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Repetitive paired-pulse TMS at I-wave periodicity markedly increases corticospinal excitability: A new technique for modulating synaptic plasticity

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Abstract

Objective: We hypothesised that facilitatory I-wave interaction set up by paired-pulse transcranial magnetic stimulation delivered with I-wave periodicity (iTMS) may reinforce trans-synaptic events and provide a means for modulating synaptic plasticity and cortical excitability. Our objective was to determine whether prolonged iTMS can increase corticospinal excitability, and whether this form of stimulation can have lasting aftereffects.

Methods: Paired stimuli of equal strength with a 1.5 ms inter-stimulus interval were delivered for 30 min at a rate of 0.2 Hz. Motor threshold and motor evoked potential (MEP) amplitude to single-pulse TMS was compared before and after intervention.

Results: Paired-pulse MEP amplitude increased linearly throughout the period of iTMS, and had increased five-fold by the end of the stimulation period. Single-pulse MEP amplitude was increased a mean of four-fold for 10 min after stimulation. Motor threshold was unaffected.

Conclusions: iTMS is an effective method for increasing excitability of the human motor cortex, and probably acts by increasing synaptic efficacy.

Significance: Reinforcement of trans-synaptic events by iTMS may provide a means to investigate and modulate synaptic plasticity in the brain.

Keywords: Paired-pulse TMS; I-wave; Synaptic plasticity

1. Introduction

A number of transcranial stimulation techniques have been described that can modify levels of excitability and inhibition in human motor cortex both during and for varying periods after stimulation. Repetitive single-pulse transcranial magnetic stimulation (rTMS) at low (~1 Hz) or high (~10 Hz) frequencies can have inhibitory or facilitatory effects respectively (Chen et al., 1997; Huang and Rothwell, 2004; Maeda et al., 2000; Pascual-Leone et al., 1994; Wu et al., 2000). Short trains of 50 Hz stimuli delivered at theta frequencies (5 Hz) can increase or decrease corticospinal excitability (Huang and Rothwell, 2004; Huang et al., 2004, 2005), and paired associative stimulation (PAS) also increases corticospinal excitability (Ridding and Taylor, 2001; Stefan et al., 2000, 2002). Repeated paired-pulse TMS at an interstimulus interval (ISI) of 3 ms has been shown to increase intracortical inhibition (Khedr et al., 2004). These effects have been attributed to forms of long-term potentiation or depression (LTP/LTD), based in part on findings in animal studies (Wang et al., 1996) and pharmacological studies in the human that implicate effects on synaptic transmission (Ziemann et al., 1998a). Transcranial direct-current stimulation (TDCS) can also evoke persisting changes in corticospinal excitability, in this case by changing

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membrane excitability or modulating homeostatic plasticity (Lang et al., 2003). Each of these interventions have merit, and the interest in refining and developing them follows from the potential they have to improve motor performance and motor learning by modulating cortical plasticity, and the implications this has for rehabilitation and for enhancing the acquisition of motor skills in normal individuals and in those with developmental learning difficulties.

Single-pulse TMS of the motor cortex elicits a brief train of high-frequency descending volleys at a periodicity of ~1.5ms (Day et al., 1989). These volleys result from indirect (trans-synaptic) activation of corticospinal neurons via excitatory cortical interneurones. It has been shown that by delivering a supra-threshold stimulus (S1) that elicits I-waves followed by a sub-threshold stimulus (S2) at intervals corresponding to I-wave periodicity, the amplitude of the motor evoked potential (MEP) is increased, and this is thought to be due to facilitatory I-wave interaction (Tokimura et al., 1996; Ziemann et al., 1998b). The favoured mechanism is that cortico-cortical discharges produced by S2 arrive at the corticospinal neuron during periods of increased firing probability caused by S1 (Hanajima et al., 2002; Ziemann et al., 1998b).

In the present study, we hypothesised that facilitatory I-wave interaction set up by paired-pulse TMS delivered with I-wave periodicity (iTMS) may reinforce trans-synaptic events and provide a means for directly targeting synaptic plasticity. Our aims were to determine whether prolonged iTMS can alter corticospinal excitability, and whether this form of stimulation can have lasting aftereffects.

2. METHODS

2.1. Subjects

Eight neurologically-normal healthy right-handed individuals (3M; 23–40 years of age) gave informed consent to participate in the study. The study was undertaken with the approval of the University Human Ethics Committee.

