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RESEARCH ARTICLE

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Paired-pulse repetitive transcranial magnetic stimulation of the human motor cortex

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Abstract In nine healthy humans we modulated corticospinal excitability by using conditioning-test paired-pulse transcranial magnetic stimulation in a repetitive mode (rTMS), and we compared its effect to conventional single-pulse rTMS. We applied 80 single pulses or 80 paired pulses to the motor cortex at frequencies ranging from 0.17 to 5 Hz. The conditioning-test intervals were 2, 5, or 10 ms. Motor evoked potential (MEP) amplitudes from the abductor digiti minimi (ADM) as target muscle and extensor carpi radialis (ECR) indicated the excitability changes during and after rTMS. During paired-pulse rTMS at a facilitatory conditioning-test interval of 10 ms, we observed a facilitation of MEPs at 1, 2, and 5 Hz. A similar facilitation was found during single-pulse rTMS, when stimulus intensity was adjusted to evoke MEPs of comparable size. Using an inhibitory conditioning-test interval of 2 ms, paired-pulse rTMS at frequencies of 1 and 2 Hz caused no change in MEP size during the train. However, paired-pulse rTMS at 5 Hz caused a strong enhancement of MEP size, indicating a loss of paired-pulse inhibition during the rTMS train. Since no facilitatory effect was observed during single-pulse rTMS with an adjusted stimulus intensity, the MEP enhancement during 5 Hz rTMS was specific for "inhibitory" paired-pulse rTMS. After 5 Hz rTMS MEPs were facilitated for 1 min, and this effect was not substantially different between paired-pulse rTMS and single-pulse rTMS. The correlation between ADM and ECR was most pronounced at 5 Hz rTMS. We conclude that paired-pulse rTMS is a suitable tool to study changes in corticospinal excitability during the course of rTMS. In addition, our data suggest that short trains of paired-pulse rTMS are not superior to single-pulse rTMS in inducing lasting inhibition or facilitation.

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Introduction

The excitability of the human motor cortex has been investigated by two different modes of transcranial magnetic stimulation (TMS). With paired-pulse techniques, interactions between the first and the second pulse occur. Typically, a subthreshold conditioning stimulus induces a short-term modulation of the motor evoked potential (MEP) from a suprathreshold stimulus. Short interstimulus intervals (ISIs; 1–4 ms) inhibit, and longer ISIs (6–15 ms) facilitate the MEP amplitude. For this type of modulation an initial stimulus below motor threshold intensity is required (Kujirai et al. 1993; Ziemann et al. 1996). Spinal epidural recordings (Nakamura et al. 1997; Di Lazzaro et al. 1998) related this inhibition to the number and amplitude of corticospinal volleys (see Amassian and Deletis 1999 for review). This TMS-induced modulation must at least in part be generated cortically, presumably by intracortical interneurons modulating the activity of corticospinal neurons (Kujirai et al. 1993; Ziemann et al. 1996).

Series of repetitive TMS (rTMS) induce modulations of cortical excitability outlasting the stimulation period by minutes (Pascual-Leone et al. 1994; Wu et al. 2000). rTMS with frequencies of 2 Hz and faster induces a facilitation of MEPs (Pascual-Leone et al. 1994; Jennum et al. 1995). Repetitive stimuli at 0.9 Hz, but not at 0.1 Hz, have been claimed to induce a decrease in MEP size (Chen et al. 1997; Tergau et al. 1997).

rTMS has been claimed to be beneficial in depression (George et al. 1995), Parkinson's disease (Sommer et al. 1998; Siebner et al. 1999a), dystonia (Siebner et al. 1999b), and epilepsy (Tergau et al. 1999). For prospective clinical application stimulation methods have to be optimized for lasting effects and for selective induction of facilitation and inhibition.

We therefore combined paired pulses and repetitive stimulation and hypothesized that paired-pulse rTMS is

superior to single-pulse rTMS in inducing lasting inhibition and facilitation.

Materials and methods

We investigated nine healthy subjects with an average age of 27.8 (range 24–30) years. All were familiar with TMS and had given informed consent. The protocol was approved by the local ethics committee. We used two Magstim Rapid stimulators, a custom-built bistimulation module, and a figure-of-eight coil with an outer diameter of 7 cm in each wing (Magstim, Dyfed, UK; maximum magnetic output of each stimulator 1.4 Tesla via bistimulation module). Before starting the experiments in humans we tested the stimulators with a probe coil and found that this set-up generates constant magnetic intensities with pairs of pulses in all investigated combinations of intensities. In humans, stimuli were delivered over the optimal representation of the right abductor digiti minimi muscle (ADM) which was determined using suprathreshold TMS over the presumed location of the left central sulcus, with the coil held tangentially to the skull and the handle pointing about 45° posterolaterally. Motor thresholds were determined by delivering single TMS over the optimal representation of the ADM and by reducing the stimulus intensity step-by-step. The resting motor threshold (RMT) was defined as the lowest intensity at which at least five out of ten consecutive MEPs were ≥50 μV in amplitude while the investigated muscle was at rest. Audio-visual EMG feedback was provided to monitor muscle relaxation. The lowest intensity at which five out of ten consecutive MEPs were ≥200–300 μV in amplitude during voluntary abduction of the small finger was set as active motor threshold (AMT; Rossini et al. 1994; Rothwell et al. 1999).

