Influence of Short-Interval Intracortical Inhibition on Short-Interval Intracortical Facilitation in Human Primary Motor Cortex

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Shirota Y, Hamada M, Terao Y, Matsumoto H, Ohminami S, Furubayashi T, Nakatani-Enomoto S, Ugawa Y, Hanajima R. Influence of short-interval intracortical inhibition on short-interval intracortical facilitation in human primary motor cortex. J Neurophysiol 104: 1382–1391, 2010. First published May 26, 2010; doi:10.1152/jn.00164.2010. Using the paired-pulse paradigm, transcranial magnetic stimulation (TMS) has revealed much about the human primary motor cortex (M1). A preceding subthreshold conditioning stimulus (CS) inhibits the excitability of the motor cortex, which is called short-interval intracortical inhibition (SICI). In contrast, facilitation is observed when the first pulse (S1) is followed by a second one at threshold (S2), named short-interval intracortical facilitation (SICF). SICI and SICF have been considered to be mediated by different neural circuits within M1, but more recent studies reported relations between them. In this study, we performed triple-pulse stimulation consisting of CS-S1-S2 to further explore putative interactions between these two effects. Three intensities of CS (80–120% of active motor threshold: AMT) and two intensities of S2 (120 and 140% of AMT) were combined. The SICF in the paired-pulse paradigm exhibited clear facilitatory peaks at ISIs of 1.5 and 3 ms. The second peak at 3 ms was significantly suppressed by triple-pulse stimulation using 120% AMT CS, although the first peak was almost unaffected. Our present results obtained using triple-pulse stimulation suggest that each peak of SICF is differently modulated by different intensities of CS. The suppression of the second peak might be ascribed to the findings in the paired-pulse paradigm that CS mediates SICI by inhibiting later I waves such as I3 waves and that the second peak of SICF is most probably related to I3 waves. We propose that CS might inhibit the second peak of SICF at the interneurons responsible for I3 waves.

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a useful tool to stimulate the human brain noninvasively (Day et al. 1989). A single electrical stimulation of the primary motor cortex (M1) elicits periodic, multiple discharges or multiple descending volleys in the corticospinal tract in animals (Patton and Amassian 1954). Similarly, TMS over M1 elicits multiple descending volleys in humans (Day et al. 1989; Di Lazzaro et al. 1998a). The first response is called a D (direct) wave; the later waves are designated as I (indirect) waves. The I waves follow the D wave periodically at intervals of ∼1.5 ms and are named I1–I3 waves in the order of their latency. The D wave is probably evoked by direct activation of the pyramidal tract neurons or their axon, and I waves are considered to be produced by activation of interneurons within M1, which in turn activate pyramidal tract neurons (Patton and Amassian 1954). A single pulse TMS evokes I waves preferentially (Day et al. 1989; Nakamura et al. 1997).

Furthermore, the paired-pulse paradigm enables us to investigate inhibitory and facilitatory circuits within M1 (Kujirai et al. 1993; Tokimura et al. 1996; Ziemann et al. 1998) probably by modulating different components of I waves. Short-interval intracortical inhibition (SICI) can be elicited by a conditioning stimulus (CS) followed by a test stimulus (S1) (Di Lazzaro et al. 1998b; Hanajima et al. 1998; Kujirai et al. 1993; Ziemann et al. 1996b). At interstimulus intervals (ISIs) of 1–5 ms, the motor evoked potential (MEP) produced by S1 is inhibited by CS. Furthermore, at ISIs of 2–4 ms, SICI is evident for the I3 wave, and to a lesser extent, the I2 wave but not for the I1 wave (Di Lazzaro et al. 1998b; Hanajima et al. 1998). The SICI at these ISIs is considered to reflect synaptic inhibition within M1 (Fisher et al. 2002; Hanajima et al. 2003; Roshan et al. 2003), which is mediated by gamma-aminobutyric acid (GABA) (Kujirai et al. 1993; Ziemann et al. 1996a). Interestingly, variation in the CS intensity results in the U-shaped SICI curve with the most enhanced SICI occurring at CS intensity of 90–110% active motor threshold (AMT) (Ort et al. 2003; Peurala et al. 2008; Ziemann et al. 1996b).

By contrast, short-interval intracortical facilitation (SICF) is elicited by a test stimulus (S1) followed by a second pulse (S2) set at around the resting motor threshold (RMT) (Tokimura et al. 1996). Three peaks of facilitation were observed: ISIs of 1.1–1.5, 2.3–2.9, and 4.1–4.4 ms (Ziemann et al. 1998). Because the intervals between the successive peaks are ∼1.5 ms, SICF is considered to represent an interaction between I waves; in fact, we previously showed that additional I2 waves were elicited at the first peak of SICF (Hanajima et al. 2002). Another study showed that the S1 and S2 pulses interacted along the later I wave pathway (Ilic et al. 2002). According to the notion that the later I wave pathway consists of chains of interneurons (Amassian et al. 1987), both authors propose that the second pulse excites the interneurons that are hyperexcitable or subliminally depolarized in the presence of S1. Although the information available is insufficient, the second or the third peak of SICF might represent additional production of later I waves; e.g., I3 or I4 waves are elicited additionally at the second or the third peak of SICF. These two peaks become greater when the intensity of the S2 increases (Chen and Garg 2000).

