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Strength-Duration Relationship in Paired-pulse Transcranial Magnetic Stimulation (TMS) and Its Implications for Repetitive TMS

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ABSTRACT

Background: Paired-pulse protocols have played a pivotal role in neuroscience research using transcranial magnetic stimulation (TMS). Stimulus parameters have been optimized over the years. More recently, pulse width (PW) has been introduced to this field as a new parameter, which may further fine-tune paired-pulse protocols. The relationship between the PW and effectiveness of a stimulus is known as the "strength–duration relationship".
Objective: To test the "strength–duration relationship", so as to improve paired-pulse TMS protocols, and to apply the results to develop new repetitive TMS (rTMS) methods.
Methods: Four protocols were investigated separately: short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), short-interval intracortical facilitation (SICF) and long-interval intracortical inhibition (LICI). First, various stimulus parameters were tested to identify those yielding the largest facilitation or inhibition of the motor evoked potential (MEP) in each participant. Using these parameters, paired-pulse stimulations were repeated every five seconds for 30 minutes (repetitive paired-pulse stimulation, rPPS). The after-effects of rPPS were measured using MEP amplitude as an index of motor-cortical excitability.
Results: Altogether, the effect of changing PW was similar to that of changing the stimulus intensity in the conventional settings. The best parameters were different for each participant. When these parameters were used, rPPS based on either SICF or ICF induced an increase in MEP amplitude.
Conclusions: PW was introduced as a new parameter in paired-pulse TMS. Modulation of PW influenced the results of paired-pulse protocols. rPPS using facilitatory protocols can be a good candidate to induce enhancement of motor-cortical excitability.

Introduction

Paired-pulse protocols have played a pivotal role in neuroscience research using transcranial magnetic stimulation (TMS). Their utility is twofold. First of all, since the first report of short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) [1], the paired-pulse protocols including these two have revealed various intra- and intercortical circuits involving the primary motor cortex (M1) (for review, see Reference 2). Second and perhaps more importantly, such circuits can be employed as building blocks for repetitive TMS (rTMS) that induces long-lasting after-effects. The first attempts in this regard were made using SICI or ICF as a component of rTMS [3–5]. Thickbroom et al. later demonstrated that repetition of short-interval intracortical facilitation (SICF)-like paired pulses induced an increase in M1 excitability for 15 min following the intervention [6]. More recent studies have also aimed at constructing novel rTMS methods based on the paired-pulse protocols [7,8]. We call these methods repetitive paired-pulse stimulation (rPPS) in this article.

Parameters such as stimulus intensity or inter-stimulus interval (ISI) must be carefully adjusted for paired-pulse protocols in order to properly test either facilitation or inhibition. Indeed, the choice of these parameters is so important that a non-optimal selection of conditioning stimulus intensity for SICI would result in no inhibition [9,10]. Results of SICF were also affected by stimulus intensity [11].

Abbreviations: ANOVA, analysis of variance; cTMS, controllable pulse parameter transcranial magnetic stimulation; FDI, first dorsal interosseous; ICF, intracortical facilitation; ISI, inter-stimulus interval; LICI, long-interval intracortical inhibition; M1, primary motor cortex; MEP, motor evoked potential; PW, pulse width; RMT, resting motor threshold; rPPS, repetitive paired-pulse stimulation; SICF, short-interval intracortical facilitation; SICI, short-interval intracortical inhibition; ThPW, threshold pulse width; TMS, transcranial magnetic stimulation.

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Pulse width (PW) is another factor which may affect the results of a paired-pulse protocol. This aspect has not been studied before, mainly because of the technical limitations of conventional stimulators. The influence of PW on neural activation, however, is established in peripheral nervous stimulation as strength–duration relationship [12]. A general principle here is "the wider, the stronger" – that is, an electric pulse with a longer duration results in a lower threshold in activating an axon. A new device has only recently enabled us to explore this parameter in the central nervous system. The device, named "controllable pulse parameter TMS" (cTMS), can manipulate the pulse shape, including the PW [13,14]. The notion of "the wider, the stronger" has been confirmed in the central nervous system by measuring the motor threshold for single-pulse TMS [15,16]. A subsequent study interestingly reported that TMS using different PWs might be associated with the activation of a different set of neural elements [17].

The aim of this study was to investigate the strength–duration relationship in several major paired-pulse protocols representing different facilitatory and inhibitory intracortical circuits, namely, SICI, ICF, SICF, and long-interval intracortical inhibition (LICI). Given that activation of an independent set of neurons is supposed to constitute a core component of each protocol, it is reasonable to hypothesize different impacts of PW on different protocols. We further pursued the question of whether individually estimated best parameters for each protocol give rise to long-lasting after-effects when used as a building block of the rPPS.