The design of the principal part of the study is illustrated in Fig. 1A. All experiments started with 20 single, suprathreshold pulses applied at 0.17 Hz (one pulse every 6 s) over the optimal motor representation of the ADM. The first 5 were rejected to minimize effects of a possible initial lack of relaxation, and the other 15 pre-rTMS trials were taken as baseline. These were followed by 80 suprathreshold single pulses or by 80 paired pulses with an ISI of 2, 5, or 10 ms. These four types of rTMS were tested using frequencies of 0.17, 0.5, 1, 2, and 5 Hz. To detect effects outlasting rTMS, another 20 suprathreshold single pulses at 0.17 Hz were applied at the end of each experiment. The intensity of the suprathreshold pulses was set to obtain MEP amplitudes of about 1.0 mV. Paired pulses consisted of a conditioning pulse (90% of AMT, equivalent to 58–75% of RMT) followed by a suprathreshold pulse. The different conditions were tested in random order with a delay of at least 1 h between experiments. These principal experiments were carried out in six subjects with an average age of 27 (range 24–28) years.

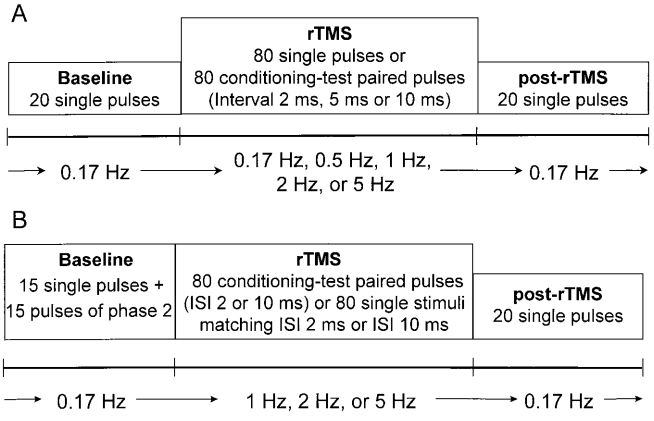

Fig. 1 **A** Diagram showing the order of stimuli in the principal experiment. Note that there are three parts: a baseline of suprathreshold test stimuli [yielding motor evoked potentials (MEPs) in the abductor digiti minimi (ADM) of about 1 mV], a repetitive transcranial magnetic stimulation (rTMS) part with single or paired stimuli at five different frequencies, and a replication of the baseline to detect post-rTMS changes in excitability. **B** Diagram showing the order of stimuli in the control experiment testing the influence of MEP size. At baseline, single suprathreshold pulses are mixed with the forthcoming rTMS type of stimulation to control for a proper adjustment of MEP amplitudes. *ISI* Interstimulus interval

MEPs were recorded from the right ADM and the right extensor carpi radialis (ECR) with surface electrodes in a belly-tendon montage at a sampling frequency of 5 kHz (filters at 10 Hz and 2.5 kHz, Synamps; Neuroscan, Herndon, Va., USA).

Data analysis

We measured peak-to-peak MEP amplitudes and latencies, and we normalized the MEP amplitudes to the pre-rTMS single-pulse amplitudes. We grouped the normalized data in bins of ten MEPs each. For all statistics MEPs during and after rTMS were analyzed separately.

To determine the time course of MEP change we used a repeated-measure ANOVA (internal factors ‘time’, ‘frequency’, and ‘type of rTMS’). To evaluate specific paired-pulse effects we normalized each subject’s MEP amplitudes from paired-pulse rTMS to the MEPs of single-pulse rTMS of the corresponding frequency, and we calculated a repeated-measure ANOVA (internal factors ‘frequency’ and ‘type of rTMS’). To elucidate frequency-dependent changes of MEP size we normalized, in each subject and for each type of rTMS, the MEP amplitudes obtained with faster frequencies to those obtained at 0.17 Hz, and we calculated a repeated-measure ANOVA (internal factors ‘frequency’ and ‘type of rTMS’). In all analyses we used *t*-tests corrected for multiple comparison (Fisher’s protected least significant difference) for *post hoc* comparisons. Oscillations of MEP amplitudes (Pascual-Leone et al. 1994; Jennum et al. 1995) were assumed if there were modulations of at least half the baseline amplitude in at least six consecutive MEPs.

We analyzed the latencies of MEPs before rTMS, of the first ten and the last ten MEPs during rTMS and of the MEPs after rTMS at the frequencies of 0.17, 2, and 5 Hz (ANOVA with ‘time’, ‘frequency’ and ‘type of rTMS’ as internal factors). In all analyses the level of significance was set at $P<0.05$. All results are given as mean ± standard deviation. To test the focality of inhibition and facilitation, we determined Pearson’s correlation coefficient of MEPs from ADM and ECR. We assumed a high correlation if the coefficient was >0.7.

