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Intracortical Inhibition and Facilitation in Paired-Pulse Transcranial Magnetic Stimulation: Effect of Conditioning Stimulus Intensity on Sizes and Latencies of Motor Evoked Potentials

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Summary: The influence of the intensity of the conditioning stimulus on intracortical inhibition (ICI) and intracortical facilitation (ICF) was assessed in a study using paired-pulse transcranial magnetic stimulation. Interstimulus intervals (ISIs) between conditioning and test stimuli were 3 msec and 13 msec. Latencies and areas of motor evoked potentials in response to the test stimulus were measured in the right extensor carpi radialis muscle. Motor evoked potential areas with ISIs of 3 msec and 13 msec showed a different dependence on the intensity of the conditioning stimulus. In contrast, the changes of motor evoked potential latencies were fairly similar with both ISIs. The findings point to a parallel action of ICI and ICF. Furthermore, the latencies seem to be a more sensitive indicator for ICF action than the size parameters of motor evoked potentials. **Key Words:** Transcranial magnetic stimulation—Motor evoked potential—Intracortical inhibition—Intracortical facilitation.

Paired-pulse transcranial magnetic stimulation (TMS) is an appropriate technique to study mechanisms of intracortical inhibition (ICI) and intracortical facilitation (ICF) in humans (Kujirai et al., 1993; Rothwell et al., 1991; Wohlfarth et al., 2000). Paired-pulse techniques have been used for clinical studies (e.g., to examine pharmacologic effects on motor cortex excitability [Wohlfarth et al., 2000]). In normal subjects, motor evoked potentials (MEPs) are reduced when a test magnetic stimulus follows a conditioning stimulus of subthreshold intensity with short (1 to 5-msec) interstimulus interval (ISIs), and they are augmented with longer ISIs (10 to 25 msec). There is evidence for the cortical origin of ICI (Di Lazzaro et al., 1998; Kujirai et al., 1993; Nakamura et al., 1997; Ziemann et al., 1996b) and for the involvement of γ-aminobutyric acid-ergic interneurons (Ziemann et al., 1997). Less is known for ICF seen with longer ISIs, although the underlying mechanisms may also be mainly cortical (Nakamura et al., 1997; Ziemann et al., 1996b). It has been suggested that ICI and ICF are mediated by separate circuits converging on a common neuronal pool—for example, the pyramidal motor neurons (Ashby et al., 1999; Ziemann et al., 1996b). Paired-pulse TMS has also been used to study pathophysiologic mechanisms of central motor disorders (Berardelli et al., 1996; Brown et al., 1996; Hanajima et al., 1996; Ridding et al., 1995; Valzania et al., 1994).

The available studies using paired-pulse TMS have focused mainly on changes of MEP sizes. Less attention has been paid to latency changes, although they could provide relevant additional information (Andersen et al.,

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1999). Moreover, the role of the intensity of the conditioning stimulus has not been investigated in great detail. This prompted us to carry out the current study, which analyzes the effect of the intensity of the conditioning stimulus on ICI and ICF, and takes into account both size and latency changes of the test MEPs.

METHODS

Seven healthy right-handed subjects (four men and three women; age range, 23 to 49 years) participated in the study. The study was approved by the local ethics committee. All subjects gave written informed consent. Subjects were seated with the right arm fixed gently in slight abduction from the trunk (20°) and flexion in the elbow (110°). Transcranial magnetic stimulation was carried out using two MagStim200 stimulators connected to the circular coil (mean diameter, 9 cm) by a Bistim module. Thus, the current flow in the coil, adjusted over the vertex to evoke optimal responses in the right extensor carpi radialis muscle, was in one and the same direction (counterclockwise when viewed from above). The motor threshold at rest was determined as the stimulus intensity eliciting three responses of at least 50 μV peak-to-peak amplitude in four stimuli (Siggelkow et al., 1999).

Motor evoked potentials were recorded from the right extensor carpi radialis muscle using conventional surface electromyographic techniques. Signals were amplified (bandpass, 10 Hz to 5 kHz) and digitized (sampling rate, 10 kHz). Epochs of a 300-msec duration (100 msec before the test stimulus and 200 msec after) were stored on disk. The electromyographic activity was monitored continuously to ensure the absence of voluntary background activity.

