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Cortico-conus motor conduction time (CCCT) for leg muscles

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ABSTRACT

Objective: To measure the conduction time from the motor cortex to the conus medullaris (cortico-conus motor conduction time, CCCT) for leg muscles using magnetic stimulation.
Methods: Motor evoked potentials (MEPs) were recorded from tibialis anterior muscles in 51 healthy volunteers. To activate spinal nerves at the most proximal cauda equina level or at the conus medullaris level, magnetic stimulation was performed using a MATS coil. Transcranial magnetic stimulation of the motor cortex was also conducted to measure the cortical latency for the target muscle. To obtain the CCCT, the latency of MEPs to conus stimulation (conus latency) was subtracted from the cortical latency.
Results: MATS coil stimulation evoked reproducible MEPs in all subjects, yielding CCCT data for all studied tibialis anterior muscles.
Conclusions: MATS coil stimulation provides CCCT data for healthy subjects.
Significance: This novel method is useful for evaluation of corticospinal tract function for leg muscles because no peripheral component affects the CCCT.

1. Introduction

Magnetic stimulation enables us to evaluate the corticospinal tract function non-invasively by measuring the central motor conduction time (CMCT) (Rossini et al., 1994; Chen et al., 2008). The CMCT is usually obtained by subtracting the motor evoked potential (MEP) latency to magnetic stimulation over the spinal enlargement (spinal latency) from that to magnetic stimulation over the primary motor cortex (cortical latency). Magnetic stimulation over the spinal enlargement activates the spinal nerve at the neuro-foramina level (Ugawa et al., 1989b). Therefore, the CMCT described above includes the conduction time through the spinal nerves running in the spinal canal (Rossini et al., 1994; Chen et al., 2008).

Maccabee et al. reported that an 8-shaped coil can activate the most proximal cauda equina at around the conus medullaris (Maccabee et al., 1996). They proposed the possibility that this stimulation method might enable us to measure the conduction time from the motor cortex to the conus medullaris [cortico-conus motor conduction time (CCCT)]. The CCCT necessarily reflects the corticospinal tract function more correctly than the conventional CMCT because peripheral components (some conduction time within the cauda equina) do not contribute to CCCT, especially in patients with peripheral neuropathy. The CCCT, however, has not been widely used yet.

A few alternative methods can be used to measure the proximal spinal nerve conduction time, such as F-wave measurement and high-voltage electrical stimulation (Ugawa et al., 1988a,b, 1989a, 1995; Claus, 1990; Eisen and Shtybel, 1990). However, F-wave measurement provides no information about the lesion sites, and high-voltage electrical stimulation is often associated with severe pain. Especially, high-voltage electrical stimulation is not tolerated by patients with skin problems (Matsumoto et al., 2005, in press).

We have developed a new method to activate the most proximal cauda equina at around the conus medullaris level using a specially devised coil [magnetic augmented translumbosacral stimulation (MATS) coil] (Matsumoto et al., 2009a,b).

The aim of this paper is to apply the MATS coil to CCCT measurement. The relation between MEP latency and body height was also studied.

2. Materials and methods

2.1. Subjects

Subjects were 51 healthy volunteers (25 men and 26 women). Their mean age and body height were 42.1 ± 15.5 (mean ± standard deviation (SD); range 24–78) years and 163.9 ± 9.3 (144–185) cm.

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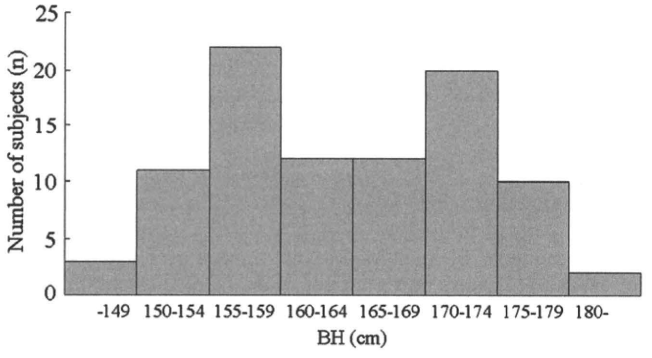

Fig. 1. Histogram of body height. There is no extremely skewed distribution of body height in our study.

The histogram of body height is shown in Fig. 1. No extremely skewed distribution of body height was observed.

Informed consent to participate in this study was obtained from all subjects. The protocol was approved by the Ethics Committee of the University of Tokyo. The experiments were conducted in accordance with the ethical standards of the Declaration of Helsinki.

2.2. Stimulation, recording and analysis

During the examination, MEPs were recorded from the tibialis anterior muscle (TA) as subjects sat comfortably on a bed. The TA muscle was selected because this muscle could be easily contracted and recorded compared to other leg muscles. Disposable silver–silver chloride disc electrodes of 9 mm diameter were placed in a belly tendon montage over TA. Signals were amplified with filters set at 20 Hz and 3 kHz and recorded using a computer (Neuropack MEB-9100; Nihon Kohden Corp., Japan).