Control experiment: influence of MEP size

As described in the results, we found an MEP increase at 1 Hz rTMS with facilitating paired pulses only. To find out whether this is related to MEP size per se or to the influence of the conditioning pulse, we undertook a control single-pulse experiment. We compared low-intensity single-pulse rTMS to inhibiting paired-pulse rTMS (ISI 2 ms), and high-intensity single-pulse rTMS to facilitating paired-pulse rTMS (ISI 10 ms). We used stimulation frequencies of 1, 2, and 5 Hz. We carefully selected single-pulse intensities to yield MEPs matching the corresponding paired-pulse condition. For detection of baseline and after-effects, each rTMS train was preceded and followed by single pulses of about 1.0 mV in amplitude (see Fig. 1B).

For analysis we used repeated-measure ANOVAs with the factors ‘type of rTMS’, ‘frequency’, and ‘bin’ to compare: (a) paired pulses with ISI 2 ms and matched single pulses, (b) paired pulses with ISI 10 ms and matched single pulses, and (c) low-intensity and high-intensity single pulses. Separate ANOVAs were calculated for results during and after rTMS. This control experiment was undertaken in five subjects (average age 29 years).

To assess a possible lack of muscle relaxation during rTMS we analyzed the average EMG amplitude in a time window of 35 ms immediately preceding each TMS stimulus artifact. We performed this analysis on all 5 Hz recordings of this control experiment (ISI 2 ms; small, adjusted, single pulses; ISI 10 ms; large, adjusted, single pulses), using the baseline pulses, the first 15 rTMS pulses for detection of an early surge in background EMG, and the last 15 rTMS pulses for detection of a gradual or late surge in background EMG.

Control experiment: transcranial electrical stimulation (TES)

As described in the results, the inhibition expected with the interval 2 ms was progressively lost and switched to facilitation during and after 5 Hz rTMS. To test whether this is related to subcortical facilitation we used TES (Multipulse Stimulator model D 185; Digitimer, Welwyn Garden City, UK) in three subjects (average age 29 years). TES is thought to act primarily at a subcortical level (Rothwell et al. 1991). At a slight voluntary contraction of the target muscle, TES is thought to activate almost exclusively D-waves, thus being independent from trans-synaptic activation (Amassian and Deletis 1999). We therefore repeated the 5 Hz, ISI 2 ms paired-pulse rTMS experiment with slight voluntary contraction of the ADM. Before and after rTMS we applied 40 paired pulses of ISI 2 ms randomly mixed with 20 TES pulses at 0.17 Hz. The TES intensity was adjusted to obtain stable MEPs of a range matching the paired-pulse MEP amplitudes. During rTMS, we replaced 4 of the first 20 and 4 of the last 20 paired pulses by TES. For analysis we compared the TES-induced MEPs during and after rTMS to baseline (*t*-tests).

Results

Excitability during rTMS

A typical example of the principal results is shown in Fig. 2. With one pair of stimuli every 6 s (0.17 Hz), inhibiting pairs of stimuli (ISI 2 ms) yielded an inhibition of the MEP amplitude, and facilitating pairs (ISI 10 ms) a facilitation. At 2 Hz the inhibition found with ISI 2 ms was preserved, while the facilitation seen with ISI 10 ms was more pronounced than at 0.17 Hz. At 5 Hz, the inhibition with ISI 2 ms was no longer detectable. The facilitation seen with ISI 10 ms was still larger than at 0.17 Hz.

Time course analysis

An overview of the principal findings is shown in Fig. 3. The MEP amplitudes during rTMS were stable at 0.17 Hz, paired pulses with ISI 2 ms showed a slight facilitation during rTMS of 0.5 Hz, and all types of rTMS yielded increased amplitudes during 1, 2, and 5 Hz rTMS. This increase was more gradual in ECR than in ADM [repeated-measures ANOVA, effect of bin, ADM, $F(7,35)=2.9$, $P<0.02$, ECR, $F(7,35)=12.0$, $P<0.0001$]. The standard deviation was large at 5 Hz due to oscillating MEP amplitudes observed only at this frequency (ADM, subject 1, all types of rTMS, subject 5, ISI 10 ms only, subject 6, single stimuli and ISI 10 ms).

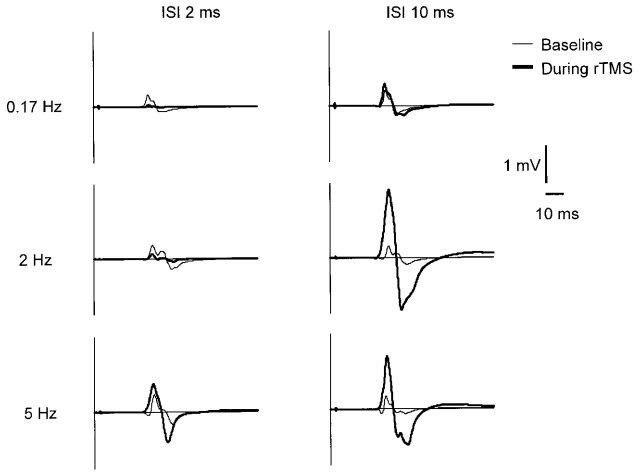

Fig. 2 Example of MEPs from ADM during rTMS from one representative subject. *Thin lines* represent the average of 15 suprathreshold single stimuli at 0.17 Hz preceding each rTMS train. *Thick lines* indicate the average of 80 MEPs from paired stimuli separated by an interval of 2 ms (*left column*) or 10 ms (*right column*). Results from frequencies of 0.17, 2, and 5 Hz are shown. Note that ISI 2 ms yields smaller amplitudes than single stimuli at 0.17 Hz and at 2 Hz, but larger amplitudes than single stimuli at 5 Hz. Amplitudes from ISI 10 ms are larger than single stimuli. This facilitation is very much accentuated in this subject at 2 Hz without a further accentuation at 5 Hz. Hence, different ISIs respond differently to increasing rTMS frequency