Subjects sat comfortably, with hands supported on a cushion, and were instructed to maintain relaxation, keep their eyes open, and to look straight ahead through a window with a view over treetops. All distracting stimuli were avoided during the study, which lasted ~50 min.

2.2. TMS

MEPs were recorded from surface electrodes placed in a belly-tendon arrangement over the first dorsal interosseous (FDI) muscle of the right hand. Signals were amplified ×1000, and filtered at 20–2 kHz, before being digitised at 1 kHz for 100 ms post-stimulus. Cortical stimuli were delivered through a 5 cm diameter figure-of-eight coil connected to a dual-pulse stimulator (Bistim 200[2], Magstim Company, UK). The coil was held tangential to the scalp with the handle directed posteriorly and angled at 45° to the *para*-sagittal plane in order to induce posterior–anterior current flow approximately at right angles to the pre-central gyrus.

The optimal scalp position for generating a MEP in the FDI was obtained by initial exploration over a 1 cm grid marked on a closely fitting cap worn by the subjects. At the optimal site, the intensity that gave rise to a small (200 μV) MEP was determined (S_{200}). Recruitment curves were generated centred on this intensity (10% of stimulator output below S_{200}, in 2% steps, to 10% above S_{200}; 2 stimuli per intensity; 5 s intervals). This abbreviated recruitment curve protocol could be completed in <2 min. Single-pulse MEP amplitude was calculated from the mean peak–peak amplitude of a series of 10 MEPs obtained from stimulation at an intensity that gave rise to an MEP of 0.5–1.0 mV at baseline (typically 5% of stimulator output above S_{200}; 5 s intervals; duration <1 min).

Two baseline single-pulse MEP amplitude measurements were performed, followed by a baseline recruitment curve measurement. After the iTMS intervention, the recruitment curve measurement was repeated (in the first 2 min post-intervention), and single-pulse MEP amplitude measurements (taking 1 min each) were then performed every 2 min for 14 min.

2.3. iTMS protocol

For iTMS, stimuli of equal strength were delivered at an ISI of 1.5 ms. Stimulus intensity was set to the stimulus intensity that, when delivered as a pair, generated a MEP of between 0.5 and 1 mV (henceforth referred to as the iMEP). A period of 30 min of iTMS was administered, during which stimuli were delivered every 5 s (360 stimuli total). None of the subjects experienced any discomfort during the period of iTMS. Peak–peak iMEP amplitude was measured for each stimulus pair.

2.4. F-waves

In three subjects, F-waves were measured with supra-maximal electrical stimulation of the ulnar nerve at the wrist (100 μs pulse width). Thirty stimuli were delivered (at 5 s intervals) before and 2 min after iTMS intervention. In one subject, F-wave measurements (30 stimuli) were taken serially at 2 min intervals for 10 min after iTMS. The number of F-waves elicited and their mean area were quantitated.

2.5. Single-pulse TMS intervention

In three subjects, MEP amplitude was compared before and after 30 min of single-pulse TMS (5 s intervals, stimulus intensity 5% above S_{200}, 360 stimuli in total).

2.6. Data analysis

Group mean data is presented as mean ± SEM. Mean amplitude of the iMEPs obtained each minute (total of 12 MEPs per minute) were calculated for each subject, expressed as a percentage of the mean data for the first minute, and averaged across subjects. For single-pulse MEP amplitude, the two baseline measurements were pooled, and the mean MEP amplitude post-intervention (for each of the data sets obtained at 2 min intervals) was expressed as a percentage of pooled baseline. Mean recruitment curves were calculated by averaging MEP amplitude from the two MEPs obtained at each intensity, expressing these as a percentage of the mean MEP amplitude across all intensities for the pre-intervention curve, and averaging across subjects. Single-pulse MEP amplitude was compared pre- and post-intervention with a one way repeated measures ANOVA and post-hoc *t* tests to test for differences at each time interval. Trends in iMEP amplitude with time during the intervention were calculated from regression analysis. The correlation between pre- and post-intervention recruitment curves was tested with Pearson's statistic.

3. Results

3.1. iTMS intervention

The intensity of the individual stimuli making up the iTMS pulses was 82 ± 0.9% of S_{200}. Single-pulse TMS delivered at this intensity did not evoke a MEP. During the first minute of iTMS, mean iMEP amplitude was 0.46 ± 0.07 mV. Throughout the period of iTMS, iMEP amplitude increased steadily (increasing by 13.5 ± 0.7% per minute; $r = 0.96$; $P = 10^{-17}$; Fig. 1), and by the end of the intervention period had increased ~five-fold to 2.16 ± 0.35 mV.

