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Corticomotor responses to triple-pulse transcranial magnetic stimulation: Effects of interstimulus interval and stimulus intensity

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Background
Paired-pulse transcranial magnetic stimuli (TMS) applied to the motor cortex enhances motor-evoked potential (MEP) responses at specific interpulse intervals (IPIs), probably from summation of I-waves by the secondary TMS pulse. This study investigated the properties of I-wave periodicity by comparing double-pulse with triple-pulse TMS at varying IPIs and stimulus intensities.

Methods
TMS was delivered to the optimal scalp position for the resting dominant first dorsal interosseous muscle at either active motor threshold (AMT) or AMT-5% stimulator output. In experiment 1, 4 conditions were tested, a double-pulse ($D^{1.5}$; IPI = 1.5 milliseconds), and triplets comprising $D^{1.5}$ with the addition of a third pulse at 1.5, 2.0, or 3.0 milliseconds ($T^{1.5}{}_{1.5}$, $T^{1.5}{}_{2.0}$, and $T^{1.5}{}_{3.0}$, respectively). Each condition was tested at 2 stimulation intensities. In a second experiment, the same protocol was repeated with a single-pulse (giving an MEP equivalent to $D^{1.5}$) replacing the first 2 pulses in each triplet.

Results
At AMT, MEP responses were significantly larger for $T^{1.5}{}_{1.5}$ and $T^{1.5}{}_{3.0}$ compared with $D^{1.5}$. Triple-pulse stimulation at AMT-5% resulted in no additional increase in MEP amplitude, or effect of IPI. Double-pulse TMS showed similar effects to the triplets when the first pulse was delivered at an intensity equivalent to $D^{1.5}$.

Conclusions
The results are consistent with an intensity-dependent facilitation of MEPs produced by triple-pulse TMS, possibly through summation of cortical I-waves. Triple-pulse TMS at I-wave periodicity may

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have application in the investigation of the cortical circuitry involved in the generation of I-waves, or form a basis for the further development of neuromodulatory TMS interventions.

Keywords transcranial magnetic stimulation; I-waves; corticomotor plasticity

When pairs of transcranial magnetic stimuli (TMS) pulses are applied to the motor cortex, the corresponding motor-evoked potential (MEP) responses are altered compared with those of single pulses. At appropriate stimulus intensities and interpulse intervals (IPIs) paired pulses can result in MEPs that are greater or smaller than the corresponding single-pulse response. A common experimental paradigm to investigate the cortical circuitry associated with these effects has been to use a conditioning stimulus (S1), followed by a subthreshold test stimulus (S2). Thus, Tokimura et al,[1] using pairs of threshold stimuli, showed a large facilitation of MEPs compared with single-pulse TMS with IPIs of around 1.1 to 1.5 milliseconds, 2.5 to 3.0 milliseconds, and 4.5 milliseconds. Ziemann et al[2] showed similar effects when a suprathreshold S1 preceded a subthreshold S2, and Illic et al[3] demonstrated a facilitatory effect of 2 subthreshold stimuli at similar time intervals. The periodicity of this effect corresponds with the firing pattern of pyramidal tract neurons of animals[4] and humans[5] after single-pulse TMS. It is thought that S1 activates pyramidal neurons indirectly via excitatory cortical interneurones, and that the transynaptic delay period provides an opportunity for S2 to summate with the excitatory postsynaptic potentials induced by S1, resulting in more neurons reaching threshold, and thus increasing the size of the descending volley. Altering cortical network behavior with multiple pulse facilitation techniques may be used as a tool to modulate the excitability of the corticomotor pathway[6,7] with potentially beneficial effects on facilitating cortical plasticity.[8]

The generation of I-waves is known to be dependent on the stimulus intensity[9] because if S1 is too low, the facilitatory effects at longer periods (3-4.5 milliseconds) corresponding to later I-wave generation are absent. In theory, the presence of multiple I-waves should provide the potential for multiple summatory effects if additional TMS pulses are delivered at appropriate intervals. To test if this is the case, we have compared double-pulse TMS responses with triple-pulse TMS at varying IPIs and stimulus intensities.

Materials and Methods

Seven healthy subjects (30-45 years old) gave informed consent to participate after approval from the local Ethics Committee. Electromyographic (EMG) recordings were made from the first dorsus interosseous (FDI) muscle of the dominant hand using 5-mm diameter electrodes (Grass) with the active electrode placed over the muscle belly and the reference electrode fixed to the metacarpophalangeal joint of the first finger.