Five single stimuli (intensity 120% of motor threshold) were applied to obtain control MEPs. Then paired stimuli with ISIs of 3 msec and 13 msec were applied randomly to study ICI and ICF (Kujirai et al., 1993). The intensity of the test stimulus was 120% of motor threshold constantly, whereas different subthreshold conditioning stimulus intensities (CSIs) were used in random order: 55%, 60%, 65%, 70%, 75%, 80%, and 85% of motor threshold. Five identical stimuli were applied for each ISI and for each intensity.

The records of MEP after single- and paired-pulse stimulation (ISIs of 3 msec and 13 msec) with CSIs 75% of motor threshold are illustrated in Fig. 1. The zero line is well defined and it is no problem to assess the MEP onset and its duration especially after rectification of MEPs. Furthermore, the increase of the interval of inte-

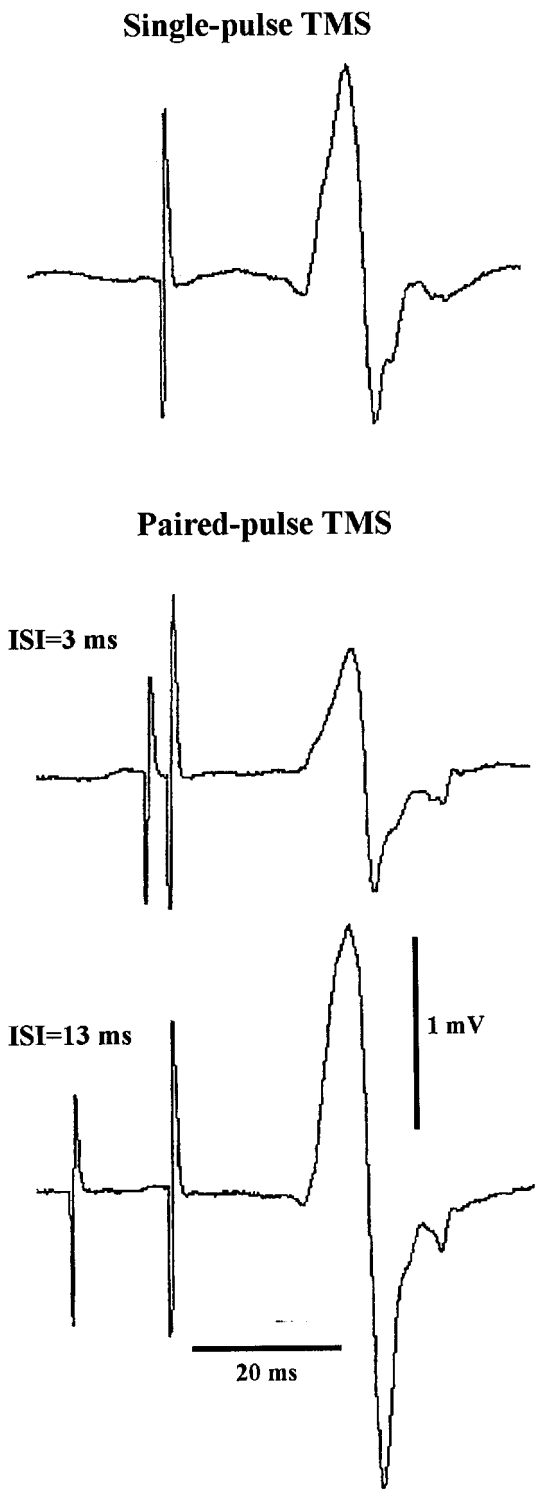

FIG. 1. The electromyographic records of motor evoked potentials after single- and paired-pulse transcranial magnetic stimulation (interstimulus intervals of 3 msec and 13 msec). The intensity of the test and conditioning stimulation is 120% and 75% of the motor threshold respectively. Note that the motor evoked potentials are time justified using the artifacts of the test stimulation.

gration, including small intervals before and after the MEP, does not affect essentially the values of its area.