The SICI and SICF are commonly considered to be mediated by different neural circuits (Chen and Garg 2000; Ortu et al.
but their effects converge on the pyramidal tract neurons or some interneurons to elicit MEP. Thus we can speculate that there is some interaction between these effects. In fact, some studies reported some relations between SICI and SICF using the paired-pulse paradigm. Peurala et al. (2008) demonstrated that measurement of SICI was contaminated by SICF when CS of higher intensity was used. Similarly, Ortu and colleagues (2008) showed that they can only assess net inhibition or facilitation by the paired-pulse paradigm because SICI and SICF were mixed when stimulus intensity became higher.

More recently, to further elucidate the putative interaction between SICI and SICF, Wagle-Shukla et al. (2009) used the triple-pulse stimulation of CS, S1, and S2. They showed that CS facilitated the peaks of SICF (Wagle-Shukla et al. 2009). Although they studied the third peak of SICF intensively, only one stimulus intensity was used for S2 and the other peaks were tested using one stimulus intensity for CS and for S2. Because the stimulus intensity and ISIs are crucial for the paired-pulse paradigm, the same might hold true in the triple-pulse paradigm. Therefore we studied a wider range of time course of SICF using several stimulus intensities for CS and S2 to clarify stimulus intensity dependency of the effect of CS on SICF under the triple-pulse paradigm. Our original hypothesis is that each peak of SICF would be modulated differently by a preceding CS and CS intensity would affect this modulation.

METHODS

Participants

Participants were 10 right-handed healthy volunteers [1 woman, 9 men; 27–46 yr old, 36.2 ± 6.6 (SD) yr old], who gave their written informed consent to participate in the experiments. No participant had neurological, psychiatric, or other medical problem, or had any contra-indication to TMS (Rossi et al. 2009; Wassermann 1998). The protocol was approved by the Ethics Committee of the University of Tokyo Hospital and was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Recordings

Participants were seated on a comfortable chair. MEPs were recorded from the right first dorsal interosseous muscle (FDI). Pairs of Ag/AgCl surface cup electrodes (9 mm diam) were placed over the muscle belly (active) and the metacarpophalangeal joint of the index finger (reference). Responses were input to an amplifier (Biotop; GE Marquette Medical Systems) through filters set at 100 Hz and 3 kHz; they were then digitized and stored in a computer for later off-line analyses (TMS Bistim Tester; Medical Try System).

TMS

TMS was given over the hand area of the motor cortex using a hand-held figure-eight coil (9 cm external diameter at each wing; Magstim, Whitland, Dyfed, UK) placed tangentially over the scalp with the handle pointing backward at ~45° laterally, which is perpendicular to the central sulcus. Monophasic TMS pulses were delivered using a magnetic stimulator (Magstim 2002; Magstim). The optimal site for eliciting MEPs in the right FDI muscle (i.e., hot spot) was determined before each experiment. The hot spot was defined as the site at which the largest responses were elicited. This position was marked using a blue pen on the scalp for repositioning the coil. Placing the coil over this position, the RMT was determined as the lowest intensity that evoked a small response (~50 μV) in the relaxed FDI.

Paired- and triple-pulse stimulation procedures

Paired- or triple-pulse stimuli were delivered using two or three magnetic stimulators (Magstim 2002; Magstim) connected with a specially designed combining module (Magstim). This device comprised a 

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\text{FIG. 1. Experimental procedures. The experimental design is exhibited schematically. In the 1st 2 experiments using paired-pulse stimulation (i.e., experiments 1 and 2), conditioned responses are compared with unconditioned ones. In experiment 1, conditioning stimulus (CS) is followed by the 1st pulse (S1) to examine short-interval intracortical inhibition (SICI). Experiment 2 used S1 followed by the 2nd pulse (S2) to study short-interval intracortical facilitation (SICF). The other 2 experiments (experiments 3 and 4) constitute triple-pulse stimulations of CS-S1-S2 compared with paired-pulse stimulations of CS-S1 (SICI paradigm). Interstimulus intervals (ISIs) between CS and S1 take negative values such as -3 and -5 ms because they precede S1.}\n\]

\[
\text{[Paired pulse]}
\]

\[
\text{Experiment 1}
\]

\[
\text{CS S1 Unconditioned}
\]

\[
\text{ISI = -3 ms}
\]

\[
\text{Conditioned}
\]

\[
\text{S1}
\]

\[
\text{Experiment 2}
\]

\[
\text{CS S1 Unconditioned}
\]

\[
\text{ISI = 1.5-5.0 ms}
\]

\[
\text{Conditioned}
\]

\[
\text{S1, S2}
\]

\[
\text{ISI = 1.5-5.0 ms}
\]

\[
\text{[Triple pulse]}
\]

\[
\text{Experiment 3}
\]

\[
\text{CS S1 Unconditioned}
\]

\[
\text{ISI = -3 ms}
\]

\[
\text{CS S1 CS alone (= SICI)}
\]

\[
\text{ISI = 1.5-5.0 ms}
\]

\[
\text{Conditioned}
\]

\[
\text{S1, S2}
\]

\[
\text{ISI = 1.5-5.0 ms}
\]

\[
\text{Experiment 4}
\]

\[
\text{CS S1 Unconditioned}
\]

\[
\text{ISI = -5 ms}
\]

\[
\text{CS S1 CS alone (= SICI)}
\]

\[
\text{ISI = 1.5-5.0 ms}
\]

\[
\text{Conditioned}
\]

\[
\text{S1, S2}
\]

\[
\text{ISI = 1.5-5.0 ms}
\]
bines the outputs from four magnetic stimulators to enable delivery of a train of four monophasic magnetic pulses at maximum through a single coil (Hamada et al. 2007, 2008). Each experiment was conducted with the target muscle (FDI) relaxed.