Methods

Participants

Twelve healthy volunteers were recruited for the main experiments (6 men and 6 women, mean age ± standard deviation was 24.4 ± 3.6 years). All participants were right-handed [18], and free from any neurological or psychiatric disorders, took no centrally-acting medications, and had no contraindications for TMS [19]. Written informed consent was obtained from each before participation. The study protocol, which conformed to the Declaration of Helsinki, was approved by the local ethics committee of the University Medical Center Göttingen.

Recordings

Motor evoked potentials (MEPs) were recorded from the first dorsal interosseous (FDI) muscle of the right hand with surface Ag–AgCl electrodes in a belly-tendon montage. The electromyography signals were amplified, band-pass filtered (2 Hz–2 kHz), and digitized at a sampling rate of 5 kHz with a micro-1401 AD converter (Cambridge Electronic Design Ltd., Cambridge, UK). All signals were stored in a computer for offline analysis. Peak-to-peak amplitude of MEP served as an index for M1 excitability.

Participants were asked to relax the right FDI during the measurements. If recordings were contaminated by voluntary muscle contraction before TMS pulse(s), they were not included in the analysis.

TMS over the M1

Magstim 200^2 (Magstim Co. Ltd., Whitland, UK) and cTMS prototype 3 (cTMS3; Rogue Research Inc., Montreal, Canada) were used to deliver TMS over the M1. Both devices were equipped with the same type of figure-of-eight coil (type P/N 9925, Magstim Co. Ltd.). The coil was held tangentially on the scalp at an angle of 45 degrees to the midsagittal plane with the handle pointing lateroposteriorly, generating a predominantly posterior-anterior current direction in the brain. At the beginning of each session, the Magstim 200^2 was used to determine the stimulation site as the site where the largest MEP was consistently elicited in the right FDI with a slightly suprathreshold stimulus. This site was marked with a pen to facilitate re-positioning of the coil. Given that the stimulation coil had the same geometry, this site was also referred to when cTMS3 was used.

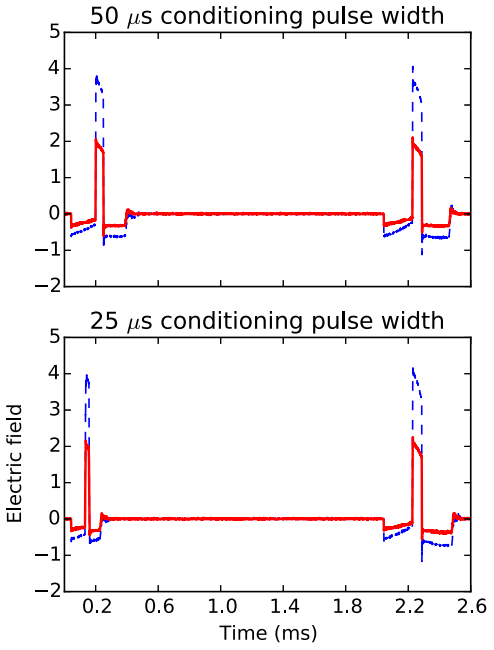

Figure 1. Induced electric field for paired-pulse stimuli at the ISI of 2 ms. The electric field induced by cTMS3 was detected with a pick-up coil placed just below the intersection of the two wings of the stimulation coil and displayed on an oscilloscope. The second pulse had a common PW of 60 μs, while the PW (of the positive phase) for the first pulse was 50 μs (top) or 25 μs (bottom). The blue, dashed lines represent induced current with an intensity of 50% machine output, while the red, solid lines represent that with 25% intensity. The X-axis shows time in ms, and the Y-axis shows the electric field strength in arbitrary units.

The electric field induced in the brain by cTMS3 had three different phases: a negative, a positive and again a negative phase, of which the PW of the positive phase is that referred to as PW in this article (Fig. 1). The ratio of the negative phase to that of the positive one was approximately 0.2 so that the stimulus as a whole was predominantly unidirectional; the positive phase in Fig. 1 is equivalent to a posterior-anterior current direction in the brain.

Before the main experiment, three types of thresholds were measured using maximum-likelihood threshold tracking with the TMS Motor Threshold Assessment Tool (MTAT2.0, http://clinicalresearcher.org/software.htm) as the minimum intensity or PW that elicits minimal MEPs (greater than 50 μV) in more than half of the trials. First, resting motor threshold (RMT) was measured using the Magstim 200^2 as a reference to make comparisons with previous studies easier. RMT for cTMS3 was measured with 60 μs PW (i.e. positive phase duration). The stimulus intensity was then set at 120% of this RMT, which we called "test intensity," when a threshold pulse width (ThPW) was defined. Similar to the determination of RMT by applying varying stimulus intensities, ThPW was estimated by changing the PW with a precision of 1 μs. The ThPW was referred to when different PWs were explored: for example, if the ThPW was 46 μs for a particular session, a PW of "ThPW-3" means 43 μs. These threshold measurements were performed for every session.