3.2. MEP amplitude

The two baseline values were not significantly different. Single-pulse MEP amplitude was significantly increased at each time point for 10 min after intervention ($F_{(7,49)} = 6.81$; $P < 0.001$), with a mean increase over the 10 min period of 398% (Table 1; Fig. 2). MEP amplitude returned to baseline 12–14 min post intervention. There was no change in MEP latency.

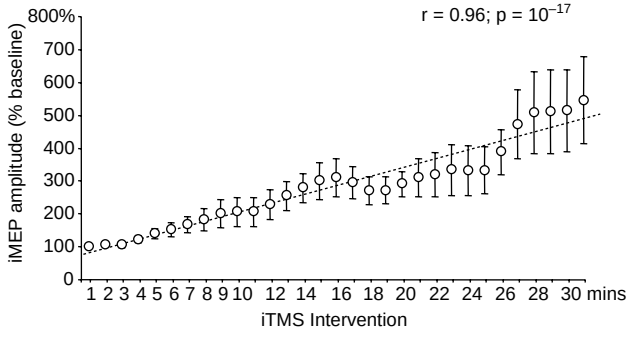

Fig. 1. Group mean iMEP amplitude during 30 min of iTMS at 0.2 Hz, normalised to 1 min, showing a steady increase in amplitude of ~500% by the end of the stimulation period.

Table 1
Percentage increase (compared to baseline) single-pulse MEP amplitude 2–14 min after iTMS intervention

	2 min	4 min	6 min	8 min	10 min	12 min	14 min
Mean	381	464	476	377	272	139	88
SEM	134	110	135	82	60	31	27
P	0.018	0.0005	0.0028	0.0004	0.0022	0.108	0.669

3.3. Recruitment curves

The post-intervention curve had a greater increase in MEP amplitude vs stimulus intensity than the pre-

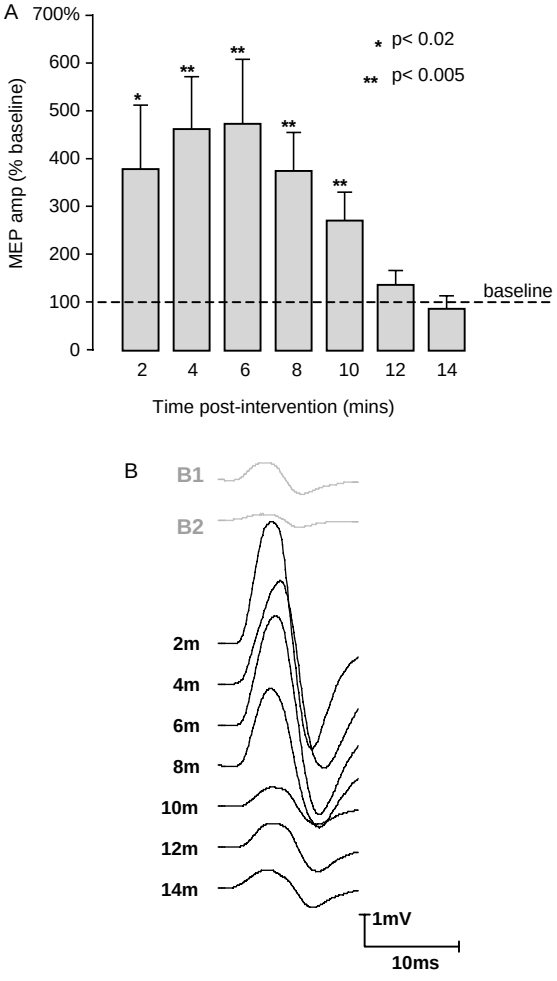

Fig. 2. (A) MEP amplitude (percent of pre-intervention baseline) across subjects ($n = 8$; mean ± SEM) at 2 min intervals after 30 min of iTMS, showing MEP amplitude significantly increased (~400%) for 10 min post-intervention. (B) Sample averaged MEP waveforms from one subject, pre-iTMS intervention (B1, B2—repeat baseline measurements) and at 2 min intervals from 2 to 14 min post iTMS, showing a substantial increase in MEP amplitude for ~10 min post-stimulation.

