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Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity

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Paired transcranial magnetic stimulation has greatly advanced our understanding of the mechanisms which control excitability in human motor cortex. While it is clear that paired-pulse excitability depends on the exact interstimulus interval (ISI) between the first (S1) and second stimulus (S2), relatively little is known about the effects of the intensities of S1 and S2, and the effects of manipulating neurotransmission through the $GABA_A$ receptor. When recording the motor evoked potential (MEP) from the resting abductor digiti minimi (ADM) muscle, using a fixed ISI of 1.5 ms, and expressing the interaction between S1 and S2 as $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$, then a systematic variation of the intensities of S1 and S2 revealed short-interval intracortical facilitation (SICF) if S1 and S2 were approximately equal to MEP threshold (RMT), or if S1 > RMT and S2 < RMT. In contrast, short-interval intracortical inhibition (SICI) occurred if S1 < RMT and S2 > RMT. Contraction of the ADM left SICI unchanged but reduced SICF. The $GABA_A$ receptor agonist diazepam increased SICI and reduced SICF in the resting ADM while diazepam had no effect during ADM contraction. Surface EMG and single motor unit recordings revealed that during ADM contraction SICI onset was at the I3-wave latency of S2, whereas SICF typically 'jumped up' by one I-wave and started with the I2-wave latency of S2. Findings suggest that SICI is mediated through a low-threshold $GABA_A$ receptor-dependent inhibitory pathway and summation of IPSP from S1 and EPSP from S2 at the corticospinal neurone. In contrast, SICF originates through non-synaptic facilitation at the initial axon segment of interneurones along a high-threshold excitatory pathway.

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Short-interval paired-pulse transcranial magnetic stimulation (TMS) in humans has been used to explore the excitability of various inhibitory (Kujirai *et al.* 1993; Di Lazzaro *et al.* 1998*b*; Hanajima *et al.* 1998; Fisher *et al.* 2002) and excitatory (Tokimura *et al.* 1996; Ziemann *et al.* 1996*c*, 1998; Di Lazzaro *et al.* 1999*b*; Hanajima *et al.* 2002) neuronal circuits at the motor cortical level. Most previous studies employed fixed intensities of the first (S1) and second stimulus (S2) within a given protocol. If S1 is below threshold for a motor evoked potential (MEP) in the target muscle and S2 is clearly above the MEP threshold, then the interaction between S1 and S2 is inhibitory at very short interstimulus intervals (ISI) of 1–5 ms (Kujirai *et al.* 1993; Fisher *et al.* 2002). However, if S1 and S2 are close to the MEP threshold (Tokimura *et al.* 1996) or S1 is clearly above the MEP threshold and S2 is below or around the MEP threshold (Ziemann *et al.* 1998; Hanajima *et al.* 2002), then MEP facilitation occurs at discrete ISIs of about 1–1.5, 2.5–3.0 and 4.0–4.5 ms. Cervical epidural recordings of the descending corticospinal volley provided strong evidence that all these interactions occur at the level of the motor cortex (Di Lazzaro *et al.* 1998*b*, 1999*b*). The exact mechanisms, however, are not fully understood. It is thought that short-interval intracortical inhibition (SICI) reflects inhibition mediated by $GABA_A$ receptors (Kujirai *et al.* 1993). Most probably, the sub-threshold S1 produces IPSP at the cortico-spinal neurones that lead to a reduced number of action potentials by the subsequent supra-threshold S2. In contrast, it is thought that short-interval intracortical facilitation (SICF) reflects direct excitation of axon initial segments of excitatory intracortical inter-neurons by S2, which had been depolarised and therefore made hyperexcitable by the preceding S1 (Hanajima *et al.* 2002). This suggests that the physiology underlying the interaction between S1 and S2 may be rather complex. Previous studies demonstrated that the exact ISI between S1 and S2 determines what kind of interaction occurs (Kujirai *et al.* 1993; Tokimura *et al.* 1996; Ziemann *et al.* 1998; Fisher *et al.* 2002). In contrast, the effects of S1 and S2 intensity have not yet been systematically explored. It was noted in preliminary experiments, for instance, that SICI at an ISI of 3 ms is maximal if S1 was approximately

80 % of resting motor threshold (RMT) and S2 clearly above RMT (Kujirai *et al.* 1993). If S1 was increased above RMT then SICI turned into SICF (Kujirai *et al.* 1993). Another paired-pulse TMS study showed, by using an ISI of 1.2 ms and a threshold-hunting protocol, that the interaction between S1 and S2 was inhibitory if S1 was < 65 % RMT, but facilitatory if S1 exceeded 65 % RMT (Awiszus *et al.* 1999). The aim of this study was to test in greater detail the effects of S1 and S2 intensity on the interaction between S1 and S2 at short ISIs of less than or equal to 5 ms. Most experiments were performed at an ISI of 1.5 ms and with the ADM at rest. In order to explore the physiology of the interaction between S1 and S2, experiments were also conducted during voluntary isometric contraction of the ADM, and single motor units (SMU) were recorded in addition. This allows the exact determination of the onset of the interaction between S1 and S2 relative to the D-wave elicited by direct activation of the proximal axon of the corticospinal neurone by anodal transcranial electrical stimulation (TES) (Hanajima *et al.* 2002). Finally, the effects of a single oral dose of the $GABA_A$ receptor agonist diazepam (DZP) were tested in the resting and active ADM in order to see to what extent the inhibitory and facilitatory interactions between S1 and S2 are affected by changes in inhibitory neurotransmission.

METHODS

Subjects

Twelve healthy volunteers (mean age, 30.2 ± 4.4 years, range, 23–36 years; 2 women, 10 men) participated in the experiments. Nine subjects were right-handed and three left-handed when tested with the Edinburgh Inventory (Oldfield, 1971). Informed written consent was obtained from all subjects. The study was performed according to the Declaration of Helsinki and was approved by the ethics committee of the J. W. Goethe University of Frankfurt, Germany.

Recording and stimulation procedures

Subjects were seated comfortably in a reclining chair. Surface EMG was recorded from the abductor digiti minimi (ADM) muscle of the dominant hand, using surface electrodes in a belly–tendon montage, with the active electrode placed over the motor point and the reference electrode on the proximal interphalangeal joint of the small finger. After amplification and 10 Hz to 2 kHz bandpass filtering (Counterpoint Electromyograph, Dantec Electronics, Skovlunde, Denmark) the EMG signal was passed through a CED micro 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) and fed into a personal computer (sampling rate 4 kHz), using customised data collection and conditional averaging software (Spike 2 for Windows, Version 3.05, Cambridge Electronic Design, Cambridge, UK) for off-line analysis.

Transcranial magnetic stimulation (TMS) was applied over the hand area of the dominant motor cortex through a figure-of-eight coil (outer diameter of each loop, 9 cm; peak magnetic field ~1.5 T) using two Magstim 200 magnetic stimulators (Magstim, Whitland, Carmarthenshire, UK) connected to the BiStim module (Magstim) throughout all measurements. The stimulating coil was placed flat on the skull with the handle pointing backwards and rotated 45° away from the mid-line. Thus, the current induced in the brain was directed approximately perpendicular towards the assumed line of the central sulcus. This is the optimal orientation for a predominantly trans-synaptic activation of the corticospinal neurone (e.g. Kaneko *et al.* 1996; Di Lazzaro *et al.* 2001). The optimal coil position for activating the contralateral ADM was determined as the site where stimulation at a slightly suprathreshold stimulus intensity consistently produced the largest MEP. This site was marked with a pen in order to assure a constant placement of the coil throughout the experiment. Resting motor threshold (RMT) was determined in the resting ADM to the nearest 1 % of maximum stimulator output using single-pulse TMS. RMT was defined as the lowest stimulus intensity which elicited MEPs > 50 μV in at least five of ten consecutive trials (Rossini *et al.* 1994). Active motor threshold (AMT) was obtained during a slight isometric contraction (5–10 % of maximum voluntary contraction) and defined as the lowest stimulus intensity which elicited a mean MEP > 100 μV from five single-trial rectified sweeps. RMT and AMT are reported as a percentage of the maximum stimulator output. The EMG was displayed continuously at a high gain (50 μV per division) of the recording device on the computer screen and played through a loudspeaker for acoustic feedback. In those experiments with the ADM at rest, trials contaminated by EMG activity were discarded from analysis.