TMS (Magstim 200, Magstim Co Ltd, UK) was delivered by using a 9-cm diameter figure-eight coil, held tangentially to the skull so as to induce current flow in a posterior-anterior direction, and positioned over the optimal scalp position for eliciting an MEP in the FDI. Resting motor threshold (RMT) to single-pulse TMS was determined by reducing the stimulator output stepwise until MEPs of more than 100 μV amplitude were evoked in 3 of 5 stimuli. Active motor threshold (AMT) was determined in the same way while subjects were asked to make a gentle (approximately 10% of maximum effort) voluntary isometric contraction of the FDI muscle (with the MEP amplitude limit >200 μV).

Double- and triple-pulse TMS was delivered by using 3 Magstim 200 units connected to a single coil via a connector box. For the first experiment (Table 1), the intensity of the individual pulses in each doublet or triplet was held the same and was set to either AMT or 5% of maximum stimulator output (MSO) below AMT. Doublets (at an IPI of 1.5 millisecond; $D^{1.5}$) and 3 triplet combinations with different IPIs were compared. For the triplets, the interval between the first 2 pulses was kept at 1.5 milliseconds, whereas the interval between the second and third pulses was varied between 1.5, 2.0, and 3 milliseconds ($T^{1.5}{}_{1.5}$, $T^{1.5}{}_{2.0}$, and $T^{1.5}{}_{3.0}$, respectively). Ten repeats for each combination of double- and triple-pulse were delivered pseudorandomly at 5-second intervals. Each experimental block (AMT and AMT-5%MSO) was conducted during the same session with a 15-minute break and with the order of testing randomized for the 2 intensities across subjects. All measurements were taken at rest.

To further compare double- and triple-pulse stimulation, the AMT triplets were converted to doublets by replacing the first 2 pulses in each triplet with a single pulse of higher intensity, with the intensity of this pulse selected so that it generated the same MEP amplitude as the doublet it replaced. For this experiment, 5 subjects returned to the laboratory on a separate occasions, and the amplitude of the MEP resulting from $D^{1.5}$ stimulation was measured (stimuli at AMT; 10 responses; 5-second intervals). The single-pulse TMS intensity required to elicit a MEP of this same amplitude was then determined ($\hat{S}$). The actual experiment consisted of the following combinations of stimuli: double-pulse stimulation at 1.5-, 2.0-, and 3.0-millisecond IPI, with the first pulse at $\hat{S}$ and the second pulse at AMT (denoted $\hat{S}D^{1.5}$, $\hat{S}D^{2.0}$,

Table 1 Double- and triple-pulse stimulation combinations for experiments 1 and 2

	S1	Δ_{1-2}	S2	Δ_{2-3}	S3	EXP1	EXP2
$D^{1.5}$	AMT	1.5	AMT	—	—	✓	✓
$T^{1.5}{}_{1.5}$	AMT	1.5	AMT	1.5	AMT	✓	✓
$T^{1.5}{}_{2.0}$	AMT	1.5	AMT	2.0	AMT	✓	—
$T^{1.5}{}_{3.0}$	AMT	1.5	AMT	3.0	AMT	✓	—
$\hat{S}$	—	—	$\hat{S}$	—	—	—	✓
$\hat{S}D^{1.5}$	—	—	$\hat{S}$	1.5	AMT	—	✓
$\hat{S}D^{2.0}$	—	—	$\hat{S}$	2.0	AMT	—	✓
$\hat{S}D^{3.0}$	—	—	$\hat{S}$	3.0	AMT	—	✓

TMS pulses S1, S2, and S3 at interpulse interval (millisecond) of Δ_{1-2} (S1-S2) and Δ_{2-3} (S2-S3). Intensity of pulses as indicated (AMT = active motor threshold, $\hat{S}$ = single pulse intensity giving same MEP amplitude as $D^{1.5}$). In experiment 1, measurements were repeated at AMT-5%MSO.

$\hat{S}D^{3.0}$); single-pulse stimulation at $\hat{S}$; double-pulse stimulation ($D^{1.5}$) at AMT; and triple-pulse stimulation ($T^{1.5}{}_{1.5}$) at AMT. Thus, there were 6 combinations, which were delivered 10 times each, pseudorandomly at 5-second intervals.

Two-way repeated measures analysis of variance (ANOVA) was used to determine the effects of condition and stimulus intensity on MEP amplitude. Where significant F values occurred, paired-sample *t* tests were performed to compare specific conditions post hoc by using Bonferroni correction for multiple comparisons as appropriate. *P* values less than .05 were considered significant and all results are presented as mean ± (SEM).