**Experimental protocol**

This study included four experiments, and the experimental design is portrayed in Fig. 1 schematically. In the first two experiments, we confirmed the stimulus intensity dependency of SICI and SICF using the paired-pulse paradigm. Briefly, in experiment 1, we studied the degree of SICI at an ISI of −3 ms with three different intensities of CS. In experiment 2, we examined the time course of SICF using two intensities of S2. Two stimulus intensities were used for the S1 to clarify the test size dependency of SICF. In the other two experiments, we used the triple-pulse paradigm. In experiment 3, which is mainly emphasized in this report, triple-pulse stimulation was performed to determine the effect of CS on SICF when both CS and S2 were given in the same trial. The order of the pulses was CS at an ISI of −3 ms, S1, and S2. We referred to the results of experiments 1 and 2 to adjust the MEP size. Finally, in experiment 4, we conducted triple-pulse stimulation of CS at −5 ms, S1, and S2. ISIs between CS and S1 were expressed in negative values, and those between S1 and S2 were in positive.

**Experiments using the paired-pulse paradigm**

**EXPERIMENT 1: CONDITIONING STIMULUS INTENSITY DEPENDENCY OF SICI.** We studied SICI at an ISI of −3 ms using three different CS intensities: 80, 100, and 120% of AMT. The S1 intensity was adjusted to evoke MEPs of 0.5 mV (S1_0.5mV) when given alone. In all, 15 responses for CS-S1 and 15 responses for S1 alone were obtained with an intertrial interval (ITI) of 6 ± 0.5 s using the conditioning-test design; measurements were performed for each CS intensity separately. The order of the sessions for three intensities was randomized among the participants. The peak-to-peak MEP amplitudes of the conditioned responses were averaged and expressed as a ratio to the mean amplitude of MEP to S1 alone (unconditioned response).

**EXPERIMENT 2: CONDITIONING AND TEST STIMULUS INTENSITY DEPENDENCY OF SICF.** The SICF was investigated at ISIs of 1.5–5.0 ms, in 0.5 ms steps. We used two S1 intensities; one of which was adjusted to evoke MEPs of 0.5 mV (S1_0.5mV). The other was adjusted to evoke MEPs of 0.2 mV (S1_0.2mV). Two S2 intensities were studied: 120 and 140% of AMT. Every combination of S1 and S2 was studied separately. Thereby four experimental sessions were performed: 120% AMT S2 with S1_0.5mV, 120% AMT S2 with S1_0.2mV, 140% AMT S2 with S1_0.5mV, and 140% AMT S2 with S1_0.2mV. The order of the sessions was randomized among the participants. Each experimental session consisted of 10 responses for each ISI (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ms) and 15 responses to S1 alone at one S1 and S2 intensity, constituting 95 trials. The ITI was 6 ± 0.5 s. The peak-to-peak MEP amplitudes of the conditioned responses were averaged for each ISI. They were expressed as its ratio to the mean amplitude of MEP elicited by S1 alone (unconditioned responses).

**Experiments using the triple-pulse paradigm**

**EXPERIMENT 3: INFLUENCE OF CS AT −3 MS ON SICF.** A single session included 10 trials of triple-pulse stimulation for SICF in the presence of CS (CS-S1-S2), 15 trials of SICF alone (CS-S1), and 15 trials of S1 alone. The ISI between CS and S1 was set at −3 ms, and ISIs between S1 and S2 were the same as those in experiment 2 (i.e., 1.5–5 ms in 0.5 ms steps). Therefore a single session contains 110 trials, the order of which was randomized, and ITI was 6 ± 0.5 s. The S1 intensity was set at S1_0.5mV. Three CS intensities (80, 100, and 120% AMT) and two S2 intensities (120 and 140% AMT) were tested separately. Consequently, six experimental sessions were performed. The order of the sessions was randomized among the participants.

The MEP sizes were averaged for each ISI and were expressed as its ratio to the mean MEP size of SICI alone (CS-S1). Then they were compared with the time course of SICF alone. In this experiment, sessions using 80% AMT CS were compared with SICF alone using S1_0.5mV, the sessions using 100% AMT CS with SICF alone using S1_0.2mV, and 120% AMT CS with SICF alone using S1_0.3mV, to adjust the test sizes considering the test size dependency of SICI, which is consistently shown in reports of previous studies (Hanajima et al. 2007; MacKinnon et al. 2005; Ziemann et al. 1996b) and our experiment 1 (see results and Table 4). SICF alone using S1_0.3mV was newly performed to obtain the control data for experiment 3, and we referred to the results of SICF alone using S1_0.5mV and S1_0.2mV in experiment 2. We considered that this adjustment is necessary because the degree of SICF was significantly different between the two test MEP sizes studied in experiment 2 (i.e., 0.2 and 0.5 mV) and because SICI decreased the MEP size to such a degree in experiment 1 (see results and Table 3).