Comparison of single-pulse and paired-pulse rTMS

As expected from the literature (Kujirai et al. 1993), ISI 2 ms yielded smaller and ISI 10 ms larger MEP amplitudes than single stimuli [ANOVA, effect of type of rTMS for ADM, $F(3,15)=13.7$, $P<0.0001$, for ECR, $F(3,15)=20.4$, $P<0.0001$]. *Post hoc* analyses confirmed that single stimuli differed significantly from ISI 2 ms and ISI 10 ms, and that ISI 2 ms differed significantly from ISI 10 ms. These differences were least pronounced with 5 Hz, and in ADM the pool of slower frequencies (0.17–2 Hz) differed from 5 Hz [interaction of type of rTMS by frequency, $F(3,15)=4.5$, $P<0.02$]. The loss of inhibition was observed in five of six subjects, the MEP amplitude during the last bin of paired-pulse rTMS with ISI 2 ms ranging from 122% to 223% of baseline. Loss of inhibition was also present to a lesser extent in the ECR. In five of six subjects the baseline single-pulse MEP amplitudes preceding 5 Hz rTMS of ISI 2 ms were larger in ADM than in ECR (mean of six subjects ± SD, 1201±660 μV in ADM and 905±913 μV in ECR).

Comparison of rTMS frequencies

We found higher MEP amplitudes during fast than during slow rTMS. In ADM the slope of the frequency-related MEP increase depended on the type of rTMS [ANOVA, interaction of frequency by type of rTMS, $F(12,60)=2.3$, $P<0.018$]. Amplitudes of ISI 10 ms already rose at 1 Hz, with a saturation at 2 and 5 Hz.

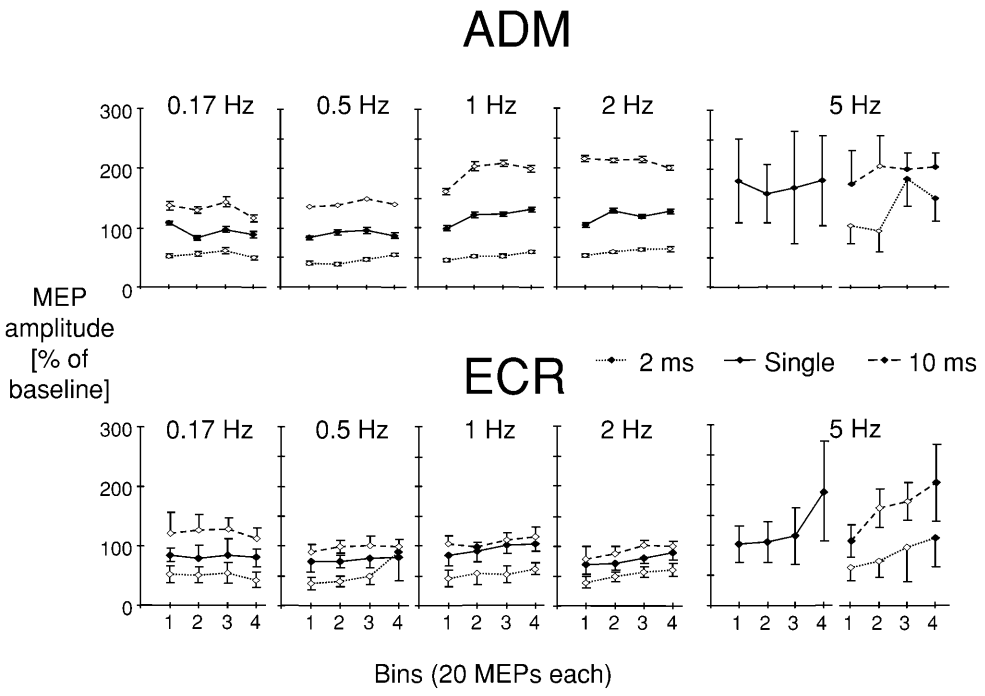

Fig. 3 MEP amplitudes recorded from ADM during rTMS. MEPs are normalized to the MEPs from 15 single-test rTMS pulses at 0.17 Hz preceding each rTMS train (see Fig. 1A for experimental design). Mean values and one standard deviation of three types of rTMS (single, ISI 2 ms, and ISI 10 ms) at five different rTMS frequencies are shown. For clarity each series of 80 stimuli or pairs of stimuli is grouped in four bins of 20 MEPs each (*1–4*). *Open data points* indicate a significant difference to the corresponding bin from single stimuli (*t*-tests corrected for multiple comparisons). Note that MEP amplitudes increased during rTMS. ISI 2 ms yielded smaller amplitudes and ISI 10 ms larger amplitudes than single stimuli; this MEP modulation faded at 5 Hz. Fast rTMS frequencies yielded higher MEP amplitudes than slow ones. In ADM, this frequency-related increase already occurred at 1 Hz for ISI 10 ms. *ECR* Extensor carpi radialis

Those with ISI 2 ms rose slightly between 0.5 and 2 Hz, but most distinctly at 5 Hz. At 1 Hz, MEPs obtained with single stimuli and with ISI 5 ms showed a stronger facilitation than ISI 2 ms, but less facilitation than ISI 10 ms frequency.