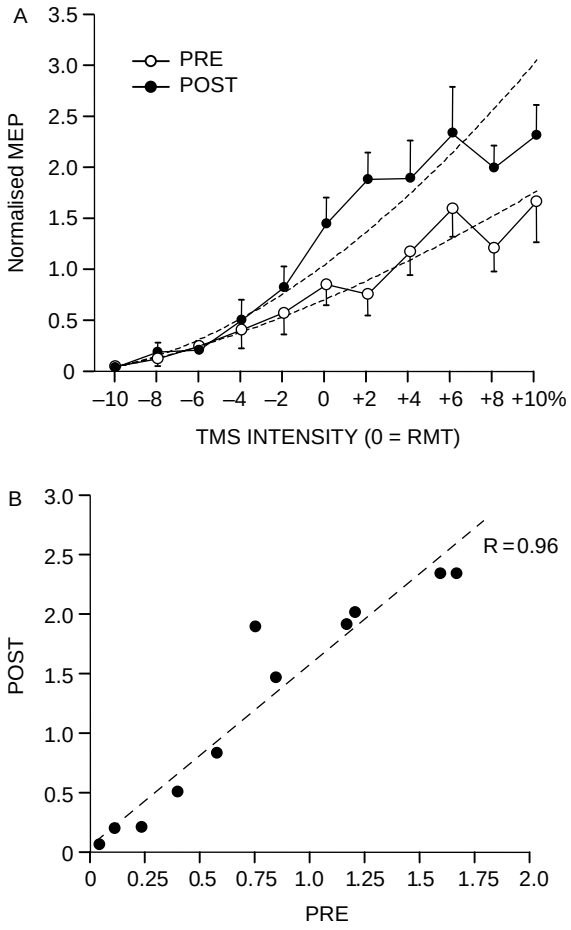

Fig. 3. (A) Group mean threshold curves pre- and post-iTMS, showing similar curve shape, but a greater rate of increase in MEP amplitude post-iTMS. (B) Relationship between pre- and post-iTMS MEP amplitudes across the TMS intensity range, showing a high level of correlation between the two threshold curves.

intervention curve (Fig. 3A). The two curves were correlated ($r=0.96$; $P=10^{-6}$; Fig. 3B) with a scaling factor of 1.5 ± 0.2 and a relative offset not significantly different to zero (0.05 ± 0.15; $P=0.8$).

3.4. F-waves

The number of F-waves recorded after iTMS intervention was essentially unchanged (group mean of six F-waves prior to iTMS and four post-iTMS). The baseline F-wave numbers were small, which is typically the case in resting hand muscles, but our aim was to determine whether there was an increase in F-wave numbers after intervention. F-wave area was unchanged by the intervention ($85\pm37\%$ of pre-intervention baseline). In the subject who underwent serial measurements, there was no increase in F-wave numbers over the 10 min period (ranging between 12 and 18 F-waves out of 30 stimuli, compared to 20/30 pre-intervention).

3.5. Single-pulse TMS intervention

There was no change in MEP amplitude after 30 min of single-pulse TMS (2.9 ± 1.1 mV pre-intervention vs 2.7 ± 1.3 mV post-intervention)

4. Discussion

Paired-pulse TMS delivered at I-wave periodicity resulted in a steadily increasing level of corticospinal excitability, as assessed by an increase in MEP amplitude, which was sustained for up to 10 min post-stimulation. Paired-pulse MEP amplitude increased five-fold during stimulation, and single-pulse MEP amplitude was increased by a mean of four-fold for 10 min after stimulation. As iTMS was designed to target synaptic events by utilising I-wave facilitation, the findings most likely reflect an increase in synaptic efficacy and suggest that iTMS is an effective method for manipulating synaptic plasticity in the motor cortex.

Previous studies have described increased or decreased corticospinal excitability during and after rTMS. Suprathreshold trains of high-frequency rTMS to motor cortex can result in significant and spreading increases in excitability, to the extent that seizures can be induced in healthy subjects, leading to the establishment of precise safety guidelines (Wassermann, 1998). On the other hand, repetitive administration of short trains of near-threshold or sub-threshold stimuli have not been associated with such side-effects, but can still increase corticospinal excitability and sustain this increase for some minutes after stimulation. However, the increases in excitability that have been described are small compared to the present results (Berardelli et al., 1998; Huang and Rothwell, 2004; Pascual-Leone et al., 1994; Peinemann et al., 2004). Perhaps the most powerful effect of rTMS on corticospinal excitability has been described with short trains of high-frequency stimuli (50 Hz) delivered at 5 Hz (theta-burst stimulation), which can induce increases in MEP amplitude of ~50–100% that persist after the end of stimulation (Huang and Rothwell, 2004; Huang et al., 2004, 2005). Similarly, paired associative stimulation can ~double MEP amplitude (Ridding and Taylor, 2001; Ridding and Uy, 2003), and is associated with an increase in the magnitude of I-wave facilitation (Pyndt and Ridding, 2004; Ridding and Taylor, 2001) suggesting that it may induce changes in synaptic efficacy. Using the present stimulation paradigm, substantial increases in excitability were induced by directly targeting I-wave facilitatory effects, and it is possible that this is a more direct approach for modulating synaptic efficacy in motor cortex.