Effects of S1 and S2 intensity at different interstimulus intervals

These experiments were conducted in six subjects with the ADM at rest. In each subject, four different ISIs of 1.5, 2.1, 3.3 and 5.0 ms were tested in pseudorandomised order and in separate sessions. These particular ISIs were selected because they had revealed different interactions between S1 and S2 in previous paired-pulse TMS experiments. The interval of 1.5 ms showed marked SICF if S1 > RMT and S2 < RMT (Ziemann *et al.* 1998), or if both stimuli were approximately equal to RMT (Tokimura *et al.* 1996), but no SICI if S1 < RMT and S2 > RMT (Fisher *et al.* 2002). In contrast, the interval of 2.1 ms showed no or much less SICF if S1 > RMT and S2 < RMT (Ziemann *et al.* 1998), or if both stimuli were approximately equal to RMT (Tokimura *et al.* 1996), while clear SICI was observed if S1 < RMT and S2 > RMT (Fisher *et al.* 2002). While these were robust between-subject effects in the previous studies, the interaction between S1 and S2 was more variable for the interval of 3.3 ms. Some subjects showed SICF while others showed no interaction (Tokimura *et al.* 1996; Ziemann *et al.* 1998). If S1 < RMT and S2 > RMT, there was clearly less SICI than for the interval of 2.1 ms (Fisher *et al.* 2002). Finally, the interval of 5.0 ms was selected because it is usually the turning point between inhibition and facilitation in the paired-pulse protocols where S1 < RMT and S2 > RMT (Kujirai *et al.* 1993; Ziemann *et al.* 1996*c*).

In all experiments, S1 and S2 were varied in steps of 10 % RMT between 60 and 140 % RMT (i.e. nine intensity steps). The paired-pulse conditions consisted of all possible combinations of S1 and S2 intensities (i.e. 9 × 9 = 81 conditions). In addition, nine single-pulse conditions were tested at the nine different intensities. Five trials were performed for each condition (i.e. 5 × 90 = 450 trials per session). The different conditions were applied in pseudo-randomised order. The correct intensities of the two magnetic stimulators were set automatically by customised software (Spike 2) via the CED 1401 laboratory interface and the remote port of the

Magstim stimulators. The intertrial interval varied randomly between 8 ± 2 s. Conditional averages of the peak-to-peak MEP amplitudes were calculated, and the interaction between S1 and S2 was expressed as the ratio of the MEP amplitude elicited by paired TMS (MEP_{S1+S2}) over the arithmetic sum of the MEP amplitudes produced by the corresponding single stimuli (MEP_{S1} and MEP_{S2}):

$$\text{Interaction} = MEP_{S1+S2}/(MEP_{S1} + MEP_{S2}).$$

Effects of DZP on the interactions between S1 and S2 at an ISI of 1.5 ms in the resting ADM

This experiment was conducted in 11 subjects. The paired-pulse TMS protocol was applied as above (nine intensity steps) at an ISI of 1.5 ms immediately prior to and 2 h after intake of a single oral dose of 20 mg DZP. The ISI of 1.5 ms was selected because it has been extensively explored in previous studies (Ziemann *et al.* 1998; Fisher *et al.* 2002; Hanajima *et al.* 2002). In particular, it was shown by replacement of one magnetic stimulus by anodal TES (Tokimura *et al.* 1996; Ziemann *et al.* 1998) and by epidural recordings of the descending corticospinal volley at the cervical spinal cord (Di Lazzaro *et al.* 1998*b*, 1999*b*) that the interactions between S1 and S2 most likely occur in the motor cortex.

Three subjects were excluded from the analysis of the DZP effects because they had already shown a significant depression of MEP amplitude to single-pulse TMS (MEP intensity curve), similar to previous findings (Boroojerdi *et al.* 2001). This, by itself, might affect the interaction between S1 and S2 (Kujirai *et al.* 1993). For the remaining eight subjects with stable MEP intensity curves, the interaction between S1 and S2 was calculated as above, separately for the pre- and post-DZP measurements. Furthermore, in order to provide a condition-by-condition comparison pre- *versus* post-DZP, each condition of S1 and S2 intensity was expressed for each subject as a weighted difference of the $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$ data:

$$(\text{Post} - \text{pre})/(\text{Post} + \text{pre}),$$

with possible values between −1 and +1. Negative values would indicate either more SICI or less SICF, depending on the value of $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$ prior to DZP intake.

As a control for the DZP experiment, four subjects were tested in an identical manner ('pre' and 'post' measurements, separated by 2 h of waiting), but without taking DZP.

Effects of DZP on the interactions between S1 and S2 at an ISI of 1.5 ms during contraction of the ADM and SMU recordings

The DZP experiment in the resting ADM was repeated in seven subjects during slight isometric contraction of the ADM (5–10 % of maximum contraction). Pauses were allowed whenever needed to avoid fatigue. Stimulus intensity was related to AMT. Otherwise, the experiment was conducted the same way as during muscle rest.

In addition to the analyses described above, the interaction between S1 and S2 was further tested by plotting the function $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ against time. To this end, the individual D-wave latency was determined by anodal TES using a Digitimer D185 electrical stimulator with a time constant of 50 μs. In each subject, $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ was then related to the individual D-wave latency of S2 which was assigned a time of zero. Finally, curves were averaged across subjects for each of the 81 conditions of S1 and S2 (Fig. 5). This approach allows the precise determination of the time course of the interactions between S1 and S2 relative to the D-wave. The onset of interaction was determined as the first consistent deviation of the $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ function away from zero. It is important to note that a valid interpretation of this analysis is possible in the active muscle only, and only at interaction onset. At this point voluntary muscle activation eliminates the requirement for temporal summation at the neuronal elements along the motor pathway to reach action potential threshold. However, only the fastest conducting corticospinal neurones are tested.

In order to verify the correctness of this analysis and its validity for a broader sample of neurones, recordings were made in three subjects from 12 SMU altogether. The anodal D-wave latency was determined for each SMU. In addition, three different paired-pulse TMS conditions were compared with the corresponding single-pulse conditions. The paired-pulse conditions were selected from the previous surface EMG data (see Fig. 3*A*). One condition tested SICI (S1 = 60 % AMT; S2 = 120 % AMT), the other two conditions tested SICF (S1 = 120 % AMT; S2 = 60 % AMT or S1 = 90 % AMT; S2 = 90 % AMT). The 60 % AMT single-pulse condition was usually not tested because it never resulted in any evoked SMU response. Conditional post-stimulus time histograms (PSTHs) were constructed from 100 trials. Conditions were applied in pseudorandom order. Trials with multiple-unit evoked responses were discarded on-line from analysis. The mean voluntary SMU firing rate during the period of 100 ms prior to stimulation was 7 s^{-1}. The timing of bins (bin width = 0.25 ms) in the PSTH was related to the anodal D-wave of S2 of the individual SMU which was assigned a value of zero. Bins were counted when they fell into an I-wave latency window. According to previous work (cf. Fig. 2 in Day *et al.* 1989), the windows of the I1-, I2- and I3-wave were set to 1.0–2.0, 2.5–3.5 and 4.0–5.5 ms, respectively, after the anodal D-wave. The interactions between S1 and S2 were analysed for each unit and SICI or SICF condition and separately for the I1-, I2- and I3-wave window by the weighted difference:

$$\text{Bin count (paired} - \text{single pulse)/bin count (paired} + \text{single pulse).}$$

Therefore, values between +1 and −1 are possible, with positive values indicating a facilitatory interaction.

Statistical procedures

The main measure of the present experiments was the interaction of S1 and S2 expressed as $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$. The effect of ISI was evaluated by counting those intensity conditions of S1 and S2 resulting in $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$ values below or above a given limit. These counts were compared across the four ISIs using Student's paired *t* test. The effects of DZP on RMT and AMT were analysed using Student's paired *t* test. The effects on the MEP intensity curve were tested with a repeated-measures two-way ANOVA with time ('pre' and 'post') and stimulus intensity (nine levels) as the within-subject factors. The effects of DZP on $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$ were analysed by counting conditions resulting in values below or above a given limit and subjecting these data to multiple *t* tests. Furthermore, for each of the 81 single conditions of S1 and S2 the weighted difference (post − pre)/(post + pre) was calculated for each subject and these data were tested against zero, using one-sample multiple *t* tests. The weighted difference SMU data were analysed according to paired-pulse condition and I-wave latency window by testing against zero, using one-sample multiple *t* tests. All multiple comparisons were corrected using Bonferroni's method. Statistical significance was assumed whenever $P < 0.05$.