**EXPERIMENT 4: INFLUENCE OF CS AT −5 MS ON SICF.** We performed the same experiments as those of experiment 3 using CS at −5 ms. Of the 10 participants who were studied in experiments 1–3, 8 (all men, 27–46 yr, 35.1 ± 7.0 yr) were enrolled in this experiment. The CS intensities of 100 and 120% AMT combined with S2 intensity of 140% AMT were used because the CS intensity of 120% AMT with S2 intensity of 140% AMT showed the most marked effect in experiment 3 (see results). The MEP sizes were averaged for each ISI and expressed as its ratio to the mean MEP size of SICI alone, then compared with the time course of SICF alone using S1_0.5mV. For this experiment, neither S1_0.2mV nor S1_0.3mV was used because no significant SICI was evoked at an ISI of −5 ms (see results).

**Statistical analysis**

In experiment 1, one-way repeated measures ANOVA was used to evaluate the effects of CS intensity on SICI. Then post hoc analyses

**TABLE 1. Motor thresholds and stimulus intensities in the paired-pulse experiments (1 and 2)**

<table>
<thead>
<tr>
<th>CS</th>
<th>S1 intensity</th>
<th>S2 intensity</th>
<th>RMT</th>
<th>AMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% AMT</td>
<td>S1_0.5mV</td>
<td>—</td>
<td>55.7 ± 3.79</td>
<td>37.0 ± 1.73</td>
</tr>
<tr>
<td>100% AMT</td>
<td>S1_0.5mV</td>
<td>—</td>
<td>55.4 ± 3.57</td>
<td>37.2 ± 1.80</td>
</tr>
<tr>
<td>120% AMT</td>
<td>S1_0.5mV</td>
<td>—</td>
<td>56.7 ± 2.09</td>
<td>37.3 ± 2.09</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>120% AMT</td>
<td>56.6 ± 3.67</td>
<td>37.2 ± 2.32</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>140% AMT</td>
<td>54.9 ± 3.52</td>
<td>37.1 ± 2.40</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>120% AMT</td>
<td>53.4 ± 3.74</td>
<td>36.1 ± 1.90</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>140% AMT</td>
<td>55.4 ± 3.77</td>
<td>37.0 ± 2.31</td>
</tr>
<tr>
<td>100% AMT</td>
<td>S1_0.5mV</td>
<td>—</td>
<td>30.7 ± 1.55</td>
<td>73.5 ± 4.84</td>
</tr>
<tr>
<td>100% AMT</td>
<td>S1_0.5mV</td>
<td>—</td>
<td>37.2 ± 1.80</td>
<td>74.5 ± 5.08</td>
</tr>
<tr>
<td>120% AMT</td>
<td>S1_0.5mV</td>
<td>—</td>
<td>44.8 ± 2.49</td>
<td>74.4 ± 5.00</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>120% AMT</td>
<td>—</td>
<td>74.1 ± 4.62</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>140% AMT</td>
<td>—</td>
<td>73.2 ± 5.28</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>120% AMT</td>
<td>—</td>
<td>66.3 ± 5.30</td>
</tr>
<tr>
<td>—</td>
<td>S1_0.5mV</td>
<td>140% AMT</td>
<td>—</td>
<td>67.6 ± 5.50</td>
</tr>
</tbody>
</table>

Values are shown as means ± SE. Motor thresholds and stimulus intensities are expressed as percentage maximal stimulator output (%MSO). CS, conditioning Stimulus; S1 and S2, first and second pulse; RMT and AMT, resting and active motor threshold, respectively.
within-subject factors were S1 (S10.5mV and S10.2mV), S2 intensity used to elucidate effects of ISI and S2 intensities on SICF. Namely, A U-shaped SICI curve was observed (Fig. 2). One-way ANOVA was applied for each experimental session using within-subject factors of condition (triple-pulse and SICF alone) and ISI. In experiments 2–4, post hoc analyses with Bonferroni’s correction for multiple comparison were applied for each S2 intensity (120% AMT and 140% AMT) to determine the test size dependency of SICF; within-subject factors were S1 (S10.5mV and S10.2mV), S2 intensity (120% AMT and 140% AMT) and ISI (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ms). Second, two-way repeated measures ANOVA was applied for each S2 intensity (120% AMT and 140% AMT) to determine the test size dependency of SICF; within-subject factors were S1 (S10.5mV and S10.2mV), S2 intensity (120% AMT and 140% AMT) and ISI (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ms). Finally, the time course of SICF for each condition was explored using Dunnett’s test.

Analyses were performed using software (Dr. SPSS II for Windows version 11.0; SPSS, Chicago, IL). Greenhouse–Geisser correction was applied if necessary to correct for nonsphericity, and a Bonferroni’s correction for multiple comparison were applied for each experimental session using within-subject factors of condition and ISI. In experiments 2–4, two-way repeated measures ANOVA revealed a significant effect of CS intensity [F(2,18) = 6.293, P = 0.007]. Post hoc analyses revealed that CS intensity of 100% AMT showed more inhibition than that of 80% AMT (P = 0.006). Table 3 shows mean MEP sizes for the unconditioned responses and the conditioned responses and showed that each CS induced significant inhibition (P = 0.020 for 80% AMT, P < 0.001 for 100% AMT and 120% AMT).

EXPERIMENT 2: CONDITIONING AND TEST STIMULUS INTENSITY DEPENDENCY OF SICF. The mean time courses of SICF are presented in Fig. 3. When S10.2mV was used, SICF (especially at 120% AMT and 0.027 for 140% AMT), corresponding to the first and the second peaks of the SICF. Furthermore, we noted S2 intensity dependency of SICF; 140% AMT S2 produced more facilitation than 120% AMT.