Results

Experiment 1

Double-pulse stimulation ($D^{1.5}$) at AMT resulted in an MEP in all subjects (0.6 ± 0.1 mV), whereas single-pulse stimulation at AMT alone did not. The triple-pulse combinations $T^{1.5}{}_{1.5}$ and $T^{1.5}{}_{3.0}$ resulted in MEPs of significantly larger amplitude than for $D^{1.5}$ (1.8 ± 0.2 mV, $P = .001$ and 1.3 ± 0.2 mV, $P = .008$, respectively), whereas the $T^{1.5}{}_{2.0}$ combination was not significantly different to $D^{1.5}$ (0.9 ± 0.2 mV, $P = .18$). Comparing the triple-pulse combinations, $T^{1.5}{}_{1.5}$ elicited significantly larger amplitude responses than either of the other combinations ($T^{1.5}{}_{1.5}$ vs $T^{1.5}{}_{2.0}$, $P = .006$; $T^{1.5}{}_{1.5}$ vs $T^{1.5}{}_{3.0}$, $P = .02$), whereas $T^{1.5}{}_{2.0}$ and $T^{1.5}{}_{3.0}$ were not significantly different. When the protocol was repeated at AMT-5%MSO, $D^{1.5}$ still evoked a motor potential, although approximately half the amplitude of that with AMT (0.3 ± 0.1 mV). In contrast, none of the triple-pulse combinations elicited an additional significant increase in MEP amplitude. These results and sample waveforms are summarized in Figures 1 and 2 and Table 1.

Experiment 2

In experiment 2 (Figure 3), the mean MEP amplitude for $D^{1.5}$ was 0.6 ± 0.1 mV. The intensity that gave a comparable MEP amplitude with single-pulse stimulation ($\hat{S}$) was on average AMT + 10.7 MSO% (± 0.9), and the mean MEP amplitude at this intensity was 0.7 ±0.1 mV, which was not significantly different with that for $D^{1.5}$.

The pattern of MEP amplitude change with the double-pulse combinations ($\hat{S}D^{1.5}$, $\hat{S}D^{2.0}$, and $\hat{S}D^{3.0}$) was similar to that observed in experiment 1 with the corresponding triple-pulse combinations ($T^{1.5}{}_{1.5}$, $T^{1.5}{}_{2.0}$, and $T^{1.5}{}_{3.0}$). The amplitude of the MEP with $\hat{S}D^{1.5}$ was significantly greater than for $\hat{S}$ alone (1.5 ± 0.3 mV; $P = .005$), as was that with $\hat{S}D^{3.0}$ (1.1 ± 0.2 mV; $P = .003$), whereas there was no significant difference for $\hat{S}D^{2.0}$ (0.8 ± 0.1 mV; $P = .76$). The stimulus combinations $\hat{S}D^{1.5}$ and $T^{1.5}{}_{1.5}$, which were

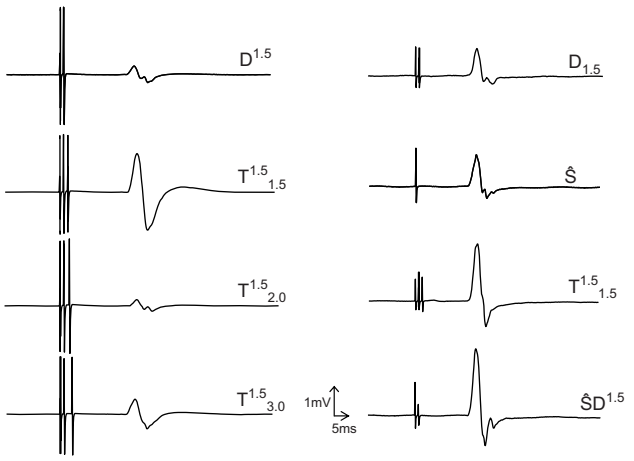

Figure 1 Examples of motor-evoked potental (MEP) responses from experiments 1 and 2 (left and right panels, respectively) in two subjects. Each trace represents an average of 10 recordings. Note the large MEP responses seen in the $T^{1.5}{}_{1.5}$ condition (left panel) and the similar responses of the $T^{1.5}{}_{1.5}$ and $\hat{S}D^{1.5}$ (right panel).