Excitability after rTMS

There was a facilitation of MEP amplitudes after 1 and 2 Hz rTMS with ISI 10 ms, although the slope of facilitation over all frequencies did not depend on the type of rTMS (interaction of frequency by type, $P>0.5$). ADM amplitudes were larger than at baseline after either type of 5 Hz rTMS [effect of frequency, $F(4,20)=4.1$, $P<0.015$, effect of type, $P>0.7$]. ADM amplitudes were larger in the first minute after rTMS than in the second [effect of bin, $F(1,5)=7.5$, $P<0.042$]. The decrease in amplitude from the first minute to the second was sharper for fast frequencies than for slow ones [interaction of frequency by bin, $F(4,20)=3.2$, $P<0.034$; see Fig. 4].

In ECR the results were similar, but less pronounced. The MEP amplitudes tended to be larger than baseline after either type of fast-frequency rTMS. MEP amplitudes tended to be larger in the first minute after rTMS than in the second, and the decrease in amplitude from the first minute to the second was sharper for fast frequencies than for slow ones [interaction of frequency by bin, $F(4,20)=4.3$, $P<0.012$].

MEP latencies

We did not find a significant change of MEP latencies with increasing frequency, and the different types of rTMS did not yield significantly different latencies (ANOVA).

Side effects and correlation of ADM and ECR

None of the subjects experienced a partial or generalized seizure, but a spread of excitation was clinically observed in subject 6 at 5 Hz frequency, and it was more pronounced with ISI 10 ms than with any other type of rTMS.

We found a high correlation of ADM and ECR amplitudes more often at fast frequencies than at slow ones (Table 1). The correlation did not depend on the relative size of ECR amplitudes as compared to ADM amplitudes. The largest MEPs in ECR were observed in subject 6, where a high correlation between ADM and ECR was rare (see Table 1). By contrast, in subjects 1 and 5, where a high correlation of muscles was frequent, ECR amplitudes were only 10–45% of the size of ADM amplitudes.

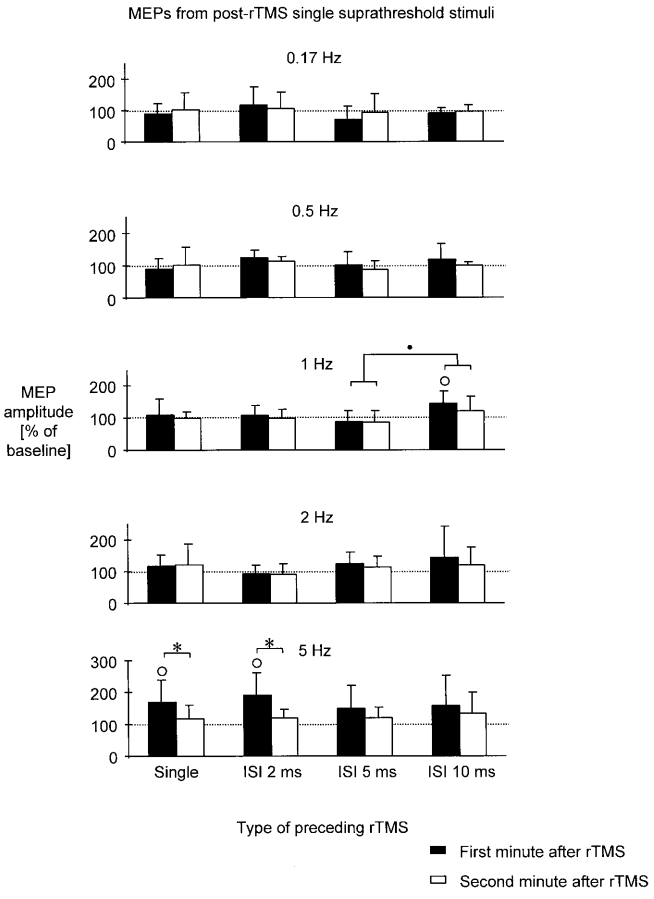

Fig. 4 MEP amplitudes of ADM evoked by single suprathreshold stimuli at 0.17 Hz after different types of rTMS. MEPs are normalized to the baseline single suprathreshold stimuli at 0.17 Hz preceding each rTMS train. Mean values and one standard deviation are shown. *Circles* indicate a significant difference to baseline, *asterisks* indicate a significant difference between bins (*t*-tests corrected for multiple comparisons), and *dots* indicate a significant difference between types of rTMS (*t*-test). Note that MEP amplitudes were facilitated for 1 min after 5 Hz rTMS of either type. rTMS of ISI 10 ms induced such facilitation already at 1 Hz

Table 1 Subjects in which abductor digiti minimi (ADM) and extensor carpi radialis (ECR) correlate. Subjects from the principal experiment in which ADM and ECR correlated during repetitive transcranial magnetic stimulation with a correlation coefficient of at least 0.7 are indicated by their number. Note that muscles correlated in more subjects at 5 Hz than at slower frequencies. (*ISI* Interstimulus interval)