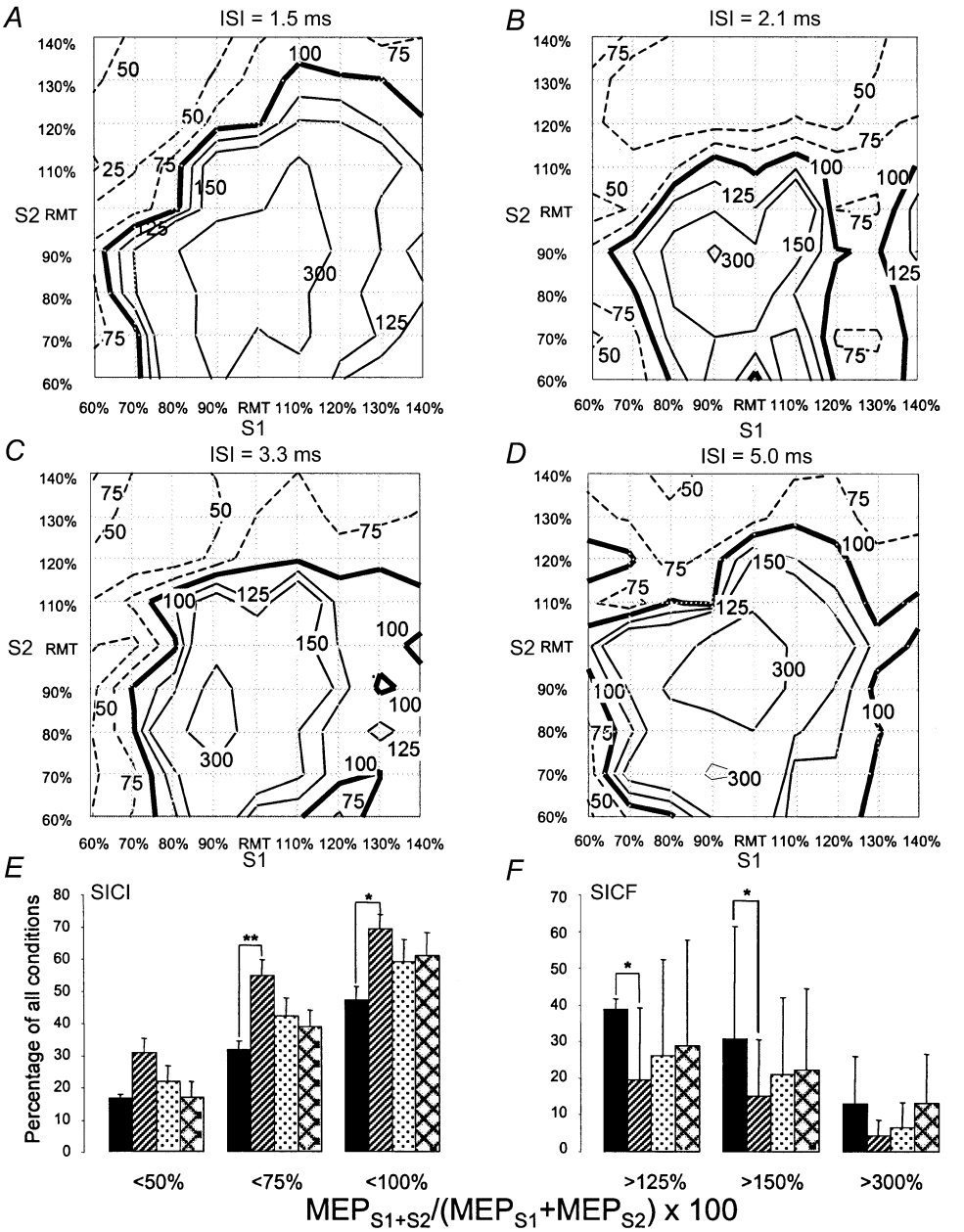

Figure 1. Short-interval paired-pulse inhibition and facilitation as a function of stimulus intensity and interstimulus interval in the resting ADM

A–D refer to interstimulus intervals (ISIs) of 1.5 (*A*), 2.1 (*B*), 3.3 (*C*) and 5.0 ms (*D*). In each diagram, stimulus intensity of the first stimulus (S1, *x*-axis) and the second stimulus (S2, *y*-axis) is related to resting motor threshold (RMT) of the abductor digiti minimi (ADM) muscle. As there are nine different stimulus intensities for S1 and S2, each diagram consists of 81 conditions. For each condition, the interaction between S1 and S2 was expressed as the percentage of ADM motor evoked potential (MEP) amplitudes produced by paired TMS (MEP_{S1+S2}) over the arithmetic sum of the MEP produced by the single stimuli (MEP_{S1} + MEP_{S2}). All data are means of six subjects and are given as contour plots. The thick continuous line in each diagram represents no interaction (100 %), dashed lines show inhibitory (< 100 %) and thin continuous lines facilitatory (> 100 %) interaction, the numbers indicate the contour line values. Note that the area of facilitation is much more extensive with the ISI of 1.5 ms compared with 2.1 ms. *E*, number of conditions (given as percentage of all 81 conditions, *y*-axis) below or above discrete interaction levels of (MEP_{S1+S2}/MEP_{S1} + MEP_{S2}) × 100 as indicated on the *x*-axis. The different bars refer to ISIs of 1.5 (black), 2.1

RESULTS

Effects of S1 and S2 intensity at different interstimulus intervals

All four ISIs resulted in an inhibitory interaction of S1 and S2, when S1 < RMT and S2 > RMT. This is in accord with previous paired-pulse experiments where S1 was typically set to around 80 % RMT and S2 to produce an unconditioned MEP of ~1 mV peak-to-peak amplitude ($S2_{1mV}$) (Kujirai *et al.* 1993). The comparison between the different ISI shows that the 'area' of SICI (i.e. the number of conditions with $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2}) \times 100 < 100$ %) was larger for ISI = 2.1 ms (Fig. 1*B*) compared to any of the other ISIs (Fig. 1*A* and *C* and *D*). This difference was statistically significant between the ISIs of 1.5 and 2.1 ms for levels of SICI < 100 and < 75 % (Fig. 1*E*, paired *t* test, corrected for multiple comparisons, $P < 0.0083$).

All ISIs resulted in a facilitatory interaction of S1 and S2, if S1 and S2 were approximately equal to RMT, in accord with previous findings (Tokimura *et al.* 1996). However, the 'area' of SICF (i.e. the number of conditions with $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2}) \times 100 > 100$ %) was larger for ISI = 1.5 ms (Fig. 1*A*) than for the other ISIs (Fig. 1*B–D*), 'expanding' to conditions with S1 > RMT and S2 < RMT. This is in line with previous reports (Ziemann *et al.* 1998; Hanajima *et al.* 2002). This difference was statistically significant between the ISI of 1.5 ms and 2.1 ms for levels of SICF > 125 and > 150 % (Fig. 1*E*, paired *t* tests, corrected for multiple comparisons, $P < 0.0083$).

RMT was not different across the different ISIs (1.5 ms, 45.0 ± 5.0 %; 2.1 ms, 45.2 ± 5.6 %; 3.3 ms, 44.0 ± 6.3 %; 5.0 ms, 44.5 ± 5.9 %; repeated-measures ANOVA, $P = 0.84$). Similarly, there was no difference of single-pulse MEP intensity curves (repeated-measures ANOVA, $P = 0.96$ (ISI), $P = 0.99$ (ISI × stimulus intensity)). These are important negative results because single-pulse MEP amplitude or TMS intensity may affect the interaction between S1 and S2 (Kujirai *et al.* 1993; Ziemann *et al.* 1996*c*).

Effects of DZP on the interactions between S1 and S2 at an ISI of 1.5 ms in the resting ADM

DZP resulted in a very slight but significant increase in RMT of, on average, 1.9 % of the maximum stimulator output (paired *t* test, $P = 0.001$; Fig. 2*A*). The MEP intensity curve with stimulus intensity adjusted to RMT remained unaffected (repeated-measures ANOVA, $F = 1.71$, $P = 0.232$ (drug); $F = 1.05$, $P = 0.411$ (drug × stimulus intensity); Fig. 2*B*). Furthermore, MEP onset latency was not affected by DZP (Fig. 2*C*). Therefore, alterations in single-pulse MEP amplitude and MEP onset latency cannot account for the effects of DZP on the interaction between S1 and S2 in the paired-pulse experiment (see below). The single-pulse results are not at variance with the depressive effects of the $GABA_A$ receptor agonist lorazepam on MEP amplitude in one previous report (Boroojerdi *et al.* 2001) because those subjects who showed a MEP depression ($n = 3$) were excluded from analysis (see Methods).

The main effects of DZP on the $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2})$ interaction were an increase of the 'area' of SICI and a decrease of the 'area' of SICF (Fig. 2*D–E*). This difference was significant for the levels of $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2}) \times 100$ < 75 %, < 100 % and > 125 % (Fig. 2*F*, paired *t* tests, corrected for multiple comparisons, $P < 0.0083$).

DZP resulted in weighted differences < −0.4 in two 'regions' of conditions of S1 and S2 (Fig. 2*G*). One such effect occurred with S1 = 60 % RMT and S2 = 130 % RMT, indicating a marked increase in SICI (Fig. 2*G*). The other occurred with S1 = RMT and S2 = 80–90 % RMT, or S1 = 70–80 % RMT and S2 = RMT (Fig. 2*G*), indicating a marked decrease of SICF (Fig. 2*G*). The asterisks in Fig. 2*G* point to interactions between S1 and S2 that were significantly different from zero (one-sample *t* tests corrected for multiple comparisons, $P < 0.0006$).

If those conditions of S1 and S2 were selected as defined in previously published paired-pulse TMS protocols (Kujirai *et al.* 1993; Tokimura *et al.* 1996; Ziemann *et al.* 1996*c*; see also Introduction), only the 'Ziemann' protocol revealed the depressive effect of DZP (Fig. 2*H*; paired *t* test, $t = 3.398$, $P = 0.011$), while the 'Kujirai' and 'Tokimura' protocols were suggestive of more SICI and less SICF, respectively, but the differences did not reach statistical significance (Fig. 2*H*, $P > 0.05$).