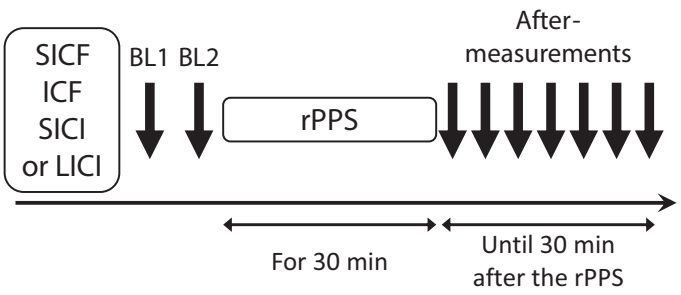

Figure 2. Overall study design. After determining the best parameters for the paired-pulse protocols, SICI, ICF, SICF, and LICI (*see* Table 1 for the parameters explored), two baselines, BL1 and BL2, were measured at a stimulus intensity which on average elicits an MEP with a 1 mV peak-to-peak amplitude. Repetitive paired-pulse stimulation (rPPS) was then performed for 30 minutes using the parameters defined above. Afterwards, MEPs were collected at the same intensity as the baselines every five minutes for 30 minutes after rPPS.

Overall study design

This study was conducted using a full-factorial, repeated-measures design. Each participant took part in four sessions, each of which used one paired-pulse protocol; the interval between the sessions was at least three days. As shown in Fig. 2, participants first received one of the four protocols (i.e., SICI, ICF, SICF or LICI) with a conditioning-test paradigm [20] to test the effects of different parameters on facilitation or inhibition. After baseline measurements, a 30 min-long rPPS was performed using the parameters found to be optimal for the particular participant in the conditioning-test paradigm. After-effects of the rPPS were measured by the MEP amplitude obtained with the same stimulus intensity as in the baselines of that session.

Paired-pulse stimulation with a randomized conditioning-test paradigm

The "best" parameters were defined as those that produced the greatest facilitation for SICF or ICF, or maximal inhibition for SICI or LICI, which in turn were characterized by the highest or lowest MEP amplitudes, respectively. As shown in Table 1, we explored a range of PWs (for the first pulse in SICI, ICF or the second pulse in SICF) as well as ISIs for each paradigm. On the other hand, the PW of the other pulses (the second one in SICI, ICF and the first one in SICF) was always 60 μs. The stimulus intensity of both of the paired-pulses was equal to the test intensity unless otherwise mentioned.

Table 1
Parameters explored in the conditioning-test paradigm.

	PW (relative to ThPW, μs)*	ISI (ms)	Total number of conditions**
SICI	–3, –6, –9, –12, –15	2, 3	11
SICF	–3, –6, –9	1.1, 1.3, 1.5, 1.7, 1.9	16
ICF	–3, –6, –9, –12, –15	10, 15	11
LICI	not applicable	100, 150, 200	8

* Refers to PW of the first pulse for SICI and ICF, and the second pulse for SICF. In LICI, two pulses with the same PW of 60 μs were used.

** Every pair of PW and ISI was investigated. In SICI for example, there were 10 paired-pulse conditions (i.e. five for PW times, two for ISI) in addition to the one single-pulse condition, which served as a baseline, giving a total of 11 conditions. Note that for LICI, two different stimulus intensities (110% and 120% RMT, *see* the main text) were tested, resulting in six paired-pulse conditions (three ISIs for each of the two intensity) and two single-pulse conditions.

ICF, intracortical facilitation; ISI, inter-stimulus interval; LICI, long-interval intracortical inhibition; PW, pulse width; SICF, short-interval intracortical facilitation; SICI, short-interval intracortical inhibition; ThPW, threshold pulse width.

The importance of the conditioning stimulus intensity is emphasized for SICI by its U-shaped effect curve; a too high as well as a too low stimulus intensity leads to less inhibition, while a moderate conditioning intensity induces maximal inhibition [9,10]. Given this relationship, we tested five different PWs (ThPW-3, ThPW-6, ThPW-9, ThPW-12 and ThPW-15) for the conditioning stimulus, which was delivered 2 or 3 ms before the test stimulus. Hence, a total of 11 conditions were tested (Table 1).

ICF was tested in a manner similar to SICI, except for the ISI, which were 10 or 15 ms. When the conditioning stimulus was below threshold, a conditioning stimulus with higher intensity resulted in more facilitation [9,21].