	0.17 Hz	0.5 Hz	1 Hz	2 Hz	5 Hz
Single	2				1, 5
ISI 2 ms	6			2	1, 2
ISI 5 ms					1, 5
ISI 10 ms			5		1, 3, 5, 6

Control experiment: influence of MEP size

Paired pulses with ISI 2 ms did not yield an MEP facilitation during 1 and 2 Hz, but a significant facilitation during 5 Hz rTMS (Fig. 5H). These results replicate those of the principal experiment. Small adjusted single stimuli remained essentially unchanged between 1 and 5 Hz, thus resulting in lower amplitudes than paired pulses at 5 Hz (ANOVA, effect of type of rTMS, P=0.02, effect of frequency, P=0.01, interaction of frequency by type of rTMS, P=0.001; Fig. 5B). Paired pulses with ISI 10 ms yielded a facilitation of amplitudes at either frequency (effect of frequency, P=0.30), they did not differ systematically from adjusted single stimuli (interaction of frequency by type of rTMS, P=0.39). MEPs after paired-pulse rTMS were not significantly different from those after single-pulse rTMS.

Large single pulses tended to show more facilitation during rTMS than small single pulses (ANOVA, effect of type, P=0.06). The effects after rTMS were similar for small and large single pulses.

A slight facilitation of the background EMG was present in a subgroup of subjects with either type of stimulation. It was about equally strong with paired pulses and with adjusted pulses (Fig. 6).

Results from TES

TMS-induced MEPs were enhanced during the course of rTMS in all subjects; the enhancements after rTMS were pronounced in one subject only (Fig. 7). TES-induced MEPs during rTMS were enhanced in one subject and enhanced to a minor degree in the two others, while TES-induced amplitude changes after rTMS were minor.

Discussion

This is the first study investigating the effects of frequency and ISI on paired-pulse TMS in a very systematic way using 80 trials of four conditions at five frequencies. The number of 80 trials was chosen in accordance with safety criteria established for single-pulse rTMS (Wassermann 1998). As our results show, this number turned out to be sufficient for evaluation of effects during stimulation, but only partially sufficient for effects after stimulation.

MEP size during paired-pulse stimulation

In the past, the main variable to manipulate inhibition or facilitation has been stimulation frequency, with a consensus that high stimulation frequencies induce a facilitation (Pascual-Leone et al. 1994; Hallett 2000) and lower stimulation frequencies an inhibition (Chen et al. 1997; Muellbacher et al. 2000). From our main experiment (Fig. 3) and from the control experiment (Fig. 5) it becomes clear that the influence of frequency and of baseline MEP amplitude exert a combined influence. To separate both effects one would have to design experiments with a large number of different stimulation amplitudes for each frequency and each ISI, an effort which