Data processing was performed off-line. Motor evoked potential sizes assessed as total voltage time integral (area [Siggelkow et al., 1999]) and latencies were measured. Values were normalized to the mean control value in each subject and then pooled for all subjects calculating mean ± standard deviation. The differences between control and test values were assessed using the Wilcoxon's matched-pair signed rank test. The effect of the different intensities of the conditioning stimuli were assessed by applying one-way analysis of variance (ANOVA). Two-way ANOVA was used to compare the effect of different CSIs on MEP parameters at both ISIs used in the study. *P* values less than 0.05 were considered significant.

RESULTS

Motor thresholds ranged from 42 to 60% of the maximal output of the stimulator (mean ± standard deviation, 51.5 ± 9.2%). The mean area and mean latency of the control MEPs were 5.72 ± 3.90 mV/msec and 17.19 ± 0.76 msec respectively.

The sizes of the test MEPs were modulated significantly ($P < 0.01$, one-way ANOVA) by CSI as illustrated in Fig. 2. In the trials with 3-msec ISIs, the dependence of MEP areas on CSI was expressed by a well-defined U-shaped curve (see Fig. 2). The reduction of MEP areas became significant above CSIs of 58.57 ± 4.76% of the motor threshold ($P < 0.05$). With the increase of CSI up to 70% of motor threshold, the reduction of MEPs was stronger. At CSIs of 70% of motor threshold, the reduction of MEPs compared with the controls was maximal and significant, with a higher level of confidence ($P < 0.01$). With further increase of CSI, the reduction of MEPs was less pronounced ($P < 0.05$), and at CSIs higher than 75% of the motor threshold, MEP reduction was not significant.

The dependence of MEP areas on CSIs at 13-msec ISIs was significantly different ($P < 0.05$, two-way ANOVA). At lower CSIs (as much as 70% of the motor threshold) the MEP areas were not affected by the conditioning stimulation. The augmentation of MEPs became significant only with CSIs higher than 75 ± 4.08% of motor threshold. Thus, the threshold of the inhibitory effect of conditioning stimulation at 3-msec ISIs was significantly lower ($P < 0.01$) compared with the threshold of the facilitator effect at 13-msec ISIs. The increase of MEP areas with the increase of CSI above 70% was not monotonous.

The dependence of MEP latencies on CSI at both ISIs was generally similar ($P > 0.15$, two-way ANOVA), although more pronounced with 3-msec ISIs (Fig. 3).

Compared with the control values (single-pulse TMS), the latencies were prolonged with CSIs lower than 65% of the motor threshold, dropped below control values with a CSI of 70%, and then remained shorter than control up to CSIs 85% of motor threshold. Some of