The control experiment (2 h waiting without DZP) showed no differences in the single-pulse and paired-pulse measures between baseline and after 2 h of waiting (data not shown). This suggests that the alterations of SICI and SICF under DZP were due to a specific drug effect.

Effects of DZP on the interactions between S1 and S2 at an ISI of 1.5 ms in the active ADM

During slight isometric contraction, SICI and SICF were expressed very similarly as during muscle rest (Figs 2*D*,

(hatched), 3.3 (stippled) and 5.0 ms (cross-hatched). Error bars are S.E.M. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$; *t* tests corrected for multiple comparisons). Note that the ISI of 1.5 ms resulted in less short-interval intracortical inhibition (SICI) and more facilitation (SICF) compared with the ISI of 2.1 ms.

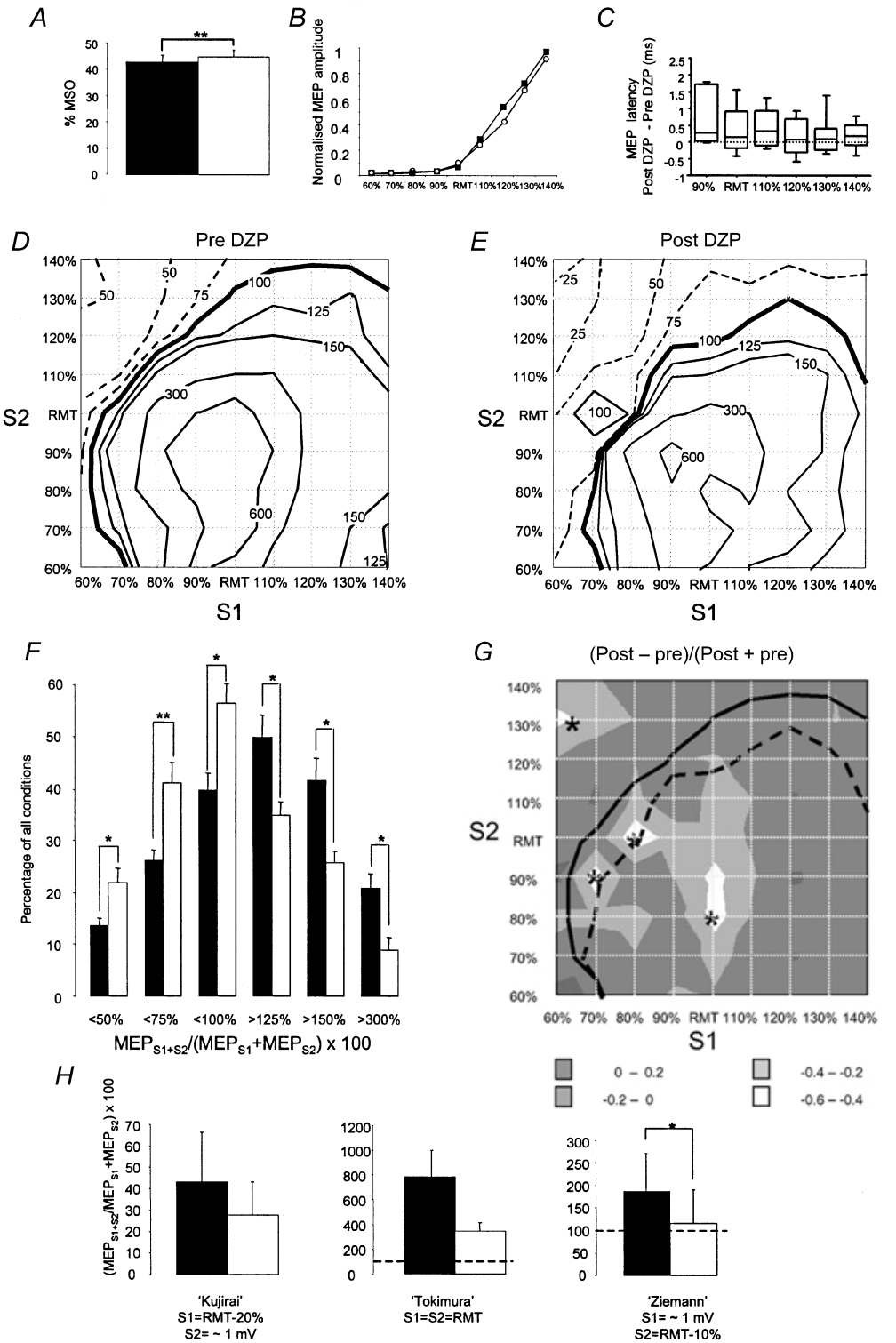

Figure 2. Effects of diazepam (DZP) on single-pulse and paired-pulse TMS measures of motor excitability in the resting ADM

All measures were taken immediately before and 2 h after a single oral dose of 20 mg of DZP and are means from eight subjects. *A*, resting motor threshold expressed as percentage of the maximum stimulator output (%MSO, *y*-axis) before (■) and after DZP (□). $^{**}P < 0.01$ (paired *t* test). *B*, motor evoked potential (MEP) intensity curves before (■) and after DZP (○). Stimulus intensity of the single TMS pulse was related to resting motor threshold (RMT, *x*-axis). MEP amplitudes are normalised for each subject to the maximum MEP before DZP, which was assigned a value of 1. *C*, changes of MEP onset latency, calculated as latency differences 'Post – pre' DZP (in ms). For the intensity of RMT – 10 %, the data are based on only five subjects because the other subjects had no visible MEP at this subthreshold intensity. *D* and *E*, contour plots

3*D*, 4*A* and 4*B*). Only the highest level of SICF > 300 % was reduced during muscle contraction, suggesting a saturation effect during muscle contraction. The $MEP_{S1+S2}/(MEP_{S1} + MEP_{S2}) \times 100 = 100\,\%$ contour line, which marks the border between SICI and SICF was not shifted by contraction (Fig. 4*B*).

DZP had no significant effect either on the single-pulse measures of motor excitability (AMT, MEP intensity curve, MEP onset latency, Fig. 3*A*–*C*), or on SICI and SICF (Fig. 3*D*–*G*). Neither were the selected paired-pulse conditions as defined in the previous protocols (Kujirai *et al.* 1993; Tokimura *et al.* 1996; Ziemann *et al.* 1996*c*) affected by DZP (Fig. 3*H*).

Time course of the interactions between S1 and S2 at an ISI of 1.5 ms in the active ADM

The time course of the function $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ was plotted relative to the anodal D-wave latency of S2 for each of the 81 conditions of S1 and S2 (Fig. 5). Consistently, the onset of SICI coincided with the I3-wave of S2 (Fig. 5, dark red bands). Only in a few exceptions, at high intensities of S2, the onset of SICI occurred at the I2-wave latency of S2 (Fig. 5, light red bands). In contrast, the onset of SICF occurred consistently at the I2-wave latency of S2 (Fig. 5, dark blue bands), and in a few conditions even at the I1-wave latency of S2 (Fig. 5, light blue bands).

The SMU recordings confirmed these findings. The representative SMU in Fig. 6*A*–*C* showed a suppression of the I3-wave of S2 in a condition testing SICI (S1 = 60 % AMT; S2 = 120 % AMT, Fig. 6*A*). In contrast, the same SMU displayed facilitation coincident with the I2-wave of S2 in conditions testing SICF (S1 = 120 % AMT; S2 = 60 % AMT; Fig. 6*B*; S1 = 90 % AMT; S2 = 90 % AMT; Fig. 6*C*). Accordingly, the analysis of all 12 SMUs demonstrated a significant inhibition of the I3-wave of S2 in the 'Kujirai' SICI condition, and significant facilitation coincident with the I2-wave of S2 in the 'Ziemann' and 'Tokimura' SICF conditions (Fig. 6*D*). Furthermore, there was a trend for a facilitation coincident with the I1-wave of S2 ($P = 0.03$, not significant after correction for multiple comparisons) in the Ziemann condition. Finally, the I1-wave of S2 remained completely unaffected in the Kujirai and Tokimura conditions (Fig. 6*D*).