In SICF, a supra-threshold stimulus is followed by a sub-threshold stimulus, as opposed to SICI and ICF. SICF is characterized by its narrow range of ISI which leads to MEP facilitation. Typically there are three different peaks of facilitation at ISIs of 1.1–1.5, 2.3–2.9 and 4.1–4.4 ms; otherwise, no facilitation or inhibition was seen [22]. We studied the first peak of SICF using five different ISIs: 1.1, 1.3, 1.5, 1.7 and 1.9 ms. Previous studies had shown that a second stimulus with higher intensity yielded greater facilitation [11,22]. To test the influence of the PW of the second stimulus on the effects along this line, three different PWs were used: the ThPW-3, ThPW-6 and ThPW-9 of each individual. A total of 16 different conditions were thus compared (Table 1).

For LICI, two identical stimuli are commonly used [23,24]. We followed this convention and mainly tested the effect of ISI, which was 100, 150 or 200 ms. Instead of changing the conditioning PW, two different stimulus intensities were investigated with 60 μs PW for both of the paired-pulses: 110% RMT as well as 120% RMT (i.e. test intensity). Only in this protocol was the stimulus intensity of the cTMS3 different from the test intensity. There were eight different conditions (Table 1).

Each protocol was tested using a randomized conditioning-test paradigm. The order of conditions was randomized, and four trials were conducted for each condition. This procedure was repeated three times, resulting in 12 MEPs for each condition. Parameters to be used in the rPPS were defined as the ISI and the PW (or intensity in case of LICI) which resulted in the largest (for ICF or SICF) or smallest (for SICI or LICI) MEP on average among the conditions described above and shown in Table 1.

Repetitive paired-pulse stimulation (rPPS)

The rPPS was conducted for 30 minutes using the "best" parameters estimated in the conditioning-test paradigm. The paired pulse was delivered every five seconds continuously [6], which gave a total of 360 pairs. MEPs elicited during the rPPS were recorded and analyzed to monitor on-line the alteration in M1 excitability.

Monitoring of M1 excitability before and after the rPPS

As an index for M1 excitability, 20 MEPs were collected using Magstim 200^2 for each time bin before and after the rPPS. Before the rPPS, TMS intensity was adjusted so that the average peak-to-peak amplitude of the MEPs was approximately 1 mV. The same intensity was used throughout the measurements. Baseline values were obtained twice before the rPPS (BL1 and BL2), and measurements were performed every five minutes from immediately following the rPPS until 30 minutes (Fig. 2).

A control experiment to estimate the stimulus intensity in terms of PW-adjusted RMT

Eight participants were tested (3 women, 21–32 years old); two had also participated in the main part. RMT was first determined

using cTMS3 with a 60 μs PW. Then ThPW was measured in the same way as in the main experiments, and finally RMT was determined again, using a PW of ThPW-6.

Data analysis

One-way analysis of variance (ANOVA) was used to test whether baseline characteristics were comparable across the sessions. RMT for Magstim and cTMS, ThPW, and absolute MEP amplitudes elicited by single-pulse TMS during the conditioning-test paradigm as well as BL1 and BL2 were compared in this way.

Normalized MEP amplitude was used as a dependent variable for the main analyses. In the conditioning-test paradigm, the mean MEP amplitude of each paired-pulse condition was normalized to that of a corresponding single-pulse TMS for each participant. MEPs collected during the rPPS were first grouped in 5-minute bins, resulting in six time bins (0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 min), each of which contained 60 MEPs (i.e. one-sixth of 360 pairs). The mean MEP amplitude of each bin was then normalized to the mean MEP amplitude of the first one minute [6]. MEPs collected after the rPPS were normalized to those in BL2.

Repeated-measures ANOVA (rmANOVA) was conducted for each part of the study. Within-subject factors were ISI and PW (or intensity in LICI) for the conditioning-test paradigm. The four paired-pulse protocols were analyzed separately. The individual parameters used in the conditioning-test paradigm are given in Table 1. PW was not included in LICI because two identical pulses were used, and two different intensities (110% and 120% RMT) were compared instead. To compare the normalized MEP amplitudes during rPPS, time (0–5, 5–10, 10–15, 15–20, 20–25, 25–30 min) and protocol (rPPS-SICI, rPPS-ICF, rPPS-SICF and rPPS-LICI) were used as within-subject factors. Regarding the after-effect of rPPS, time (0, 5, 10, 15, 20, 25 and 30 min after stimulation) and protocol (rPPS-SICI, rPPS-ICF, rPPS-SICF and rPPS-LICI) were entered as within-subject factors. Non-sphericity was corrected for with the Greenhouse–Geisser method whenever necessary.

If the results of rmANOVA were significant, a *post-hoc* analysis was conducted using the Student's *t*-test with Bonferroni correction for multiple comparisons. To test whether the MEP amplitude changed significantly after the rPPS, Dunnett's test was applied using the MEP amplitude at BL2 as the control condition.

All statistical analyses were performed with IBM SPSS Statistics version 23 (IBM Corporation, Armonk, NY, USA) at a significance level of $p = 0.05$.