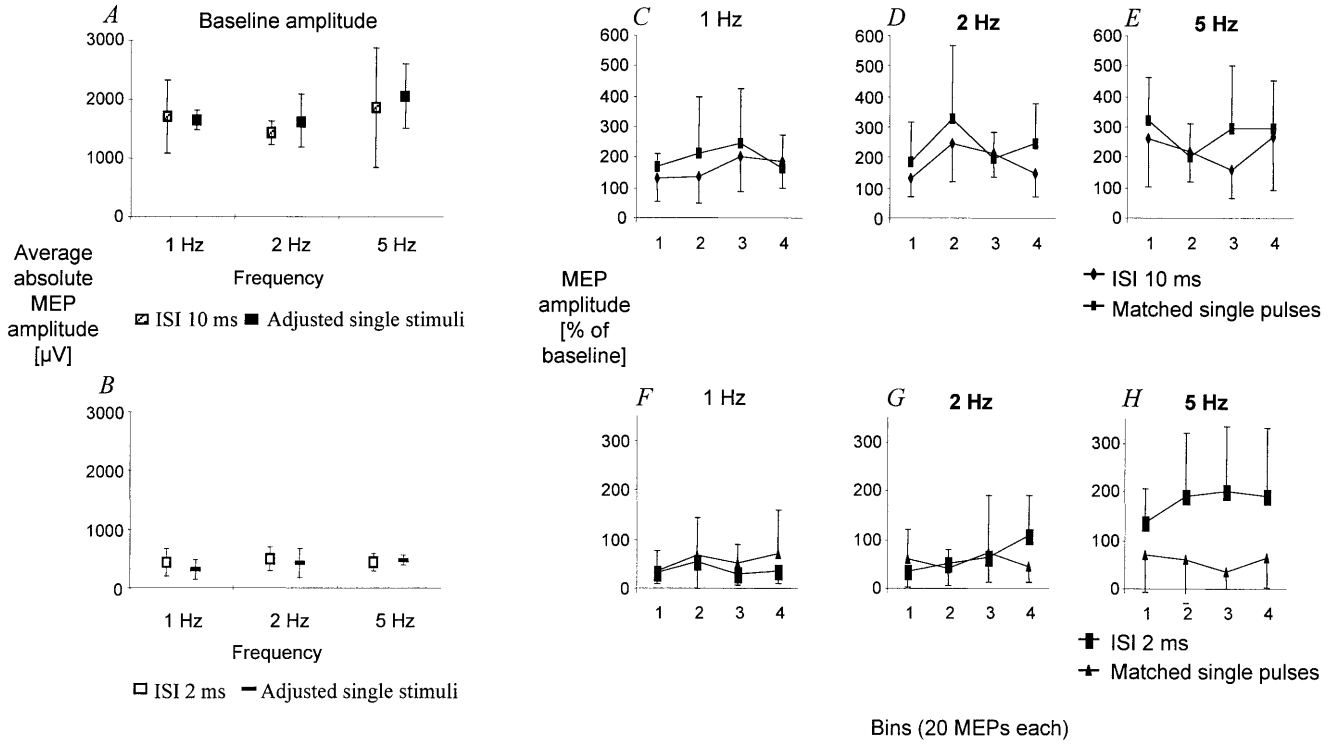

Fig. 5A–H Control experiment: comparison of paired-pulse rTMS with single pulses adjusted in MEP amplitude (mean values and one standard deviation). **A**, **B** Amplitudes before rTMS. Note that amplitudes of single stimuli well matched those of facilitating paired pulses (**A**) or of inhibiting paired stimuli (**B**). **C–H** Amplitudes during rTMS. Note that ISI 2 ms yielded smaller amplitudes than adjusted single stimuli at 1 Hz (**F**) and during early 2 Hz rTMS (**G**), but much larger amplitudes at 5 Hz (**H**). ISI 10 ms did not differ from adjusted stimuli at either frequency (**C–E**)

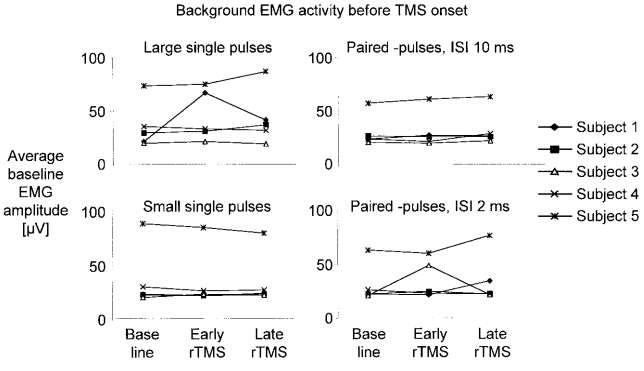

Fig. 6 Background EMG activity of the before, during early, and during late 5 Hz rTMS. Mean EMG amplitude of a 35-ms time window preceding the TMS stimulus artifact is shown. The baseline comprises 15 single pulses at 0.17 Hz frequency, the early part of rTMS consists of the first 15 rTMS traces, and the late part of rTMS comprises the last 15 rTMS traces. Four types of rTMS were analyzed (ISI 2 ms, small adjusted single pulses, ISI 10 ms, large adjusted single pulses). There is an early surge in background EMG in two cases, likely related to a startle at the onset of rTMS. In all other cases the rise in background EMG activity was modest or absent

is far beyond the scope of this study. On the other hand, matching the stimulus intensity of the test pulse to yield comparable MEP amplitudes with ISI 2 ms and ISI 10 ms would require very strong test pulses for ISI 2 ms and very weak test pulses for ISI 10 ms. Since intracortical facilitation and inhibition depend on the test pulse intensity (Ziemann et al. 1996) they would be tested in a suboptimal, distorted, and incomparable way. We, therefore, have preferred a different approach to overcome the difference in baseline MEP amplitude. We used two types of matched single stimuli, one with a low and another with a high amplitude (control experiment 1). This approach enables us to compare adjusted single pulses and paired pulses without confounding baseline MEP differences. Thus, this approach allows us to answer the basic question of superiority of paired-pulse rTMS over single-pulse rTMS addressed in this study. This control experiment (Fig. 5) made it clear that rTMS effects with amplitudes already high at baseline do not differ much with ISI 10 ms or unpaired stimuli. This is most likely explained by a ceiling effect. In the same control experiment, single stimuli with a primarily low amplitude behaved similarly at 1, 2, and 5 Hz (Fig. 5). A specific paired-pulse phenomenon occurred with ISI 2 ms adjusted to the same amplitude. It was clearly inhibiting at 1 Hz, inhibition was progressively reduced at 2 Hz, and it was clearly facilitating in all subjects at 5 Hz. This specific paired-pulse effect was also observed in the principal experiment where the inhibition seen with ISI 2 ms at lower repetition rates was progressively lost during the rTMS train. The subcortical facilitation during rTMS revealed by TES suggests that the loss of inhibition is due to a corticospinal facilitation that out-