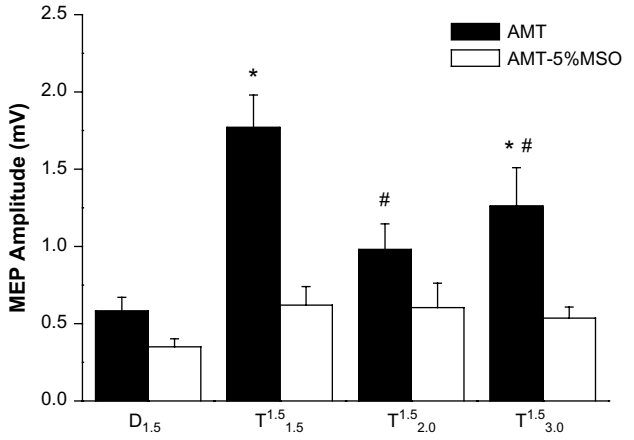

Figure 2 Motor-evoked potential (MEP) responses of first dorsus inteross (FDI) to double -and triple-pulse transcranial magnetic stimulation (TMS) at different interpulse intervals (IPIs) (n = 7 subjects). Significant differences compared with $D^{1.5}$ (*) and $T^{1.5}{}_{1.5}$ (#) shown.

designed to be directly compared ($\hat{S}$ replacing the first pair of pulses in $T^{1.5}{}_{1.5}$), gave MEP amplitudes that were similar (1.5 ± 0.3 mV vs 1.3 ± 0.4 mV, respectively).

Discussion

Triple-pulse TMS exerts a facilitatory effect on MEP amplitude that is consistent with I-wave dynamics. With IPIs at I-wave periodicity (~1.5 milliseconds) MEP facilitation was observed, whereas this was not the case with the inclusion of an intermediate interval (2.0 milliseconds), or with stimulation at an intensity below that expected to generate multiple I-waves. Comparable facilitation was observed with suitably matched paired-pulse and triple-pulse stimulation, suggesting that similar mechanisms underlie triple- and double-pulse facilitation.

Single-pulse TMS generates multiple descending volleys in the corticospinal tract at ~1.5 milliseconds periodicity, and although the mechanisms involved remain poorly understood and largely theoretical, it is generally accepted that these volleys arise from trans-synaptic, and therefore indirect (I-wave), activation of corticospinal cells.[10,11] Paired-pulse TMS delivered at multiples of I-wave periodicity appears to facilitate an interaction between I-wave volleys and result in a supralinear increase in MEP amplitude. However, the uncertainty surrounding the mechanisms of I-wave generation also means that the precise way in which paired-pulse TMS acts is not known. One concept is that the second pulse in a TMS pair (S2) is delivered during a period of some form of heightened excitability arising from successive I-wave volleys initiated by the first pulse (S1). With this model, it is not certain whether a triple-pulse stimulus paralleling I-wave dynamics could result in further facilitation, or whether the presentation of 3 stimuli at such high frequency might be less effective because of an increased contribution from refractory effects[12] or an interaction with short-latency (~3 milliseconds) inhibitory networks.[13] The current results demonstrate that the inclusion of a third pulse (S3), at appropriate timing and intensity, is strongly facilitatory.

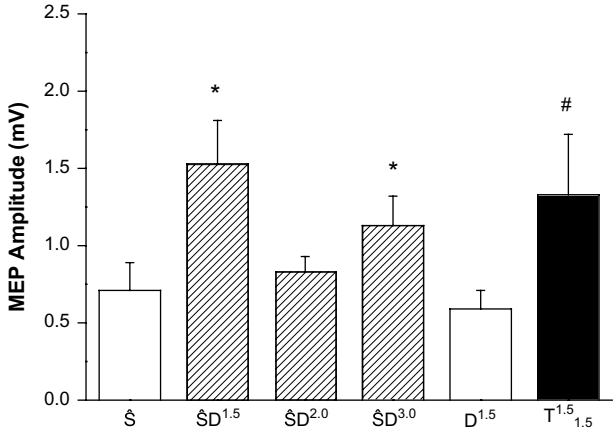

Figure 3 Comparison of motor-evoked potential (MEP) responses to double-pulse stimulation (first pulse at $\hat{S}$ intensity) at different interpulse intervals (IPIs) and triple-pulse stimulation at 1.5-millisecond IPI (n = 5 subjects). Significant differences compared with $\hat{S}$ (*) and $D^{1.5}$ (#) shown.