Results

No participant reported apparent side-effects during or after the experimental procedures. Baseline values such as the motor thresholds or amplitudes of MEP to single-pulse TMS were comparable among the four experimental sessions (all $p > 0.2$, Tables 2 and 3).

Conditioning test paradigm

The results for SICI and ICF are presented together in Fig. 3A. Varying the conditioning PW showed a mild U-curve phenomenon for SICI, which is in line with the results of previous studies exploring different stimulus intensities [9,10], such that the maximal inhibition occurred with a PW of ThPW-6 (Fig. 3A, 2 ms and 3 ms). Statistically, the rmANOVA revealed a significant main effect of PW ($F_{1.7,18.8} = 5.67$, $p = 0.015$). *Post-hoc* tests revealed that amount of inhibition obtained at ThPW-15 was significantly different from that at ThPW-6 ($p = 0.002$) and ThPW-9 ($p = 0.014$), but not at ThPW-3 ($p = 0.129$). In contrast, the amount of facilitation seen in ICF was more or less linear. Wider, and hence presumably more effective, pulses yielded more facilitation both for ISIs of 10 and 15 ms. A main effect of the PW was significant ($F_{4,44} = 22.1$, $p < 0.001$). *Post-hoc* tests revealed that facilitation at ThPW-3 was significantly larger than that at ThPW-9 ($p = 0.003$), ThPW-12 ($p < 0.001$) and ThPW-15 ($p < 0.001$), and facilitation at ThPW-6 was significantly greater than at ThPW-12 ($p = 0.035$) and ThPW-15 ($p = 0.002$). When compared with the unconditioned MEP amplitude, MEPs by the paired-pulse were significantly decreased in SICI with ThPW-9 ($p = 0.032$ and 0.023 for 2 and 3 ms, Dunnett's test) and ThPW-6 ($p = 0.036$ and 0.011 for 2 and 3 ms); MEP increase in ICF was, on the other hand, significant only with larger PW, i.e. ThPW-3 ($p = 0.005$ and 0.014 for 10 and 15 ms) and ThPW-6 ($p = 0.041$, for 10 ms).

The results of SICF (Fig. 3B) confirmed the expectation that a wider, and hence presumably more effective, second pulse would produce more facilitation. Indeed, a main effect for PW was significant ($F_{1.4,15.5} = 19.7$, $p < 0.001$), as well as that for ISI ($F_{1.4,15.4} = 14.5$, $p = 0.001$) and their interaction ($F_{8,88} = 2.26$, $p = 0.031$). Given the significant interaction, we conducted the *post-hoc* analysis separately for each PW to further explore the time course of SICF. With a PW of ThPW-3, facilitation was significantly greater at 1.3 ms ($p = 0.006$) and 1.5 ms ($p = 0.008$) than at 1.9 ms. In a similar fashion, facilitation was greater at 1.3 ms ($p = 0.029$) than at 1.9 ms with a PW of ThPW-6. With a PW of ThPW-9, there were no significant differences except for that between 1.1 ms and 1.3 ms ($p = 0.049$). A *post hoc* test regarding the PW revealed a significant difference between ThPW-3 and ThPW-9 ($p = 0.001$), indicating that a wider stimulus resulted in more facilitation.

With regard to LICI (Fig. 3C), the results are similar to those reported previously, in that maximum inhibition was seen at 100 ms ISI [25]. A main effect of ISI was significant ($F_{2,22} = 16.2$, $p < 0.001$), and *post-hoc* analysis showed that inhibition at 100 ms ISI was significantly different from that at 200 ms ISI ($p = 0.002$).

Stimulation parameters generating maximal facilitation or inhibition differed among the participants (Fig. 4), but we, nonetheless, did observe a tendency that was consistent with the observation above. For SICI, the second largest PW (i.e. ThPW-6) resulted in maximal inhibition in most participants, similar to the U-shaped phenomenon regarding the conditioning intensity [9]. ICF tended

Table 2
Baseline characteristics in the conditioning-test paradigm.

	RMT for Magstim 200²*	RMT for cTMS*	ThPW (μs)	Test MEP amplitude (mV)
SICI	36.7 ± 1.3	31.2 ± 1.2	46.3 ± 0.7	1.49 ± 0.26
SICF	38.0 ± 1.6	32.1 ± 1.3	47.9 ± 0.8	1.42 ± 0.19
ICF	37.2 ± 1.6	31.9 ± 1.5	47.8 ± 0.6	1.50 ± 0.24
LICI	38.3 ± 1.8	32.5 ± 1.4	47.3 ± 0.7	1.41 ± 0.28

* RMTs are represented as % of maximum stimulator output for each device.
cTMS, controllable pulse parameter transcranial magnetic stimulation; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; MEP, motor evoked potential; RMT, resting motor threshold; SICF, short-interval intracortical facilitation; SICI, short-interval intracortical inhibition; ThPW, threshold pulse width.
Values are expressed as mean ± standard error of the mean.