DISCUSSION

Effects of S1 and S2 intensity at different interstimulus intervals

Systematic variation of the intensities of S1 and S2 led to consistent patterns of paired-pulse interactions. If the intensities of S1 and S2 were approximately equal to RMT, SICF occurred, in line with one previous paired-pulse TMS study (Tokimura *et al.* 1996). In addition, SICF was obtained if S1 > RMT and S2 < RMT, but only with an ISI of 1.5 ms. This facilitation was absent or expressed to a much lesser extent with ISIs of 2.1, 3.3 and 5.0 ms. This is in accordance with one previous report which demonstrated SICF between a suprathreshold S1 and a subthreshold S2 only at discrete ISIs of 1.1–1.5, 2.3–2.9 and 4.1–4.4 ms (Ziemann *et al.* 1998). SICI was obtained if S1 < RMT and S2 > RMT, in agreement with previous findings (Kujirai *et al.* 1993; Ziemann *et al.* 1996*c*). SICI was most pronounced with an ISI of 2.1 ms. At this interval, SICI 'expanded' to conditions with S1 > RMT and S2 > RMT. The stronger SICI with an ISI of 2.1 ms compared with the other intervals may have been predicted from one recent study which showed, by using a threshold tracking technique, that intervals of 1.0 and 2.2–2.6 ms were particularly effective in producing SICI while intervals of 1.5 and 3.0–4.5 ms were much less effective (Fisher *et al.* 2002).

Site of the interaction between S1 and S2

The site of the paired-pulse interactions was not specifically tested in the present experiments because there

(average from 8 subjects) of the interaction between the first (S1) and second stimulus (S2) expressed as $MEP_{S1+S2}/MEP_{S1} + MEP_{S2} \times 100$ pre- (*D*) and post-DZP (*E*). The interstimulus interval was 1.5 ms. Conventions for the contour plots are the same as in Fig. 1*A*. *F*, number of conditions (given as percentage of all 81 conditions, *y*-axis) below or above discrete interaction levels of $MEP_{S1+S2}/MEP_{S1} + MEP_{S2} \times 100$ as indicated on the *x*-axis. ■ and □, pre- and post-DZP, respectively. Error bars are S.E.M. $^*P < 0.0083$, $^{**}P < 0.001$; *t* tests corrected for multiple comparisons. *G*, the data in the contour plots in *D* and *E* are shown as weighted differences (post-DZP − pre-DZP)/(post-DZP + pre-DZP). Accordingly, values between −1 and +1 are possible. Negative values indicate either an increase of short-interval intracortical inhibition (SICI), or a decrease of facilitation (SICF), depending on the value of $(MEP_{S1+S2}/MEP_{S1} + MEP_{S2} \times 100$ pre-DZP. The grey-shaded areas refer to the different ranges of weighted difference values as given in the inset. The asterisks indicate conditions that were statistically significantly different from zero ($P < 0.0006$, one-sample *t* tests, corrected for multiple comparisons). The continuous and dashed lines are the $(MEP_{S1+S2}/MEP_{S1} + MEP_{S2}) \times 100 = 100\,\%$ contour lines pre- and post-DZP from *D* and *E*, respectively, to delineate SICI and SICF 'areas'. Note that DZP led to increase of SICI and reduction of SICF. *H*, short-interval (1.5 ms) paired-pulse intracortical inhibition (SICI) according to the 'Kujirai protocol' (left diagram) and short-interval paired-pulse intracortical facilitation (SICF) according to the 'Tokimura protocol' (middle diagram) and the 'Ziemann protocol' (right diagram) pre- (■) and post DZP (□). The particular conditions of the first (S1) and second stimulus (S2) in the different protocols are given below each diagram. Error bars indicate S.E.M. $^*P < 0.05$ (paired *t* test).

exists already a wealth of consistent evidence from previous studies for placing the site of this interaction into the motor cortex. When either the magnetic S1 or magnetic S2, or both stimuli were substituted by anodal TES, SICI (Kujirai *et al.* 1993) and SICF (Tokimura *et al.* 1996; Ziemann *et al.* 1998) disappeared. The implication is that anodal TES activates the corticospinal system preferentially directly at the proximal corticospinal axon, some nodes of Ranvier distant to the cell body, and therefore is resistant to changes in excitability of the corticospinal neurone (Di Lazzaro *et al.* 1999*a*). Even stronger evidence in favour of a cortical site of the paired-pulse interactions came from epidural cervical spinal cord recordings of the descending corticospinal volley elicited

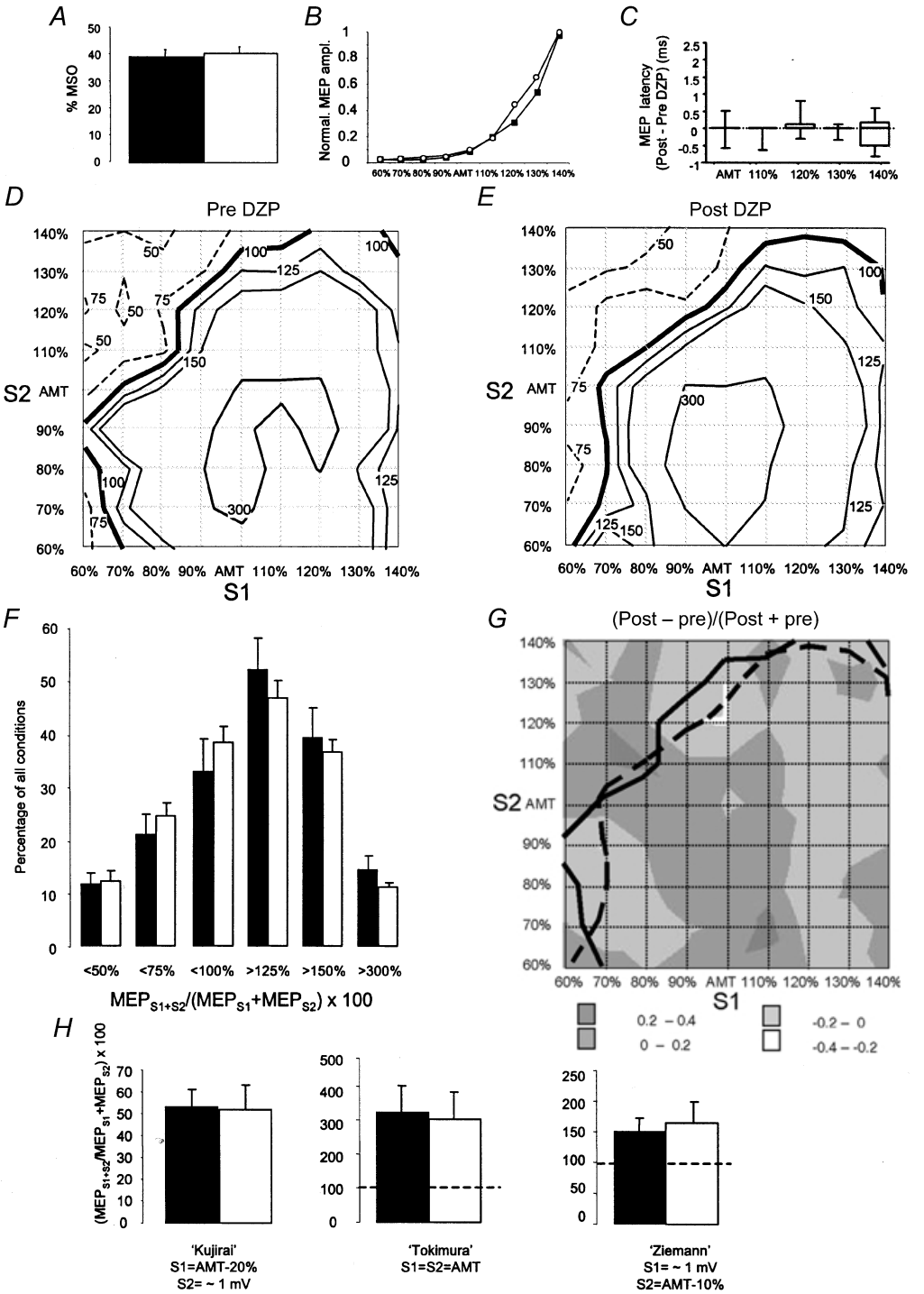

Figure 3. Effects of diazepam (DZP) on single-pulse and paired-pulse TMS measures of motor excitability in the active ADM

Conventions and arrangement are the same as in Fig. 2. Note that DZP no longer produced significant effects during slight isometric contraction of the ADM.

by TMS of the hand area of motor cortex. These recordings showed that SICI and SICF were associated with a marked decrease or increase, respectively, of the I2-wave and later I-waves, while the I1-wave remained unaffected (Di Lazzaro *et al.* 1998*b*, 1999*b*). Still, it cannot be fully excluded that some interaction between S1 and S2 in the present experiments took place at a subcortical or even spinal level, in particular when both stimuli were above motor threshold.

Effects of DZP and voluntary contraction on the interactions between S1 and S2 at an ISI of 1.5 ms

SICI and SICF remained largely unaffected by voluntary contraction, with the exception of the highest SICF values which were reduced by contraction (Figs 2*D*, 3*D* and 4). At first sight, the SICI data are at variance with one previous study which showed a reduction of SICI during contraction (Ridding *et al.* 1995). However, a close look reveals that there was a slight (non-significant) trend toward less SICI during contraction also in the present experiments (e.g. 42 *vs.* 52 % SICI in the 'Kujirai' condition during rest *vs.* contraction, Figs 2*H* and 3*H*). Reduction of the highest SICF values during contraction (S1 and S2 approximately equal to motor threshold) is consistent with previous findings (Tokimura *et al.* 1996).