While we found substantial changes in single and paired-pulse MEP amplitude, the recruitment curves indicate that motor threshold was unaffected. This is based on the observation that the recruitment curves performed before

and after intervention were significantly scaled but not significantly offset. Threshold measurement is typically based on the stimulus intensity that gives rise to a preset MEP amplitude, however, this is confounded in a situation, such as the present, in which MEP amplitude has increased. If there was a true threshold change, as well as an overall increase in MEP amplitude, then the recruitment curves would have been offset (change in threshold) as well as scaled (change in amplitude), which was not the case.

A range of pharmacological studies have provided evidence that motor threshold depends on membrane excitability rather than on synaptic transmission (Ziemann et al., 1995, 1996a, b, c), suggesting that the changes we observed in MEP amplitude were a result of modulation of synaptic efficacy. These changes are likely to have occurred at cortical level, given that spinal excitability, as determined by F-wave measurements, was not increased by the intervention. Previous studies have used an increase in I-wave facilitation as evidence for a cortical effect (Ridding and Taylor, 2001; Ridding and Uy, 2003). As intracortical networks generating I-waves were the target of our intervention, it seems likely that increased excitability of these networks was responsible for the increase we observed in MEP amplitude. Finally, a similar period of 30 min of single-pulse TMS which activated spinal motor-neurons did not lead to an increase in MEP amplitude.

We designed the iTMS technique with the aim of modulating synaptic plasticity, and this approach may have some parallels with the concept of Hebbian plasticity and learning (Hebb, 1949). Hebb's postulate was that modifications in synaptic efficacy could arise from correlated firing of presynaptic and postsynaptic neurons. The leading experimental models for such a mechanism are long-term potentiation (LTP) and long-term depression (LTD) (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973), which are thought to be the basis for the synaptic changes that underlie learning and memory. While rTMS and PAS are thought to exert their effects through LTP/LTD-like mechanisms, these methods target synaptic transmission indirectly. We propose that reinforcement of trans-synaptic events by iTMS may provide a more direct way to investigate and modulate synaptic plasticity.

I-wave facilitation formed the basis for our iTMS intervention. We found an increase in iMEP amplitude during the intervention, providing evidence that iTMS does increase the excitability of cortical networks involved in I-wave facilitation. However, we also observed an after-effect on single-pulse MEP amplitude that was of a comparable magnitude. Thus, it is appears that by targeting networks involved in I-wave facilitation we have modulated the underlying mechanisms of I-wave generation. How a single cortical stimulus gives rise to multiple descending I-waves is not fully understood, but these I-waves reflect repeated trans-synaptic activation of corticospinal neurons. Taken together, the iMEP and MEP findings therefore suggest that the excitatory effects we observe with iTMS may be a consequence of modulation of these trans-synaptic events.

Our present approach resulted in a profound excitatory effect, without adverse side effects during or after stimulation. However, even though subjects reported being comfortable throughout the procedure, the magnitude of the excitability changes we induced suggests some caution is warranted in implementing this technique. Present safety guidelines, which do not address frequencies above 25 Hz, cannot easily be extrapolated to this form of intervention incorporating two pulses at a short inter-pulse interval and 5 s rest periods. However, 5 s breaks between trains of 20 Hz rTMS of maximal allowed duration have been found to prevent a cumulative increase in cortical excitability (Wassermann, 1998).

We have described here a novel and effective technique for non-invasively raising corticospinal excitability in the human motor cortex, probably by modulation of synaptic efficacy. Further studies are required to characterise the physiological effects of this intervention and to optimise the stimulation parameters. Whether these physiological effects can result in improved motor function or learning, and whether such approaches could be beneficial in recovery of function after brain injury, are of particular interest for future studies.

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