Table 3
Baseline characteristics for MEP measurements before and after rPPS.

	Stimulus intensity (%MSO)	MEP amplitude at Baseline 1 (mV)	MEP amplitude at Baseline 2 (mV)
SICI	45.8 ± 2.4	1.02 ± 0.04	1.02 ± 0.05
SICF	47.0 ± 2.3	1.05 ± 0.05	1.05 ± 0.04
ICF	45.8 ± 2.2	1.04 ± 0.05	1.01 ± 0.04
LICI	45.8 ± 2.3	1.08 ± 0.04	1.03 ± 0.06

MSO, maximal stimulator output for Magstim 200²; rPPS, repetitive paired-pulse stimulus. The other abbreviations are the same as those in Table 2.
Values are expressed as mean ± standard error of the mean.

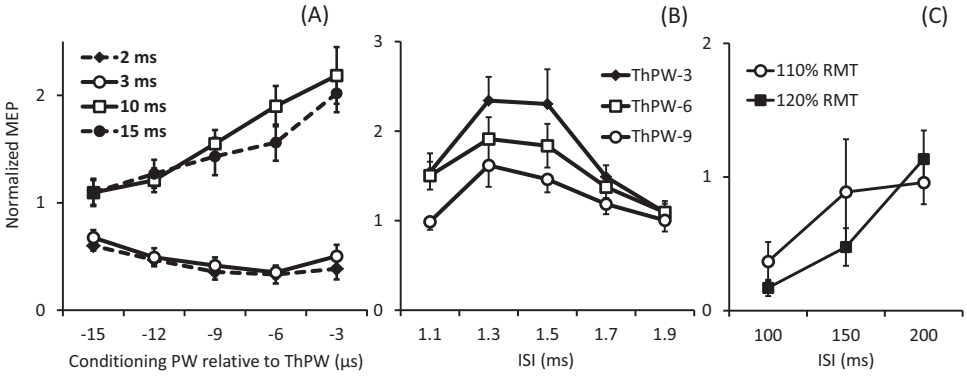

Figure 3. Conditioning-test paradigm. The Y-axes indicate the normalized MEP amplitude. (A) Results for SICI and ICF are presented together. Shorter ISIs (2 and 3 ms) represent SICI and longer ones (10 and 15 ms) ICF. The X-axis indicates the PW of the conditioning pulse relative to the ThPW. In SICI, the PW of ThPW-6 yielded the greatest inhibition, while ICF resulted in more facilitation with larger PW. (B) The results of SICF are shown separately for different PWs (ThPW-3, ThPW-6 and ThPW-9) of the second subthreshold pulse. A larger PW results in more facilitation. (C) LICI at 100 ms showed the greatest inhibition, at both 110% RMT and 120% RMT stimulus intensities.

to have more participants who showed maximal facilitation with increasing PW, as did the SICF. The results of LICI were more homogeneous: ten responded best at 100 ms and the other two at 150 ms, both with 120% RMT.

Effects during rPPS

MEP amplitudes increased during rPPS, when the protocol was SICF or ICF (Fig. 5). There was a statistically significant main effect for time ($F_{5,55} = 4.00$, $p = 0.04$), and we observed a trend for an effect of the protocol ($F_{3,33} = 2.58$, $p = 0.07$); interaction between time and protocol was not significant ($F_{5.5,\ 61.0} = 0.96$, $p = 0.45$). Normalized MEP amplitudes during rPPS-ICF differed significantly from those during rPPS-LICI ($p = 0.009$) and rPPS-SICI ($p < 0.001$). Similarly, MEPs during rPPS-SICF were significantly greater than those during rPPS-LICI ($p = 0.020$) and rPPS-SICI ($p < 0.001$).

After-effects of rPPS

In accordance with the findings during rPPS, normalized MEP amplitudes were increased after rPPS-SICF and rPPS-ICF (Fig. 6). rmANOVA revealed a significant main effect of protocol ($F_{3,33} = 9.77$, $p < 0.001$) and time ($F_{6,66} = 4.24$, $p = 0.01$), while the interaction of the two was not significant ($F_{5.6,61.1} = 1.84$, $p = 0.11$). Indeed, rPPS using both facilitatory protocols induced more potentiation of MEP than rPPS-LICI or rPPS-SICI ($p < 0.001$ for all combinations, i.e., rPPS-ICF vs. rPPS-LICI, rPPS-ICF vs. rPPS-SICI, rPPS-SICF vs. rPPS-LICI and rPPS-SICF vs. rPPS-SICI). Moreover, rPPS-SICF was found to induce more potentiation than rPPS-ICF ($p = 0.011$). Dunnett's method was employed to determine at which time point rPPS-SICF and rPPS-ICF yielded greater MEP amplitudes than the BL2. After rPPS-ICF, MEP amplitudes at 0 min were greater than that at BL2 ($p = 0.018$), while after rPPS-SICF the MEP amplitudes at 0, 5, 10, 15 and 20 min were significantly greater than those at BL2 ($p = 0.011$, 0.004, 0.006, 0.004, 0.048, respectively).

