recovery at 60 min indicates an effect that is longer-lasting than reported previously (Touge et al., 2001).

A potential limitation of the data concerns the repositioning of the coil after sham stimulation, which might have introduced a systematic difference in the effectiveness of the 1 Hz treatment that followed. However, random error or bias in the replacement of the coil after sham would be unlikely to produce less apparent inhibition relative to baseline, because moving off the optimal site after priming would produce smaller MEPs in the post-treatment test phase, effectively mimicking inhibition. A suboptimal position for the baseline phase that was “corrected” after sham priming would have resulted in a group difference in the baseline MEP amplitude, which we ruled out statistically (see Results).

This study was designed to ask whether theta modulation of the 6 Hz priming stimulation might be more effective than constant frequency stimulation, but this was not the case. The lack of any discernable difference between the priming types suggests that any frequency in the 4–8 Hz range, through which the FM stimulation was modulated, might be as effective. We also note that the theta burst paradigm used for in vitro studies delivers high-frequency (e.g., 100 Hz) electrical stimulation in bursts occurring at the theta frequency. How well our paradigm approximated these conditions, if at all, is a matter of conjecture at this point. Nevertheless, frequency modulation remains worth considering as an adjuvant technique in rTMS treatment studies.

It is also notable that increasing the length of the priming trains did not increase the MEP depression produced by subse-
quent 1 Hz rTMS. This suggests that the effect of the combined treatments in experiment 1 may have reached a “floor,” below which additional MEP depression was not possible. However, the study was limited by the fact that we only performed the test stimulation for a period of 60 min, during which there was little if any wearing off of the depression under the primed conditions. Perhaps differences in the duration of the effect would have emerged in a longer observation period.

A remarkable feature of the motor cortex depression with 1 Hz rTMS is the lack of any readily detectable behavioral correlate. In previous studies (Wassermann et al., 1996; Chen et al., 1997a), we examined a variety of neuropsychological and clinical measures, but the only motor finding to emerge was an increase in finger-tapping frequency. Other manipulations that depress the MEP (for instance, the commonly prescribed Na+ channel blocker anticonvulsants phenytoin (Chen et al., 1997b) and carbamazepine (Ziemann et al., 1996)) also have generally mild or nonexistent motor side effects at clinically effective doses, suggesting that the motor system compensates easily for changes in the excitability of the cortical output neurons. In testing simple reaction time, it was our intention to screen only for behavioral effects at the clinically significant level. More detailed or rigorous testing (for instance, a task requiring targeted movements without feedback) might have disclosed effects on motor behavior.

Although the primary purpose of this study was to look for a priming effect on 1 Hz rTMS depression, our findings also provide some support, albeit circumstantial, for the analogy between the depressant effect of 1 Hz rTMS in the human cortex and LTD in animals. LTD of motor cortex neurons by 1 Hz rTMS is plausible and has been invoked as an explanation (Touge et al., 2001). TMS activates the muscles via a trans-synaptic pathway (Thompson et al., 1989; Rothwell et al., 1991, 1994) that these and other results have shown is susceptible to modification by 1 Hz rTMS. Recently, Rioult-Pedotti et al. (2000) demonstrated learning-related potentiation in horizontal connections onto layer II–III motor cortex pyramidal neurons along with conventional LTP and LTD in the same synapses. They proposed that such synaptic changes are responsible for the rapid reorganization of motor outputs in response to changes in input (i.e., motor learning). It is possible that 1 Hz rTMS causes alterations in these or other synapses in the motor cortex, possibly those directly onto corticospinal neurons, resulting in decreased neuronal excitability or synaptic efficacy along the pathway from cortex to spinal cord. The augmentation of the depressant effect of 1 Hz rTMS by priming at 6 Hz is the first physiological evidence in favor of an LTD-like mechanism for this change.

Touge et al. (2001), in arguing against LTD as the basis of rTMS depression, noted that the effect on MEPs disappears in voluntarily activated muscles, because if synapses directly in the path of the stimulus from cortical horizontal axon to muscle were modified, the MEP produced by a stimulus of a given intensity should be smaller after treatment regardless of whether neurons in the pathway are depolarized by voluntary drive. The more likely explanation, according to this view, is a decrease in the excitability (e.g., mild hyperpolarization or stabilization) of the corticospinal neurons themselves that could be overcome by sufficient voluntary drive, thereby restoring MEP amplitude. LTD, however, need not occur “in series” with the path of the stimulus from cortex to muscle to affect neuronal excitability. For instance, collateral intracortical pathways, such as those studied by Rioult-Pedotti et al. (1998, 2000), could exert an influence on the resting excitability of cortical output cells that would be swamped by voluntary drive. One might also note that in vitro LTD has not been tested under conditions analogous to voluntary activation of the modified pathway.

It has been observed that under some conditions, priming stimulation of synapses in vitro, especially by very strong stimulation, can lead to enhancement of long-term potentiation (Abraham and Bear, 1996; Abraham and Tate, 1997). A similar effect has been observed in humans during rTMS studies (Wassermann et al., 1996a; Chen et al., 1998), in which accidental seizures occurred unexpectedly after closely spaced series of stimulation trains that would have been safe if delivered in isolation. Our findings with subthreshold 6 Hz priming indicate that even very mild stimulation can alter the cortical response to later stimulation. This has implications for protocols designed to increase cortical responsiveness (for example, those used in the experimental treatment of depression and Parkinson’s disease) (Wassermann and Lisanby, 2001) and for the safety of high-frequency rTMS in general.

Regardless of the mechanism of 1 Hz rTMS depression or 6 Hz priming, these results have considerable methodological importance. Low-frequency rTMS is being adopted successfully as a technique for making “temporary lesions” for the study of cognitive processes (Kosslyn et al., 1999; Hilgetag et al., 2001), sensory (Knecht et al., 2003), and motor cortical (Muellbacher et al., 2002) processes, to take only a few leading examples. Clinicians, too, are beginning to use the same procedures to depress pathologically overactive brain areas, most successfully in schizophrenia (Hoffman et al., 2003), but also in focal dystonia (Siebner et al., 1999), Tourette syndrome (Munchau et al., 2002), epilepsy (Tergau et al., 1999; Theodore et al., 2002), hemineglect after stroke (Brighina et al., 2003), and depression (Klein et al., 1999). Priming stimulation, which has been safe and easy to administer in our hands, could greatly improve the effectiveness of low-frequency rTMS as a tool for the laboratory and clinic.

References