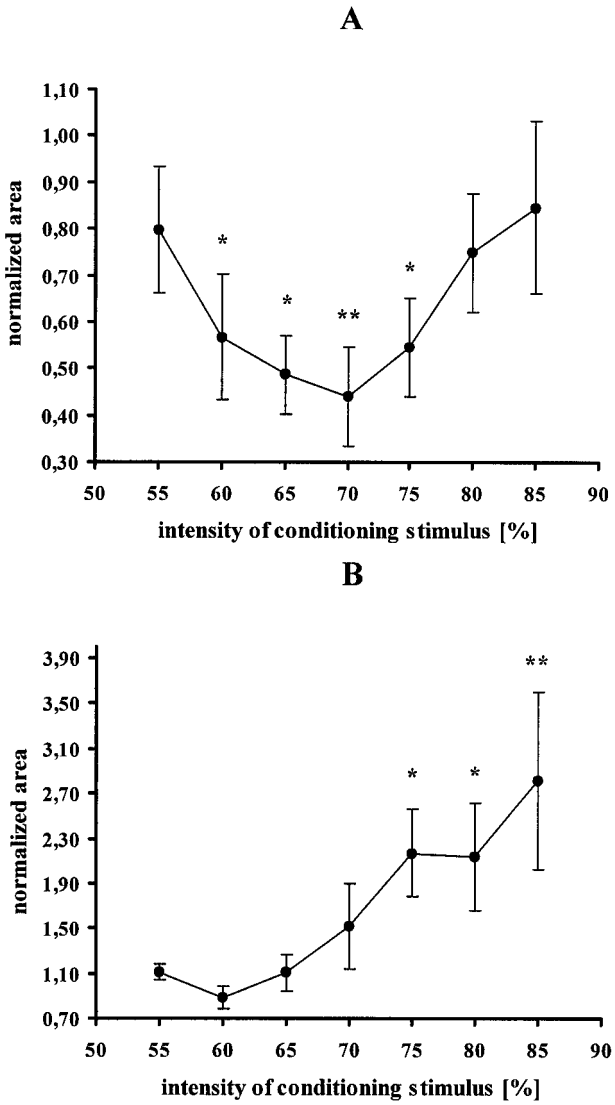

FIG. 2. (A, B) The dependence of motor evoked potential (MEP) areas on conditioning stimulation intensity (percentage of motor threshold) at interstimulus intervals of 3 msec **(A)** and 13 msec **(B)**. The values of MEP areas are normalized to the control values (unconditioned MEPs). The intensity of the test stimulation is 120% of motor threshold. The dependence of MEP areas on the conditioning stimulus intensities are significantly different for both interstimulus intervals ($P < 0.05$, two-way analysis of variance). The asterisks indicate significant differences (Wilcoxon's test) to control values: $*P < 0.05$ and $**P < 0.01$.

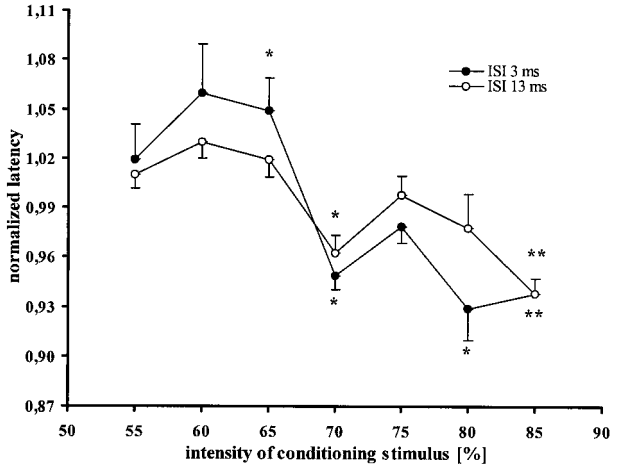

FIG. 3. The dependence of motor evoked potential (MEP) latencies on conditioning stimulus intensities (percentage of motor threshold) at interstimulus intervals of 3 msec and 13 msec (filled and open circles respectively). The values of MEP latencies are normalized to the control values (unconditioned MEPs). The intensity of the test stimulation is 120% of motor threshold. The dependence of MEP latencies on conditioning stimulus intensities are not significantly different for both interstimulus intervals ($P > 0.15$, two-way analysis of variance). Asterisks indicate significant differences (Wilcoxon's test) to control values: $*P < 0.05$ and $**P < 0.01$.

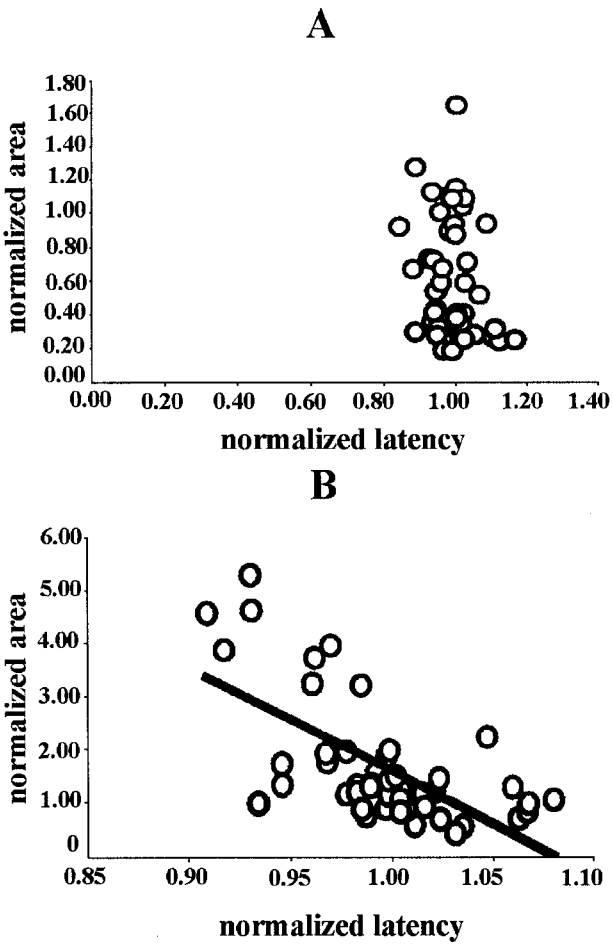

FIG. 4. (A, B) Relationship between normalized conditioned motor evoked potential areas and normalized conditioned motor evoked potential latencies in the trials with 3-msec interstimulus intervals **(A)** and 13-msec interstimulus intervals **(B)**. In the trials with 13-msec interstimulus intervals, a significant linear regression exists ($r = 0.64$, $P < 0.01$).