Three-way repeated measures ANOVA revealed the main effect of S1 [F(1.9) = 10.54, P = 0.009], S2 intensity [F(1.9) = 24.02, P = 0.001], and ISI [F(1.63,14.67) = 8.70, P = 0.005]. Significant interaction was also found between S1 and ISI [F(2,46,22.11) = 6.10, P = 0.005], but no interaction between other combinations of factors was found to be statistically significant. Two-way repeated measures ANOVA applied for each S2 intensity disclosed the main effect of S1 [F(1.9) = 5.84, P = 0.039 for 120% AMT and F(1.9) = 6.58, P = 0.030 for 140% AMT] and ISI [F(1.53,17.38) = 7.42, P = 0.010 for 120% AMT and F(1.94,17.44) = 7.03, P = 0.006 for 140% AMT], and significant interaction between S1 and ISI [F(7.63) = 3.55, P = 0.003 for 120% AMT and F(2,00,17.97) = 4.97, P = 0.019 for 140% AMT]. Post hoc analyses showed significant difference between S10.5mV and S10.2mV at ISIs of 1.5 ms (P < 0.001 for 120% AMT and 0.027 for 140% AMT) and 3.0 ms (P = 0.034 for 120% AMT and 0.007 for 140% AMT), corresponding to the first and the second peaks of the SICF. Finally, the time course of SICF for each condition was explored using Dunnett’s test.

Motor thresholds and stimulus intensities are shown in Tables 1 and 2. Mean MEP amplitudes for S1 alone and CS-S1 in each of the experimental session are shown in Tables 3 and 4.

**RESULTS**

Motor thresholds and stimulus intensities are shown in Tables 1 and 2. Mean MEP amplitudes for S1 alone and CS-S1 in each of the experimental session are shown in Tables 3 and 4.

**Experiments using the paired-pulse paradigm**

**EXPERIMENT 1: CONDITIONING STIMULUS INTENSITY DEPENDENCY OF SICI.** Figure 2 shows the CS intensity dependency of SICI. A U-shaped SICI curve was observed (Fig. 2). One-way repeated measures ANOVA revealed a significant effect of CS intensity [F(2,18) = 6.293, P = 0.007]. Post hoc analyses revealed that CS intensity of 100% AMT showed more inhibition than that of 80% AMT (P = 0.006). Table 3 shows mean MEP sizes for the unconditioned responses and the conditioned responses and showed that each CS induced significant inhibition (P = 0.020 for 80% AMT, P < 0.001 for 100% AMT and 120% AMT).

**EXPERIMENT 2: CONDITIONING AND TEST STIMULUS INTENSITY DEPENDENCY OF SICF.** The mean time courses of SICF are presented in Fig. 3. When S10.2mV was used, SICF (especially at 1.5 and 3.0 ms) were more enhanced than those with S10.5mV. Furthermore, we noted S2 intensity dependency of SICF; 140% AMT S2 produced more facilitation than 120% AMT.

Three-way repeated measures ANOVA revealed the main effect of S1 [F(1.9) = 10.54, P = 0.009], S2 intensity [F(1.9) = 24.02, P = 0.001], and ISI [F(1.63,14.67) = 8.70, P = 0.005]. Significant interaction was also found between S1 and ISI [F(2,46,22.11) = 6.10, P = 0.005], but no interaction between other combinations of factors was found to be statistically significant. Two-way repeated measures ANOVA applied for each S2 intensity disclosed the main effect of S1 [F(1.9) = 5.84, P = 0.039 for 120% AMT and F(1.9) = 6.58, P = 0.030 for 140% AMT] and ISI [F(1.53,17.38) = 7.42, P = 0.010 for 120% AMT and F(1.94,17.44) = 7.03, P = 0.006 for 140% AMT], and significant interaction between S1 and ISI [F(7.63) = 3.55, P = 0.003 for 120% AMT and F(2,00,17.97) = 4.97, P = 0.019 for 140% AMT]. Post hoc analyses showed significant difference between S10.5mV and S10.2mV at ISIs of 1.5 ms (P < 0.001 for 120% AMT and 0.027 for 140% AMT) and 3.0 ms (P = 0.034 for 120% AMT and 0.007 for 140% AMT), corresponding to the first and the second peaks of the SICF. Finally, the time course of SICF for each condition was explored using Dunnett’s test.

**TABLE 3. Test and CS-S1 MEP sizes in the paired-pulse experiments (1 and 2)**

<table>
<thead>
<tr>
<th>CS</th>
<th>S1</th>
<th>S2</th>
<th>Test MEP Size, mV</th>
<th>CS-S1 MEP Size, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% AMT</td>
<td>S10.5mV</td>
<td></td>
<td>0.50 ± 0.04</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>100% AMT</td>
<td>S10.5mV</td>
<td></td>
<td>0.52 ± 0.03</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>120% AMT</td>
<td>S10.5mV</td>
<td></td>
<td>0.52 ± 0.05</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>—</td>
<td>S10.5mV</td>
<td>120% AMT</td>
<td>0.52 ± 0.03</td>
<td>—</td>
</tr>
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<td>S10.2mV</td>
<td>140% AMT</td>
<td>0.47 ± 0.04</td>
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<tr>
<td>—</td>
<td>S10.2mV</td>
<td>140% AMT</td>
<td>0.23 ± 0.03</td>
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<tr>
<td>—</td>
<td>S10.2mV</td>
<td>140% AMT</td>
<td>0.20 ± 0.03</td>
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 MEP, motor evoked potential.