The small effects of voluntary contraction on SICI and SICF suggest that the intracortical pathways mediating the voluntary drive and the paired-pulse interaction are largely independent and converge only at a distal point such as the corticospinal neurone (Fig. 7). Epidural recordings of the corticospinal volley showed that voluntary contraction increased the size and number of I-waves (Di Lazzaro *et al.* 1998*a*). This suggested an increased excitability of the corticospinal neurone during contraction. If one assumes a fixed range of excitabilities of the corticospinal neurone, voluntary contraction would shift the excitability toward the maximum excitability. SICI could then still be transmitted without much alteration while the highest values of SICF would saturate.

Systematic variation of S1 and S2 intensity showed that the system underlying SICI can be activated by very low-intensity S1 at $\leqslant$ 60 % AMT (Figs 1, 2*D*, 3*D* and 5). In contrast, there was no indication that S1 at intensities of 60–70 % RMT or AMT was capable of producing any facilitatory interaction. This confirms that the lowest threshold system activated by TMS in the hand area of human motor cortex is inhibitory (Davey *et al.* 1994; Ziemann *et al.* 1996*c*; Awiszus *et al.* 1999). In the resting ADM, DZP significantly increased SICI, in particular with conditions of very low-intensity S1 (60–70 % RMT, Fig. 2*D*, *E* and *G*). These are non-optimal for producing SICI (Kujirai *et al.* 1993; Fisher *et al.* 2002), and therefore escape a possible 'floor effect' which may have been the reason for the lack of effects of benzodiazepines on SICI in previous experiments (Inghilleri *et al.* 1996; Ziemann *et al.* 1996*a*). The increase of SICI by DZP in the present

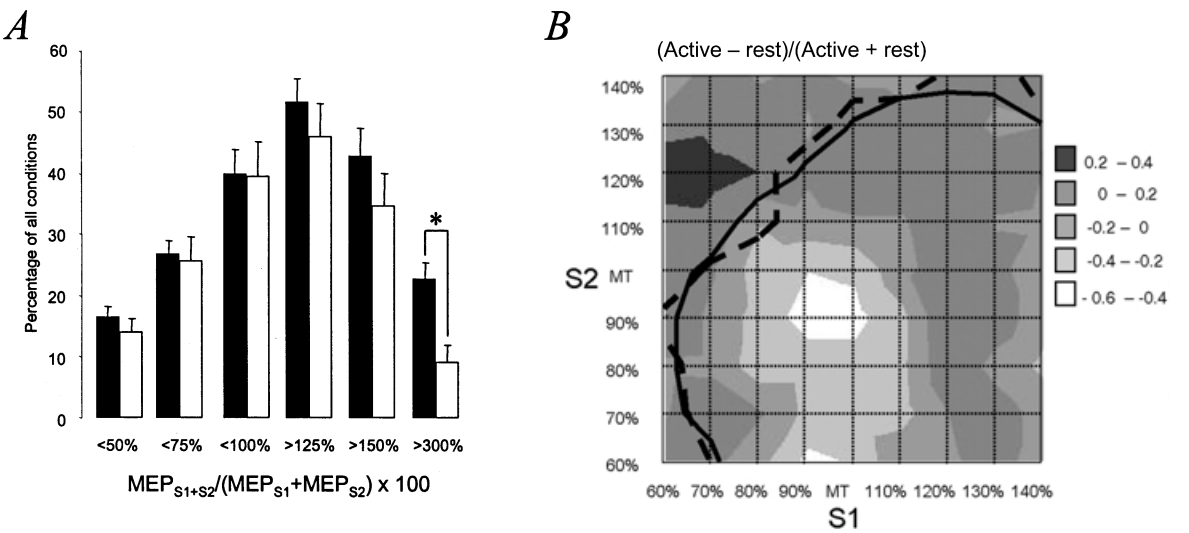

Figure 4. Effects of voluntary ADM contraction compared to rest on paired-pulse TMS measures of motor excitability

A, number of conditions (given as percentage of all 81 conditions, *y*-axis) below or above discrete interaction levels of (MEP$_{S1+S2}$/MEP$_{S1}$ + MEP$_{S2}$) × 100 as indicated on the *x*-axis. ■ and □, resting and active ADM before DZP intake, respectively. Data are from the seven subjects who participated in both experiments. Error bars indicate S.E.M. Note that voluntary contraction did not affect SICI but resulted in a significant decrease in the highest SICF level. *$P < 0.01$. *B*, mean weighted differences ($n = 7$ subjects) of each condition of S1 and S2 comparing the paired-pulse interaction (MEP$_{S1+S2}$/MEP$_{S1}$ + MEP$_{S2}$) in the active and resting ADM. The continuous and dashed lines are the (MEP$_{S1+S2}$/MEP$_{S1}$ + MEP$_{S2}$) × 100 = 100 % contour lines at rest and during contraction, respectively, to delineate SICI and SICF 'areas'. Note that contraction decreased the highest values of SICF but did not result in a shift of the 100 % contour line.

experiments confirms the early notion that SICI is mediated by the $GABA_A$ receptor (Kujirai *et al.* 1993; Ziemann *et al.* 1996*b*). During isometric contraction, DZP completely lost its effect on SICI (Fig. 3*D*–*H*) although contraction by itself prior to DZP intake did not significantly affect SICI (see above). The most parsimonious explanation for this dissociation is that voluntary contraction is capable of modifying the $GABA_A$ receptor of corticospinal neurones by reducing its sensitivity to regulation by benzodiazepines while its sensitivity to GABA is maintained. A similar rapidly developing dissociation was described in rat hippocampal dentate granule cell $GABA_A$ receptors during epileptic seizures (Kapur & Macdonald, 1997).

Systematic variation of S1 and S2 also showed that the excitatory system mediating SICF comes into play only if the intensity of S1 is ⩾ 80 % RMT or AMT (Figs 1, 2*D*, 3*D* and 5), similar to previous observations (Awiszus *et al.* 1999). This suggests the existence of, at least, two largely independent systems which may converge at the corticospinal neurone, the low-threshold inhibitory system and a high-threshold excitatory system (Fig. 7). Facilitation in the excitatory system must have the potential to 'overrule' the inhibitory system. Otherwise, SICF would not be possible. SICF was strongly reduced by DZP in the resting ADM (Fig. 2*D*–*H*). This was an expected finding because S1 always activates the inhibitory system (which is enhanced by DZP) in parallel with the