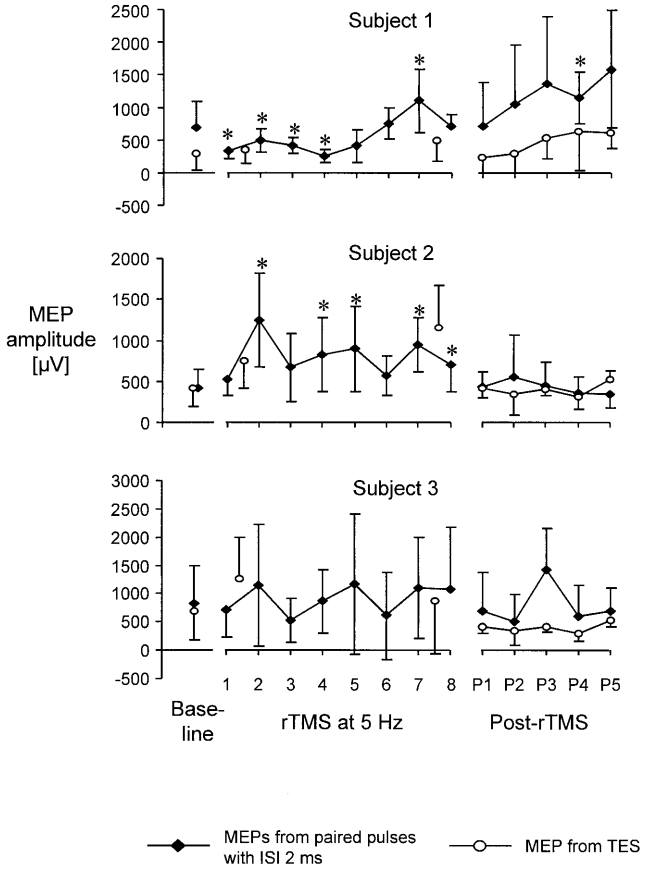

Fig. 7 MEPs amplitudes from the transcranial electrical stimulation (TES) control experiment. Paired-pulse TMS with a conditioning-test interval of 2 ms (*filled squares*) has been mixed with single TES pulses (*open circles*). Stimulation frequency was 0.17 Hz at baseline and after rTMS, and 5 Hz during rTMS. MEPs during and after rTMS are grouped in bins of 6–10 MEPs from TMS and 3–4 MEPs from TES, according to randomization. *Asterisks* indicate a significant difference from baseline (*t*-test, $P<0.05$). Note that MEPs from TMS increase during rTMS, again confirming the loss of inhibition with ISI 2 ms at 5 Hz, while MEPs from TES show a marked facilitation in subject 2 and only a modest facilitation in subjects 1 and 3

balances intracortical mechanisms. Alternatively, the enhanced pre-rTMS background EMG may indicate an enhanced voluntary motor drive, which is known to reduce intracortical inhibition (Ridding et al. 1995). This suggests that the loss of inhibition may be at least in part a cortical effect.

The peripheral feedback from the stimulated arm was likely stronger with high-intensity rTMS than with low-intensity rTMS. Also, subclinical rhythmic oscillations in the motor network, induced either directly by rTMS or indirectly by rhythmic peripheral feedback, were more likely to occur with high-intensity rTMS than with low-intensity rTMS (Narici et al. 1987; Salmelin and Hari 1994). Hence, peripheral feedback and rhythmic oscillations may have contributed to the stronger facilitation with high-intensity rTMS (Fig. 5).

MEP size saturation during stimulation was more gradual in ECR than in ADM. This is consistent with previous studies (Pascual-Leone et al. 1994). Also, the loss of inhibition observed with ISI 2 ms at 5 Hz was more pronounced in ADM than in ECR. Most likely, facilitation had a steeper onset and saturated more rapidly for the target muscle ADM than for the ECR. A novel finding is that the correlation of MEP size between both muscles during rTMS was higher at fast frequencies and most often found with the ISI 10 ms. It appears that high frequencies may be suitable to synchronize earlier neighboring pools of motor neurons.

Effects outlasting rTMS

Eighty stimuli at 5 Hz induced excitatory after-effects. This is in good agreement with the findings of Berardelli and colleagues (1998). They used trains of 20 suprathreshold TMS pulses at 5 Hz, resulting in a facilitation of MEPs 900 ms after rTMS. They assumed that the mechanism of cortical facilitation may either consist in an enhanced excitability of corticospinal cells or in a reduced activity of cortical inhibitory circuits. This assumption was based on negative results with transcranial electrical stimuli and with spinal reflexes. Also here, the loss of inhibition after rTMS of ISI 2 ms at 5 Hz appears to be largely a cortical effect, since a subcortical facilitation after rTMS was much less pronounced than during rTMS.

To keep conditions comparable we used the number of 80 stimuli for all frequencies and all types of rTMS. For frequencies slower than 5 Hz, only rTMS with ISI 10 ms induced a lasting facilitation of MEP size. Otherwise, the number of 80 stimuli was not sufficient to produce inhibitory after-effects. This is well in accordance with the literature, where longer trains of rTMS were necessary to produce a lasting inhibition of cortical excitability (Chen et al. 1997; Siebner et al. 1999b; Muellbacher et al. 2000). In another series of experiments (Sommer et al. 1998) we used 900 conditioning-test pairs at 1 Hz and found an inhibition of MEP size after inhibiting pairs and a facilitation after facilitating pairs. Whether similar effects can be obtained with long trains of single-pulse rTMS of different intensities is currently being studied.

In summary, the data presented here confirm different mechanisms for inhibition and facilitation, with inhibition requiring distinctly more stimulation than facilitation. Paired-pulse rTMS turned out to be a suitable tool to study changes in corticospinal excitability during the course of rTMS. Short trains of paired-pulse rTMS were not superior to single-pulse rTMS in inducing lasting inhibition or facilitation.

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