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course of SICF in each condition was explored. Significant facilitation was found at several ISIs including 1.5 and 3.0 ms (Fig. 3).

Experiments using the triple-pulse paradigm

**EXPERIMENT 3: INFLUENCE OF CS AT — 3 MS ON SICF.** Figure 4 shows that the mean time courses of SICF depict a complex relation between SICI and SICF.

In the sessions using S2 of 120% AMT (Fig. 4, A–C), the first and the second peaks were facilitated in the presence of 80% AMT or 100% AMT CS (Fig. 4, A and B). By contrast, the second peak was inhibited in the presence of 120% AMT CS (Fig. 4C). In sessions using S2 of 140% AMT (Fig. 4, D–F), 80% AMT CS produced almost no changes in SICF (D). It is particularly interesting that 100% AMT CS and 120% AMT CS suppressed the second peak of SICF (Fig. 4, E and F). The first peak was also suppressed to a lesser extent (Fig. 4, E and F). Representative responses from one participant are portrayed in Fig. 5. In the left traces, which show SICF alone using S10.3mV and 140% AMT S2, at both the first (ISI = 1.5 ms) and the second (ISI = 3 ms) peaks, the MEP sizes are much larger than that elicited by S1 alone (S10.3mV). In contrast, the right traces from the triple-pulse stimulation using 120% AMT CS, S10.5mV, and 140% AMT S2, demonstrate that the second peak is suppressed; the MEP at the second peak (ISI = 3 ms) is only slightly larger than that of SICI alone (CS and S1). Because of the SICI (compare CS and S1 with S10.5mV in Fig. 5), the sessions using 100% AMT CS and 120% AMT CS were not compared with SICF alone using S10.5mV but with SICF alone using S10.2mV or S10.3mV. In fact, using 100% AMT and 120% AMT for CS, CS-S1 MEP sizes were around 0.2 and 0.3 mV, respectively (Table 4). A two-way repeated measures ANOVA revealed a significant interaction between condition and ISI in the session using 120% AMT CS and 140% AMT S2 [F(7,63) = 2.96, P = 0.010; Fig. 4F]. In other sessions, no significant interaction was found. Post hoc analyses revealed a significant difference at ISIs of 2.5 ms (P = 0.021) and 3.0 ms (P = 0.031), corresponding to the second peak of SICF.

**EXPERIMENT 4: INFLUENCE OF CS AT — 5 MS ON SICF.** At CS-S1 ISI of — 5 ms, CS did not induce significant inhibition (Table 4). The time course is presented in Fig. 6, and the second peak of SICF was suppressed. Statistical analysis revealed significant interaction between condition and ISI in the session using CS intensity of 120% AMT and S2 intensity of 140% AMT [F(2.215, 7.63) = 4.15, P = 0.032; Fig. 6B]. Post hoc analysis showed a significant difference at the ISI of 3.0 ms (P = 0.047), similar to *experiment 3*.

**DISCUSSION**

In this study, we demonstrated that different peaks of SICF were modulated by the preceding CS differently and that this modulation was stimulus intensity dependent. When CS intensity was below or around AMT and S2 was relatively low (i.e., 80 or 100% AMT CS and 120% AMT S2 in *experiment 3*), the first and the second peak were facilitated. This facilitation, however, did not reach statistical significance at variance with the previous report (Wagle-Shukla et al. 2009). In contrast, it is particularly interesting that the second peak of SICF was suppressed in the condition using a higher CS intensity (120% AMT). When S2 became stronger (i.e., 140% AMT), 100% AMT CS also suppressed the second peak (Fig. 4E). Especially, the suppression of the second peak of SICF was statistically significant in the condition which used 120% AMT CS and 140% AMT S2 (Fig. 4F).

**Facilitation of SICF by CS below or around AMT**

We noted a facilitatory effect of CS on the first and second peaks of SICF in conditions using 80 or 100% AMT CS and 120% AMT S2, although this facilitation was not statistically significant. The finding is, at least in part, in line with the preceding study (Wagle-Shukla et al. 2009). They found that the CS preceding the SICF paradigm “disinhibits” neural

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**TABLE 4. Test and CS-S1 MEP sizes in the triple-pulse experiments (3 and 4)**

<table>
<thead>
<tr>
<th>CS</th>
<th>S1</th>
<th>S2</th>
<th>Test MEP Size, mV</th>
<th>CS-S1 MEP Size, mV</th>
</tr>
</thead>
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<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
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<td></td>
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<tr>
<td>—</td>
<td>S10.5mV</td>
<td>120% AMT</td>
<td>0.31 ± 0.05</td>
<td>—</td>
</tr>
<tr>
<td>80% AMT</td>
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<td>120% AMT</td>
<td>0.49 ± 0.02</td>
<td>0.38 ± 0.03</td>
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<tr>
<td>100% AMT</td>
<td>S10.5mV</td>
<td>120% AMT</td>
<td>0.48 ± 0.04</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>120% AMT</td>
<td>S10.5mV</td>
<td>120% AMT</td>
<td>0.51 ± 0.04</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>100% AMT</td>
<td>S10.5mV</td>
<td>140% AMT</td>
<td>0.50 ± 0.03</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>120% AMT</td>
<td>S10.5mV</td>
<td>140% AMT</td>
<td>0.53 ± 0.04</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td><strong>Experiment 4</strong></td>
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<tr>
<td>100% AMT</td>
<td>S10.5mV</td>
<td>140% AMT</td>
<td>0.47 ± 0.05</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>120% AMT</td>
<td>S10.5mV</td>
<td>140% AMT</td>
<td>0.48 ± 0.05</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.47 ± 0.06</td>
<td>0.43 ± 0.11</td>
</tr>
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</table>
circuits responsible for late I-waves resulting in an overall facilitation and proposed that the disinhibition is mediated by another group of inhibitory interneurons. Likewise, we consider that the facilitation seen at the lower CS intensity might be an observation similar to that by Wagle-Shukla et al. (2009) possibly caused by a “disinhibition” mechanism. We do not have a clear explanation for the lack of statistical significance though. The number of subjects might be too small to reveal significance, or some methodological difference such as stimulation parameters might explain the difference.