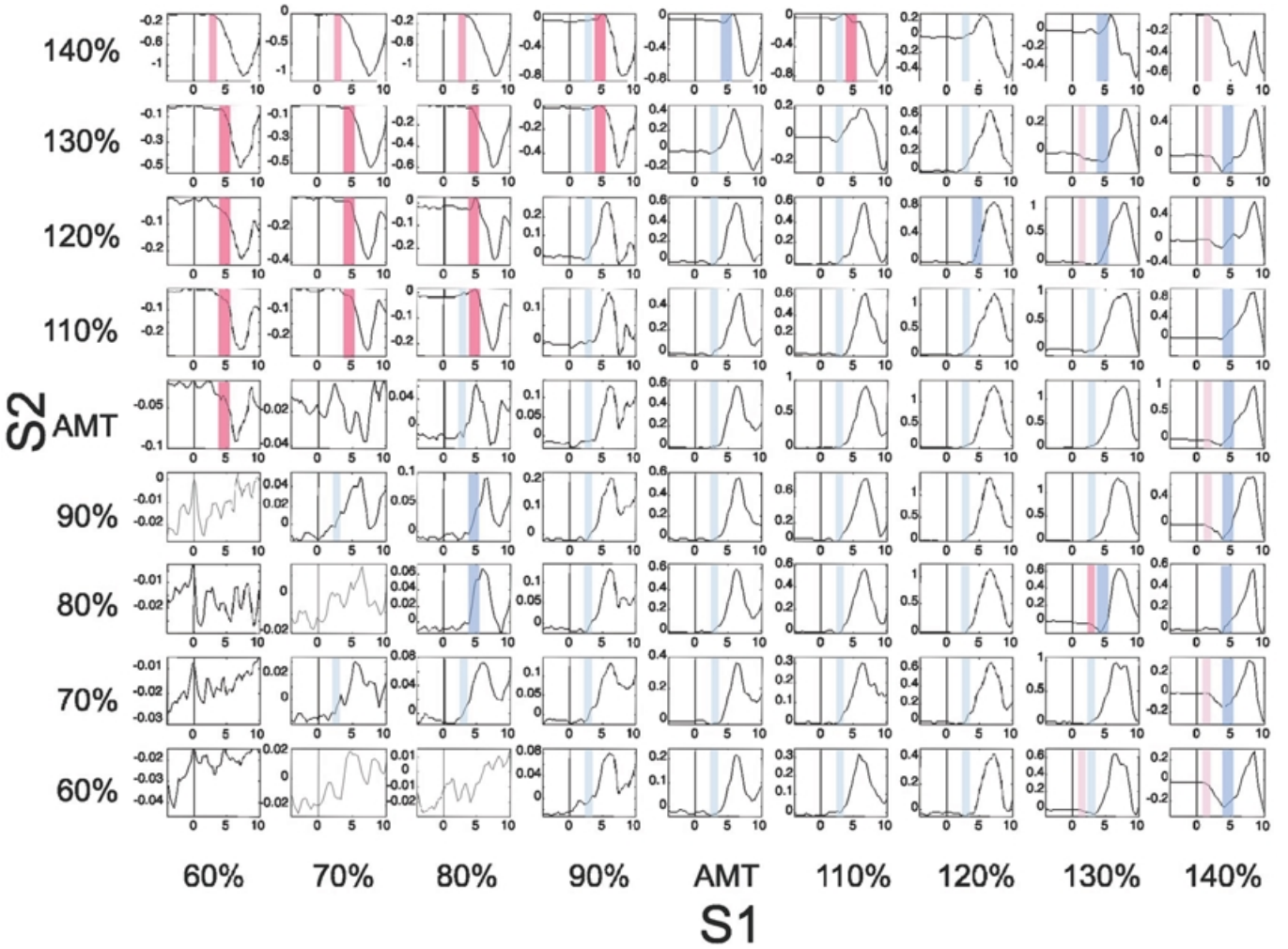

Figure 5. Time course of the interaction $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ during slight isometric contraction of the ADM

The intensities of S1 relative to AMT are given on the *x*-axis, those of S2 on the *y*-axis. One diagram was constructed for each of the 81 conditions of S1 and S2 intensity. Each diagram displays the time course of the mean $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ (in mV) from seven subjects. Before averaging, each individual curve was related to the individual anodal D wave latency of S2 in the active ADM which was assigned a time of zero. The onset of a clear deviation of the $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ curve away from zero was marked by a box. Three boxes with fixed different timings 1.0–2.0, 2.5–3.5 and 4–5.5 ms after the D-wave latency of S2 were used, in order to indicate an onset of the interaction between S1 and S2 falling into the range of the I1, I2 or I3 latency of S2. Note, that the onset of SICI (indicated by red boxes) was mainly during the I3-wave of S2. In contrast, the onset of SICF (blue boxes) was mainly during the I2-wave of S2.

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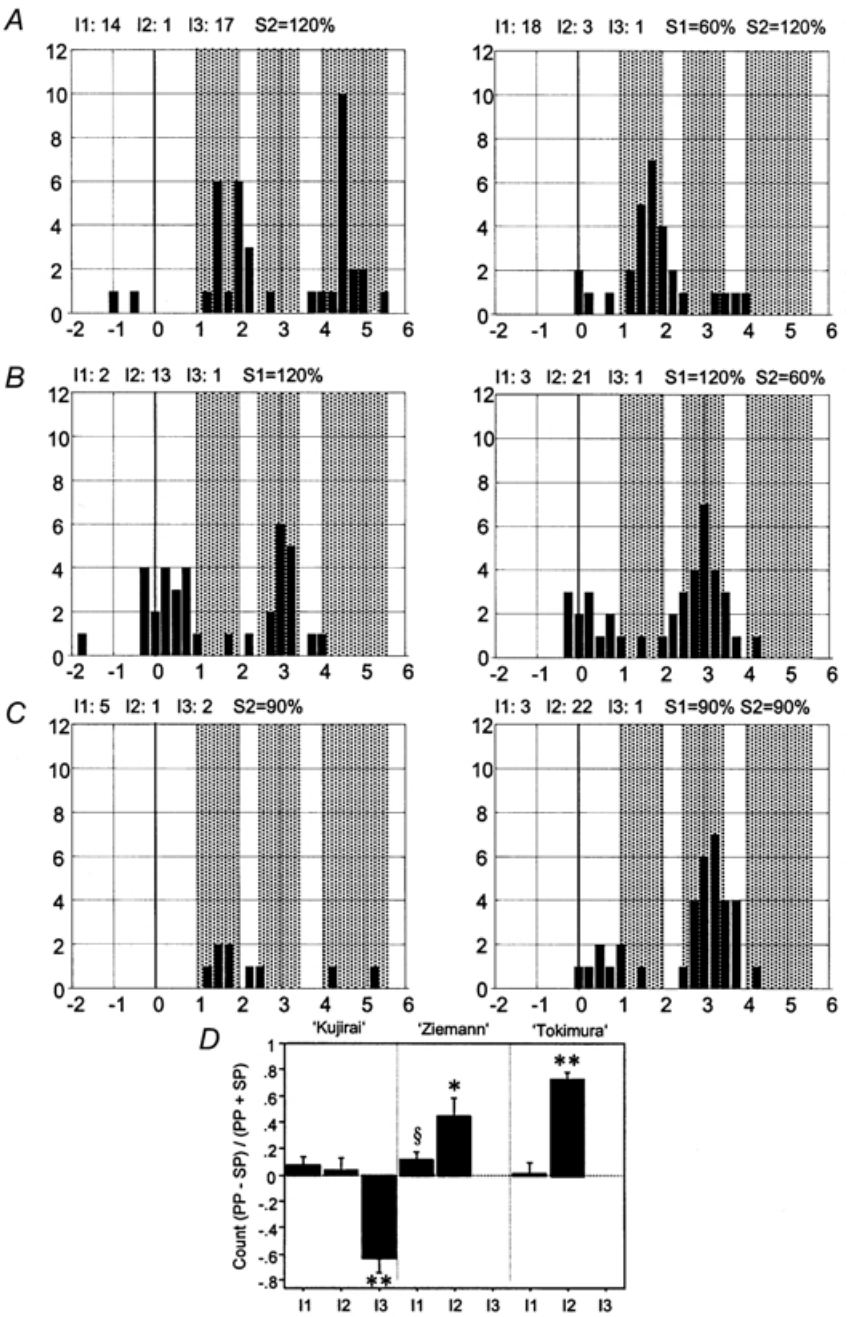

Figure 6. Interaction between S1 and S2 in SMU recordings

A–C, one representative SMU tested for SICI (*A*, S1 = 60 % AMT; S2 = 120 % AMT), and SICF (*B*, S1 = 120 % AMT; S2 = 60 % AMT; *C*, S1 = 90 % AMT; S2 = 90 % AMT). Each PSTH (bin width = 0.25 ms) was constructed from 100 trials. Time zero refers to the anodal D-wave latency of S2 of this unit. The left PSTHs refer to single-pulse stimulation by the higher intensity pulse, the right PSTHs to the corresponding paired-pulse stimulation. The dotted boxes indicate intervals of 1.0–2.0, 2.5–3.5 and 4–5.5 ms after the D-wave which refer to the range of I1-, I2- and I3-wave latencies of S2, respectively. Counts of SMU discharge in the range of the three I-wave latencies are given above each diagram. Note that SICI (*A*) was produced by a complete inhibition of the I3-peak of S2, whereas SICF (*B* and *C*) resulted from a facilitation coincident with the I2 latency of S2. *D*, summary display of the data from all 12 SMU. The interaction of S1 and S2 is expressed as the weighted difference between the paired-pulse (PP) and single-pulse (SP) conditions on the *y*-axis. Note that SICI (Kujrai protocol) resulted consistently from a depression coincident with the I3-wave of S2. In contrast, SICF (Ziemann and Tokimura protocols) resulted consistently from a facilitation coincident with the I2-wave of S2. §$P = 0.03$ (non-significant after correction for multiple comparisons); *$P < 0.015$; **$P < 0.001$.

excitatory system. The complete lack of effect of DZP during isometric ADM contraction (Fig. 3*D–H*) confirms previous recordings of the descending corticospinal volley from the cervical spinal cord (Di Lazzaro *et al.* 2000) and is consistent with the idea (see above) that voluntary contraction decreases the sensitivity of the $GABA_A$ receptor to benzodiazepine regulation.

Time course of the interactions between S1 and S2 at an ISI of 1.5 ms in the active ADM

We have proposed here a novel way to analyse the time course of paired-pulse interactions during isometric contraction of the target muscle by using the surface EMG derived $MEP_{S1+S2} - (MEP_{S1} + MEP_{S2})$ curve related to the anodal D-wave. The results of this analysis were fully compatible with the SMU data (Figs 5 and 6). The analysis based on surface EMG data has, however, the advantage that a larger range of stimulus intensities can be tested which would result in multiple-unit responses in SMU recordings.