Control experiment

RMT at ThPW-6 was 41.3 ± 4.7% (mean ± standard deviation) of the maximum stimulator output, whereas 120% RMT at 60 μs was 37.4 ± 4.2%. In other words, the intensity used in the main experiment for stimuli with a PW of ThPW-6 was on average 90.7% (range: 84.2%–94.7%) RMT for that PW.

Discussion

In this study we explored the strength–duration relationship in paired-pulse TMS using the conditioning-test paradigm and utilized the results to optimize rPPS protocols. With respect to the degree of facilitation or inhibition, variations in the PW had an effect similar to variations of intensity. When used as a component of rPPS, SICF and ICF seemed to be effective in the potentiation of M1 excitability.

Strength–duration relationship has been less studied in the central nervous system than in the peripheral nervous system. A hyperbolic relationship between the PW and the threshold has been proposed in peripheral nervous system stimulation. This would imply that the key parameter for activation of neural tissue is not the peak

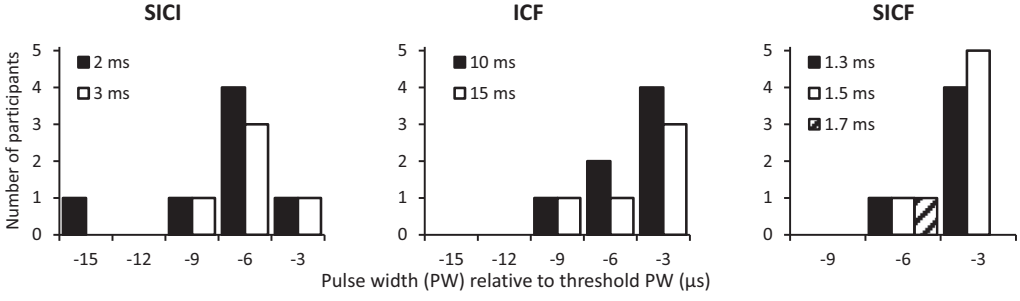

Figure 4. Variability in the best parameters for SICI, ICF and SICF. Each graph shows the number of participants who responded best at each parameter in each of the three (SICI, ICF or SICF) protocols. The X-axis gives PW relative to the individual ThPW. The Y-axes show the numbers.

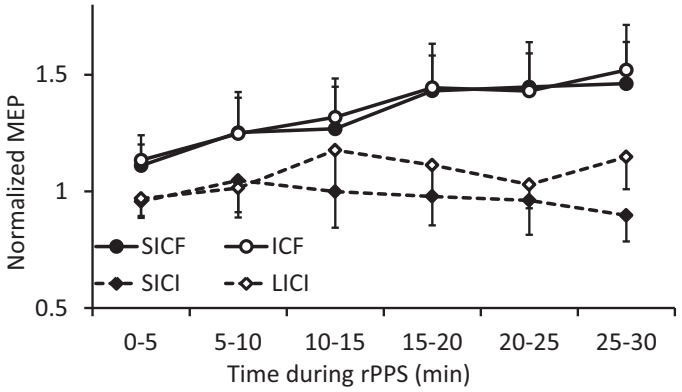

Figure 5. Time course of MEP amplitude during rPPS. MEP amplitudes during rPPS are averaged within bins of five minutes for each protocol and each participant, and normalized to the mean MEP amplitude for the first one minute, which consists of 12 MEPs. MEP amplitude increases during the facilitatory protocols, i.e. SICF or ICF.

induced current (equivalent to intensity), but the total amount of charge applied, which can be estimated as the product of induced current and its duration for a pulse with a rectangular shape [12,26]. The amount of charge can be increased with cTMS either by increasing intensity or by using pulses of longer duration. With conventional TMS devices, on the other hand, the amount of charge can only be altered by changing the stimulus intensity. In this study, the intensity was kept constant at the test intensity (except for a part of LICI testing), while changes in PW resulted in different amounts of charge applied to the brain. Given the present results that showed that increasing PW had an effect similar to increasing the intensity in the conventional setting, we would argue that paired-pulse protocols might also be dominated by this charge rule. With a constant induced peak current (i.e. intensity), a longer pulse duration would apply more charge to the brain, resulting in effects similar to an increase in intensity. More studies are required to develop a more precise formula describing the relationship between PW and the extent of facilitation or inhibition produced by a given paired-pulse protocol. Influence of conditioning intensity should be also taken into consideration; it may interact with manipulation of PW.