Suppression of the second peak of SICF by CS above AMT

The main and novel point in this report lies in the significant suppression of the second peak of SICF in the presence of CS above AMT (i.e., 120% AMT) while there was no significant modulation of the first peak of SICF with this stimulus intensity. Although the first peak tended to be modulated by CS, the fact that significant difference was found only in the second peak suggests that the first peak was less susceptible to CS in comparison to the second peak. Thus we consider that the
peaks of SICF were differently modulated by the preceding CS and that our findings might be produced by some I-waves interaction in each SICF peak.

We reported earlier that the first peak of SICF consists of the summation of S1 and S2 applied 1.5 ms later along the I2 wave pathway (Hanajima et al. 2002). The S1 activates interneurons for I2 waves, which then deliver subliminal depolarization of interneurons along its pathway 1.5 ms later. The following S2 activates these subliminally depolarized interneurons when the S2 is applied at a preferred time (i.e., 1.5 ms later than the S1). This activation produces additional I2 waves, and leads to the facilitation occurring at I2 latency. Similarly, the second peak of SICF is probably generated at I3 latency from S1 in the following way: the S1 activates interneurons for I3 waves, which subliminally depolarize interneurons along its pathway 3 ms later; then S2 directly activates these interneurons. On the other hand, I3 waves are more suppressed by CS in the paired-pulse paradigm than I1 or I2 waves (Di Lazzaro et al. 1998b; Hanajima et al. 1998). Considering these two arguments, our results are explained as follows: in the presence of CS, S1 produces less activation of interneurons for I3 waves, which in turn engenders less subliminal depolarization of interneurons along its pathway 3 ms later. Consequently, S2 coming 3 ms later cannot activate additional interneurons sufficiently; then the second peak of SICF does not emerge.

Furthermore, this suppression was similarly significant when CS was applied at −5 ms, although SICI itself was not evoked at an ISI of −5 ms, in line with previous findings (Hanajima et al. 2007). In fact, SICI is considered to be produced by GABAergic inhibitory postsynaptic potential (IPSP) elicited by CS (Kujirai et al. 1993; Ziemann et al. 1996a). The IPSPs last more than 10 ms (McCormick 1989; Williams and Stuart 2003). We already demonstrated that I3 waves were inhibited at an ISI of 10 ms (Hanajima et al. 1998). The IPSP produced by CS would be partly effective at 5 ms later, which might explain SICF suppression by CS at an ISI of −5 ms.

In contrast to the second peak of SICF, why was the other peak less susceptible to CS? The first peak of SICF is believed to result from the interaction between I1 and I2 waves, both of which are less suppressed by CS than later I waves in the paired-pulse paradigm (SICI). In this context, the smaller susceptibility of I2 waves to SICI might simply explain less modulation of the first peak in the presence of CS. As for the third peak, which is considered to be mediated by another interaction of I waves, we did not find a significant peak in experiment 2. A previous study demonstrated that 90% RMT S2 did not produce the third peak but that 100% RMT S2 did (Chen and Garg 2000). The present results agree with this study because our 140% AMT, that is the intensity of the higher S2, corresponded to ~90–95% RMT as calculated by Tables 1 and 2. Stimulus intensity dependency of the third peak has remained to be studied.