The onset of SICI coincided with the I3-wave of S2, while the I1-wave was never affected (Figs 5 and 6). Sometimes, SICI started already at the I2-wave of S2, but this effect was small and inconsistent (Fig. 5). These findings are compatible with previous epidural recordings of the

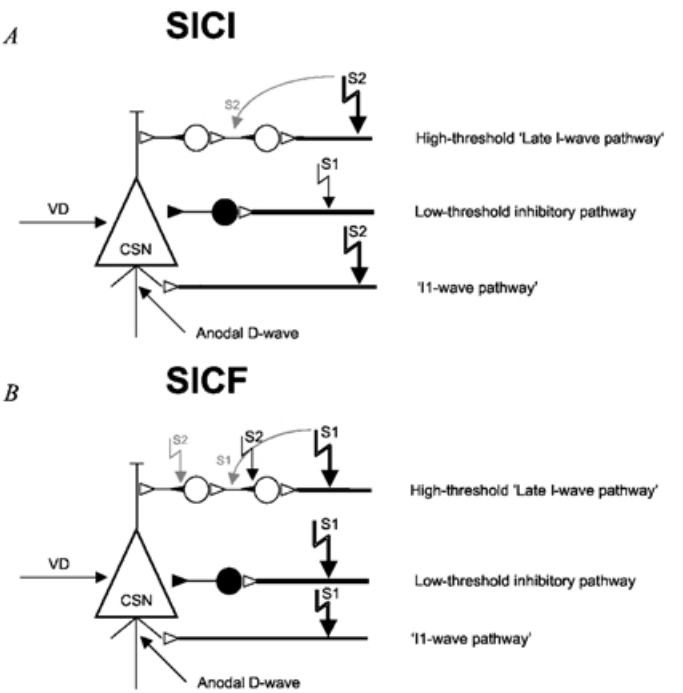

Figure 7. Connectivity model to explain SICI and SICF

The connectivity model is derived from Fig. 4 in Amassian *et al.* (1987). The model is a gross simplification of nature but it is sufficient to explain all experimental data. It assumes that there exists one low-threshold inhibitory pathway, and high-threshold excitatory 'I1- and late I-wave pathways'. CSN, corticospinal neurone; VD, voluntary drive. ● denotes a GABAergic inhibitory interneurone, the ○s are excitatory interneurones. *A*, for SICI, a low-intensity S1 (indicated by the small filled arrow) and a high-intensity S2 (indicated by the large filled arrow) are used. S1 only activates the low-threshold inhibitory pathway. S2 given 1.5 ms after S1 only activates the I1- and late I-wave pathways, while the low-intensity pathway is refractory. The IPSP and EPSP from the inhibitory pathway and the 'late I-wave pathway' summate at the CSN at a delay of three I-wave intervals relative to the anodal D-wave latency. In some instances, S2 may activate the axon of the second-order interneurone, in particular if high intensity is used (indicated by the grey curved arrow). In this case, the EPSP from S2 would interact with the IPSP from the inhibitory pathway at the SCN two I-wave intervals later than the anodal D-wave latency. *B*, for SICF, a high-intensity S1 and a low-intensity S2 are used. S1 activates all pathways. S2 cannot activate any axon due to refractoriness. However, the initial axon segment of the second-order interneurone in the 'late I-wave pathway' (indicated by the small filled triangle adjacent to the cell soma) is hyperexcitable due to the EPSP from S1 and can be excited directly by S2. Therefore, the site of excitation by S2 'jumps up' by one I-wave latency, and the facilitatory interaction between S1 and S2 lags the anodal D-wave latency by only two I-wave intervals. In some instances, S1 may activate in addition the axon of some second-order interneurones (indicated by the grey curved arrow). In this case, the initial axon segment of first-order interneurones is hyperexcitable due to the EPSP from S1 and can be excited by S2. The facilitatory interaction between S1 and S2 would then lag the anodal D-wave latency by only one I-wave interval.

descending corticospinal volley which showed that the I1-wave was never inhibited and the I2-wave was only inhibited at an ISI of 1 ms, but not at ISI ⩾ 2 ms. The sparing of the I1-wave is consistent with several other observations which also showed that this wave is much less sensitive to modulation than later I-waves (Nakamura *et al.* 1997; Hanajima *et al.* 1998; Tokimura *et al.* 2000). These dissociated effects are best explained by segregated pathways for the I1-wave and the later I-waves, with very direct access of the 'I1-wave pathway' to proximal parts of the corticospinal neurone (Amassian *et al.* 1987; Sakai *et al.* 1997; Fig. 7). In contrast to SICI, the onset of SICF typically coincided with the I2-latency of S2, and in some instances even with the I1-latency of S2 (Figs 5 and 6). This is entirely consistent with recent SMU data (Hanajima *et al.* 2002). Those authors managed, by variation of the coil orientation, to elicit selectively an I1- or I3-wave in a given SMU. Paired-pulse TMS using a suprathreshold S1 and a subthreshold S2 at an ISI of 1.5 ms resulted in a facilitation of the I3-wave of S1 (coincident with the I2-wave of S2), and no effect on the I1-wave of S1 but the appearance of an I2-peak (coincident with the I1-wave of S2).

Mechanisms of SICI and SICF

The major question is by which mechanisms SICI and SICF occur. A crucial argument can be based on the observation of the 'jumping up' of SICF by one or even two I-wave latencies compared to SICI, even if a fixed intensity of S2 was used (e.g. S2 = 120 % in Fig. 5).

One elegant way to explain this jumping up would be to assume that: (1) SICI occurs through an interaction at the corticospinal neurone by summation of IPSP mediated by the low-threshold inhibitory pathway and EPSP mediated by the higher-threshold excitatory 'late I-wave pathway' (Fig. 7*A*). Low-intensity S1 will activate the low-threshold inhibitory pathway only, and higher-intensity S2 will activate the high-threshold 'late I-wave pathway' only. S2 will not activate the low-threshold inhibitory pathway because, according to the low intensity of S1 required to obtain optimal SICI (Kujirai *et al.* 1993; Ziemann *et al.* 1996*c*; Fisher *et al.* 2002), S1 had most probably already activated most or all parts of this pathway which is then refractory for subsequent S2 activation at an interval of 1.5 ms (Amassian *et al.* 1998). The onset of SICI coincides with the conduction time in the excitatory late I-wave pathway, which is approximately three I-wave latencies later than the anodal D-wave of S2; (2) SICF occurs due to an interaction of S1 and S2 along the high-threshold excitatory late I-wave pathway (Fig. 7*B*). In contrast to the pure SICI situation, the higher-intensity S1 will now activate at least some axons of the late I-wave pathway. If S2 is given 1.5 ms after S1, and the intensity of S1 ⩾ S2, then S2 cannot excite any axon, due to the refractory period of corticocortical axons (Amassian *et al.* 1998). However, S2 may directly excite the initial axon segment of those excitatory interneurones which had received an EPSP from S1, and therefore, may be hyperexcitable at the time of S2 (Amassian *et al.* 1990; Deletis *et al.* 2001; Fig. 7*B*). This model explains why the onset of SICF jumps up by one I-wave latency compared with SICI because S2 activates hyperexcitable interneurones one I-wave latency ahead of the site of excitation when S2 is given alone.

Several properties of SICF can be predicted from this model. Evidence from previous studies suggested that the neuronal time constant of the initial axon segment is probably very short. Chronaxies of about 300 μs were reported for interneurones in rat visual cortex (Nowak & Bullier, 1998) and of 60–130 μs in intrinsic collaterals of pyramidal tract cells in cat motor cortex (Asanuma *et al.* 1976). If values of approximately 300 μs were correct for the axons of interneurones in the late I-wave pathway, then shifting the ISI between S1 and S2 away from the I-wave interval (~1.5 ms) should result in a significant reduction or even lack of SICF. S2 would hit initial axon segments which were no longer hyperexcitable due to the rapid decay of the EPSP at the initial axon segment. Indeed, previous studies showed that SICF occurred only at discrete ISI which are approximately multiples of the I-wave interval (Tokimura *et al.* 1996; Ziemann *et al.* 1998). ISI intermediate between I-wave intervals should even result in SICI for many conditions of S1 and S2 because of the lack of facilitatory interaction along the late I-wave pathway. This was demonstrated here for the ISI of 2.1 ms (Fig. 1*B*).

In conclusion, the present experiments showed that the interactions between the effects of the first and second stimulus of paired TMS at an ISI of 1.5 ms may be inhibitory or facilitatory, depending on the intensities of the two stimuli. Examining the exact onset of the interactions between the first and second pulse relative to the anodal D-wave latency by using SMU recordings and a novel analysis of the surface EMG supported the view that the inhibition most likely occurred through summation at the corticospinal neurone of IPSP elicited by the first pulse mediated through a low-threshold $GABA_A$ receptor dependent inhibitory pathway, and EPSP elicited by the second pulse mediated through a high-threshold excitatory late I-wave pathway. In contrast, facilitation originated mainly non-synaptically through direct excitation of the axon initial segment of interneurones along the late I-wave pathway by the second pulse which were made hyperexcitable through EPSP by the first pulse. Using the dimension of stimulus intensity in paired-pulse TMS may help to advance our understanding of disordered paired-pulse cortical excitability in neurological diseases such as epilepsy or movement disorders which had exhibited a rather unspecific deficiency of SICI in previous conventional paired-pulse TMS protocols (Ziemann, 1999).

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