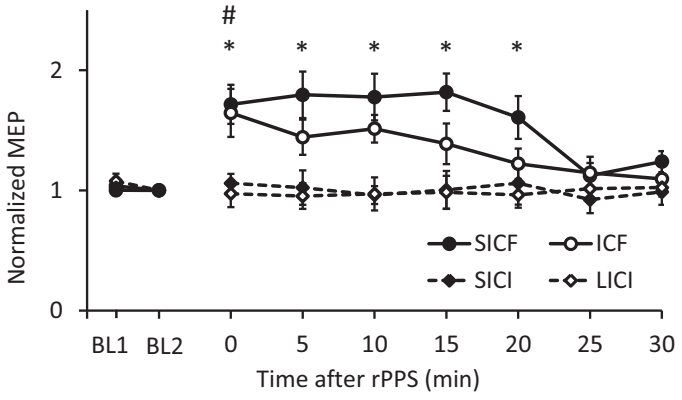

Figure 6. Time course of MEP amplitudes following rPPS. Twenty MEPs were collected at each time point and normalized to the mean MEP amplitude obtained at BL2 to plot the time course following rPPS. MEPs were initially greater than the baseline after facilitatory rPPS. Asterisks (*) denote a significant MEP increase after rPPS-SICF, compared with the BL2. The hash symbol (#) indicates a significant MEP increase after the rPPS-ICF. BL, baseline 1; BL2, baseline 2.

Several studies have already investigated the after-effects of rPPS, some of which have reported promising results. We followed the methods reported previously [3,6], and found that rPPS-ICF as well as rPPS-SICF resulted in an increase of MEP amplitudes following the intervention. Other studies have already reproduced the long-term potentiation-like effect of rPPS-SICF [7,27], to which we have added the finding that a SICF protocol based on strength–duration relationship could induce similar after-effects. On the other hand, rPPS-ICF was reported to show an MEP increase at a higher repetition rate than ours, i.e. every 5 s or 0.2 Hz [4]. The different total number of repetitions or other factors might have contributed to these results.

The precise mechanisms underlying rPPS-SICF or rPPS-ICF are not yet fully elucidated. Interestingly, however, the after-effects of rPPS-SICF may not be caused by I-wave facilitation, which is presumably the basis of SICF [28]. Nevertheless, rPPS-SICF did not affect MEPs elicited by cervico-medullary junction stimulation [28,29], providing some evidence for supra-spinal mechanism. Regarding rPPS-ICF, glutamatergic interneurons within the M1 could have played a cardinal role. ICF is probably mediated by those interneurons, and repetitive activation of them might have resulted in sustained enhancement of cortical excitability. In order to explore a network-level change caused by rPPS, it would be interesting to associate the influence of rPPS with electrophysiological measures other than MEP amplitude. The amount of inhibition for SICI or LICI, or facilitation for SICF or ICF could be good candidates for this purpose. Moreover, a variety of neurotransmitters is assumed to be involved in the intracortical circuits tested by paired-pulse protocols, but a possible link between the pharmacological impact on facilitation or inhibition and rPPS after-effects has not been established yet. Given that the after-effects of non-invasive brain stimulation techniques were affected by the administration of drugs which are associated with neurotransmitters [30], further pharmacological as well as physiological studies are warranted to establish a mechanistic description of rPPS.

The “best” parameters to yield maximal facilitation or inhibition differed among the participants, which is in line with several recent studies that showed variable responses to non-invasive brain stimulation among participants [8,31,32]. Differences in interneuron network architecture within M1 have been proposed as a plausible morphological substrate in this regard, but a convincing explanation for the variable response to a paired-pulse protocol must be elucidated in future studies. To make things more complicated, the best parameter on one day might not be the best one on another day [33].

There are some limitations in this study. First of all, rPPS protocols could be improved by manipulating factors other than those that we tested here. While ISI and PW were adjusted on an individual basis, the interval between the pairs was fixed at 5 s based on previous studies [6,27], and stimulus intensity was set at the test intensity throughout. Adjusting these factors might yield even better results. Second, as mentioned above, the mechanisms underlying the after-effects of rPPS-SICF and rPPS-ICF remain yet to be revealed. Specifically, a cortical origin of rPPS after-effects was not explicitly demonstrated here, although at least rPPS-SICF probably increased MEPs at a supraspinal level [28]. As to the PW-dependent alteration in the amount of facilitation or inhibition found in the conditioning-test paradigm, it is still difficult to estimate a quantitative relationship between the PW and the results of paired-pulse protocols.

In conclusion, cTMS has opened up new opportunities to explore the strength–duration relationship of paired-pulse TMS in addition to that of the single pulse. Furthermore, paired-pulse protocols can be promising tools to probe efficient rTMS methods for inducing long-lasting changes in the human central nervous system.

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