**Effects of CS intensity on SICF**

In the present study, CS intensity had considerable influence on SICF: CS below or around AMT disinhibited, albeit non-significantly, the first and second peak of SICF, whereas CS above AMT significantly suppressed the second peak of SICF and tended to suppress the first peak of SICF, too. The results bear out the importance of CS intensity in the triple-pulse paradigm. We favor the view that the suppression of the second
peak of SICF is ascribed to the direct effect of inhibitory interneurons on I3 wave and that the “disinhibition” of SICF peaks by lower CS intensity is mediated by different groups of inhibitory interneurons (Wagle-Shukla et al. 2009). In this context, there may be at least three explanations to account for the stimulus intensity dependency of the modulation of SICF. One possibility is that the cortical neurons stimulated by CS are different depending on the stimulation intensity. CS with lower intensity could activate interneurons responsible for disinhibition more preferentially and higher CS elicits firing of interneurons that mediate SICI, leading to inhibition of I waves. Furthermore, considering the fact that disinhibition was observed only when CS intensity was low, activation of the interneurons mediating disinhibition may show a ceiling effect with higher stimuli. Another possibility relates to a contamination of SICF elicited by higher intensity CS (Peurala et al. 2008). It is possible that the contamination of SICF with a preceding CS results in refractoriness of neurons responsible for SICF, leading to the suppression of SICF peaks. This possibility is, however, less likely in view of the argument of Peurala et al. (2008) that it should be necessary for the CS to be >90% RMT to elicit a contaminating SICF effect because the CS needs to evoke many I waves enough for succeeding temporal summation (Peurala et al. 2008). Here our highest intensity of CS (i.e., 120% AMT) was approximately equal to 80% RMT as calculated by Tables 1 and 2, so that the CS is insufficient to evoke a huge SICF effect as the S1 in the SICF paradigm. Furthermore, CS intensity of 100% AMT, less contaminated by SICF (Peurala et al. 2008), tended to suppress the second peak of SICF using 140% AMT S2, although the effect was not statistically significant. Thus the contamination of SICF alone does not simply account for the suppression of the second peak of SICF by CS. Nevertheless future studies are needed to determine the effect of the contamination of SICF in the triple-pulse paradigm. Finally, variation in S2 intensity might be partly responsible for the present findings. Because the significant suppression of the second peak of SICF was observed in the condition using the higher intensity S2 (140% AMT), we cannot exclude the possibility that the parameters of the present study evoked much stronger SICF than in the previous study (Wagle-Shukla et al. 2009) and favored findings of inhibition of SICF, similar to a ceiling effect.

**Tripulse paradigm in comparison with paired-pulse paradigm**

It is particularly interesting that a clear suppression of the second peak of SICF was the most prominent with the CS of 120% AMT. Tripulse stimulation using 100% AMT CS and 140% AMT S2 showed a tendency for suppression of the second peak (Fig. 4E, experiment 3), but it was not statistically significant. By contrast, the most effective suppression was found at 100% AMT using the paired-pulse paradigm (Fig. 2, experiment 1); in fact, we found a U-shaped curve of SICI in experiment 1, compatible with previous studies (Orth et al. 2003; Ziemann et al. 1996b). Consequently, an apparent discrepancy exists between the paired- and triple-pulse paradigms. Recent reports described that SICI was contaminated by SICF at higher CS intensity above AMT and that only a net inhibition could be observed at these conditioning intensities (Ortu et al. 2008; Peurala et al. 2008). Importantly, these recent studies attributed the cause of the U-shaped curve or the lack of suppression by higher CS intensity mainly to contamination of SICF, not to reduction in SICI. Thus it is possible that inhibitory effects of CS are identical or may be stronger when a higher CS is used. In fact, IPSP is increased with higher stimulation intensity (Williams and Stuart 2003). Besides, one previous study has shown that inhibitory effect of a higher CS on I3 wave is stronger when I3 wave was elicited preferentially (Hanajima et al. 1998). On the basis of these arguments, our present results from triple-pulse stimulation might be interpreted as follows: a higher CS produces more inhibitory effect, but it is blurred in the usual paired-pulse paradigm testing SICI because of the contamination of SICF. On the other hand, the triple-pulse paradigm performed in the present study could possibly reveal an inhibitory effect of CS in its stronger end by demonstrating the suppression of SICF.

**FIG. 7. A proposed hypothesis. A hypothesis to explain the present results are depicted schematically. , a corticospinal neuron; and , interneurons. A chain of facilitatory interneurons () produce later I waves when a single pulse transcranial magnetic stimulation (TMS) is applied. Inhibitory interneurons () A mainly synapsing with interneurons for I3 wave are activated when a preceding CS was applied, mediating SICI. On the other hand, SICF occurs when S2 follows S1, by temporal summation. When a CS with a higher intensity is used, it also activates some of the facilitatory interneurons, and SICI is contaminated by SICF. We propose another group of inhibitory interneurons ( ), which have lower threshold than interneurons A, to account for the present findings. In the triple-pulse paradigm, a lower intensity CS could possibly activate interneurons B predominantly, leading to facilitation of SICF by inhibiting interneurons A, which finally dis-inhibits I3 waves production. On the other hand, a strong CS can preferentially activate interneurons A than B, resulting in the suppression of the second peak of SICF through inhibition of I3 waves.**
Technical consideration

Because we have conducted the experiments over several days, some other factors than intracortical process might have affected the present results, such as fatigue or intraindividual variability. On the other hand, however, it was also necessary to divide the experiments into several portions since too long experiments would also have made the subjects fatigued.

Proposed hypotheses and conclusion

On the basis of the discussion in the preceding text, CS can modulate peaks of SICF differently, and this modulation might be dependent on stimulus intensity. CS below or around AMT might disinhibit the first and the second peaks and CS above AMT can inhibit the second, and possibly the first, peaks. We have shown that stimulus intensity had much influence on the effect of CS on SICF. We favor a model that some inhibitory interneurons send inputs to other interneurons that mediate SICI, which itself is similar to the model proposed by Wagle-Shukla et al. (2009). This hypothesis is shown schematically in Fig. 7. The former inhibitory interneurons (neuron B in Fig. 7) had a lower threshold for TMS and exert disinhibition by inhibiting the latter inhibitory interneurons (neuron A in Fig. 7). These two groups of interneurons mainly affect I3 waves. We depicted a chain of interneurons in Fig. 7, but it is also possible that different I waves are produced by different groups of interneurons because the mechanism of I wave generation is unknown. In that model, neurons A and B described in the preceding text might predominantly influence interneurons that mediate I3 wave in the same way. The effects of SICI and SICF converge mainly on the production of I3 waves.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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