these changes reached significance, as indicated in the figure. The decrease of MEP latencies with the increase of CSIs above 70% was not monotonous. Thus, the changes of MEP latencies were polyphasic and in one and the same direction for ISIs of 3 msec and 13 msec. The polyphasic modulation of MEP latencies was evident for all investigated subjects.

Linear regression analysis showed a negative correlation ($r = 0.64$, $P < 0.01$) between MEP sizes and latencies in the trials with 13-msec ISIs whereas there was no significant correlation in the trials with 3-msec ISIs (Fig. 4).

DISCUSSION

The different thresholds of ICI and ICF indicate that these two mechanisms use different neuronal circuits (Ziemann et al., 1996b), converging finally at the corticospinal system. The neuronal elements involved in ICI are more sensitive to TMS reacting to lower CSIs. Both mechanisms, however, appear to be simultaneously active, and their effects may be summated. The parallel action of both intracortical systems is the most reasonable explanation of the observed disassociation between MEP sizes and latencies in our study. Therefore, at CSIs below the ICF threshold (as much as 70%), the ICI affects the test MEPs even at 13-msec ISIs, increasing the latencies without significant changes in MEP sizes. With the further increase of conditioning intensity, the ICI loses its power when the ICF becomes predominant and the MEPs at 3 msec are reversed gradually to the control (unconditioned) values. At CSIs above 75%, the MEP areas at 3 msec are not significantly different compared with the controls, but their latencies are significantly shorter. This finding, and the significant linear regression between MEP sizes and latencies in the trials with 13-msec ISIs, indicates that the latencies of the ICF effect is shorter compared with those of the ICI. Most probably the pathway of ICF may be shorter than that of ICI, involving less synapses.

A shorter action of ICI than ICF may account for the finding that ICI is predominant with very short ISIs, and

ICF prevails with longer ISIs, provided that a CSI of 70% of the motor threshold is used, as in most studies. Moreover, our data show that the generally used CSI of 70% of the motor threshold is ideal for the study of ICI but not for the study of ICF, which can be better reproduced with ICIs of 75 to 80%. Additionally, the MEP latencies are more sensitive to indicating the onset of ICF than MEP sizes that are still reduced because of ICI. The faster action of ICF would result in shorter MEP latencies without the MEP areas being changed (increased), even at 3-msec ISIs.

Another interesting finding of our study is the polyphasic modulation of MEP latencies at both ISIs used, and not monotonous changes of MEP areas at 13-msec ISIs with the increase of the conditioning intensity. The possibility that this is an artifact resulting from the intraindividual variations of ICI and ICF thresholds is unlikely, because essential variations and overlapping of ICI and ICF threshold ranges are required. In our study we found relatively small intraindividual variations, without overlapping of inhibitory (55 to 65% of motor threshold) and facilitatory (70 to 80% of motor threshold) threshold ranges. Furthermore, the polyphasic modulation of MEP latencies was observed for all investigated subjects. Most possibly, a nonlinear summation of ICI and ICF influences is taking place. The underlying mechanisms for such a nonlinear summation are not clear, but the involvement of additional cortical neuronal circuits, although possible, is not obligatory. The cross-influences between the inhibitory and facilitatory circuits may result in a such nonlinear summation. Actually, Ziemann et al. (1996a), studying the effect of γ-aminobutyric acid agonists on motor cortex excitability, postulated that the facilitatory interneurons are under the control of inhibitory interneurons. The existence of similar reciprocal influences from facilitatory toward inhibitory interneurons seems quite possible, because the reciprocity of neuronal connections is a well-known principle, taking place on spinal as well as on cortical levels.

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