What might the third pulse be facilitating? A possibility is that S1 and S2 combine to generate a certain I-wave volley that S3 then interacts with. This concept seems to be supported by the observation that a paired-pulse stimulus, in which the intensity of the first pulse ($\hat{S}$) is increased to amplitude-match the MEP to that generated by the first 2 pulses in a triple-pulse stimulus, results in a similar increase in MEP amplitude. The implication is that S1 and S2 can be replaced with $\hat{S}$ to similar effect. However, the similarity in motor evoked responses might occur despite differences in I-wave components generated by single- and double-pulse stimulation. Epidural recordings have shown that paired-pulse TMS at AMT intensity facilitates later I-waves, and that I1 is not significantly increased in amplitude,[14] whereas single-pulse TMS at stronger intensity results in an increase in the amplitude of I1, as well as recruitment of additional I-waves.[9] Thus there are likely to be differences in how paired- or single-pulse TMS may condition a subsequent stimulus, with the triple-pulse combination possibly favoring later I-waves.

An alternative, but closely related, possibility is that S3 interacts with I-waves generated both by S1 and S2, perhaps as a result of parallel activation of different fractions of the interneuronal network by S1 and S2. With this model, a triple-pulse stimulus may follow serial I-wave dynamics and interact with early and later I-waves generated by preceding pulses in the triplet. With either a cumulative model (S3 interacting with the summed effect of S1 and S2) or a parallel model (S3 facilitating responses from S1 and S2), triple-pulse stimulation can be expected to target later or multiple combinations of I-waves.

The possibility exists that subcortical mechanisms may have contributed to the observed variation in MEPs via modulation of disynaptic inhibitory networks, because it is known that direct corticospinal projections occur to Ia interneurones.[15] Di Lazzarro et al[14] suggested that the lack of MEP facilitation of paired-pulses at 2-millisecond IPI in a number of subjects (despite an increase in amplitude of I-waves at this interval) could be explained by activation of spinal Ia inhibitory inputs under these specific conditions. Moreover, Mercuri et al[16] showed that subthreshold TMS could result in a reduction in the disynaptic phase of reciprocal inhibition of a human forearm flexor muscle when the cortical stimulus was delivered at an appropriate interval relative to a peripheral nerve shock. Thus, it is conceivable that triple-pulse TMS might enhance or diminish such Ia inhibitory inputs depending on the intensity and timing of each stimulus.

Lower-intensity (AMT-5%MSO) double-pulse TMS facilitated MEP amplitude but, in contrast to stimulation at AMT, the inclusion of a third pulse had little further effect at any IPI. Although it is possible that at this lower intensity insufficient later I-waves are generated, and thus unavailable for facilitation with S3, this does not readily explain the facilitation observed with double-pulse stimulation. A possible mechanism might be that, at lower stimulus intensities, refractoriness is more important than facilitation, so that although there is some interaction between S1 and S2, S2 itself fails to produce further I-waves because of a refractoriness of axons after S1. An alternative explanation is that S1, at low intensity, might not only generate some I-wave volleys that could be facilitated by S2, but also might activate inhibitory interneuronal networks responsible for short-interval cortical inhibition (SICI), which has a time-course of approximately 2 to 5 milliseconds.[17] Thus, the effect of the third pulse, delivered 3.0 to 4.5 milliseconds after S1, could have been inhibited. It is of interest that paired-pulse inhibitory protocols involving subthreshold S1 reduce later I-waves,[9] and it is the later I-waves that appear to mediate I-wave facilitation.[18]

By taking advantage of the transsynaptic mechanisms that probably underlie paired-pulse I-wave facilitation, repeated low-intensity double-pulse stimulation at I-wave periodicity can lead to an increase in corticospinal excitability,[6,7] making I-wave facilitation a basis for interventional TMS. The current results suggest that triple-pulse stimulation may also be a promising, and perhaps more effective, form of intervention, given the doubling of the MEP amplitude that was achieved by adding a third pulse to the double-pulse combination. Even though there was little difference in MEP amplitude between triple-pulse versus matched paired-pulse stimulation, there are likely to be differences in the I-waves targeted by these stimulus patterns. The possible preferential targeting of later or multiple I-waves by triple-pulse stimulation raises the possibility that it could be effective as an intervention targeting trans-synaptic events. Recently, Hamada et al,[19] compared repetitive double-pulse versus quadruple-pulse TMS at I-wave periodicity, and found similar increases in single-pulse MEP responses in the first few minutes after both interventions, but that the effects were longer lasting after quadruple-pulse TMS. They suggested that paired-pulse and quadruple-pulse TMS may exert their effects through different populations of descending volleys, which would concur with our findings.

To conclude, triple-pulse TMS at I-wave periodicity has a powerful facilitatory effect on MEP production under resting conditions. The results suggest that multiple or later I-waves may be targeted by triple-pulse stimulation, and this may have application in the investigation of the cortical circuitry involved in the generation of I-waves, or form a basis for the further development of neuromodulatory TMS interventions based on I-wave dynamics.

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