Magnetic stimulation was conducted using a monophasic stimulator (Magstim 200; The Magstim Co. Ltd., UK). For cortical magnetic stimulation, a double-cone coil (The Magstim Co. Ltd., UK) was placed over the Cz (international 10–20 system), with induced currents flowing mediolaterally over the contralateral leg motor area (Terao et al., 1994, 2000). The MEP onset latency was measured in the active condition (cortical latency).

Fig. 2 portrays the placement of MATS coil (diameter 20 cm, 0.98 T; The Magstim Co. Ltd., UK) when recording MEPs from the right TA. The MATS coil was always placed from the midline to the contralateral side of the body (the opposite side from the recorded muscle) to prevent some non-target parts of the coil from activating distal peripheral nerves for the target TA. The most proximal cauda equina at around the conus medullaris was activated using the MATS coil, whose edge was positioned over the first lumbar (L1) spinous process for inducing currents to flow upward in the body (Matsumoto et al., 2009b). For the neuro-foramina level stimulation, the edge of MATS coil was positioned over the fifth lumbar (L5) spinous process for inducing currents to flow 45° downward from horizontal direction (Matsumoto et al., 2009a). This direction of induced currents (45°) was optimal to elicit MEPs because the induced currents should flow along the anatomical course of spinal nerves (Ugawa et al., 1989b; Epstein et al., 1991; Mills et al., 1993; Maccabee et al., 1996; Ruohonen et al., 1996; Matsumoto et al., 2009a). In L1 and L5 level stimulation, the onset latencies of MEPs were measured in the relaxed condition (L1 and L5 level latencies).

To obtain the minimal and reproducible MEP latency, the stimulus intensity was increased gradually and several MEPs evoked by stimulation at several different intensities were superimposed. The CCCT, conventional CMCT, and cauda equina conduction time (CECT) were obtained (92 sides). The CCCT was obtained by subtracting the L1 level latency from the cortical latency, the conventional CMCT by subtracting the L5 level latency from the cortical latency, and the CECT by subtracting L5 level latency from L1 level latency. Linear regression analysis was conducted to investigate the relation between each conduction time and body height.

The MEP sizes were compared between the simulation positions (60 sides). The base-to-peak amplitude of MEP was mea-

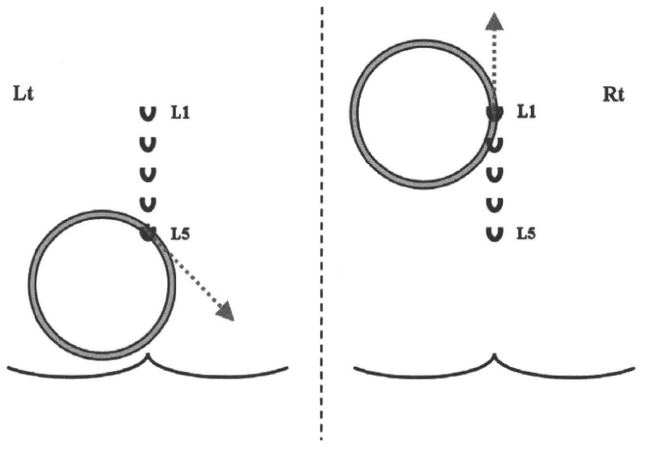

Fig. 2. MATS coil stimulation method. This figure shows positions of MATS coil when MEPs are recorded from right TA. For the most proximal cauda equina stimulation, the edge of MATS coil is positioned over the first lumbar spinous process for inducing currents to flow upward. For neuro-foramina level stimulation, the edge of the MATS coil is positioned over the fifth lumbar spinous process for inducing currents to flow 45° downward from a horizontal direction.

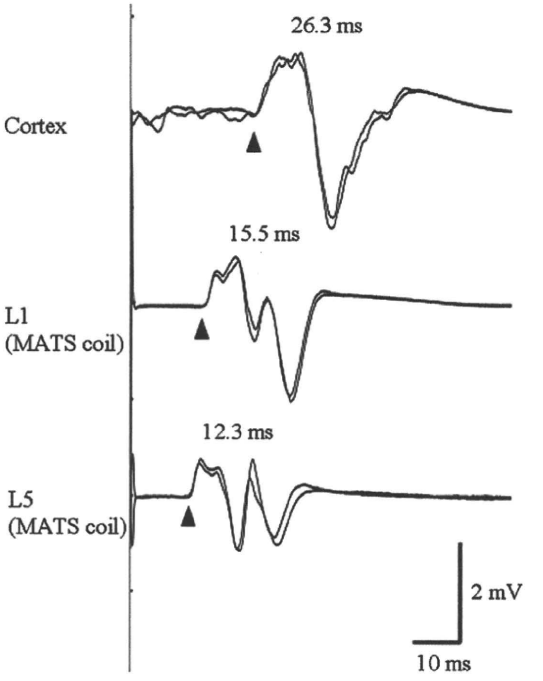

Fig. 3. Representative MEPs in a normal subject. The conventional CMCT is obtained by calculating the latency difference between MEPs to cortical and L5 level stimulation. Similarly, the CCCT is obtained by calculating the latency difference between MEPs to cortical and L1 level stimulation.

sured. At L1 and L5 levels, the intensity was increased gradually to the maximal stimulator output (100%). The amplitudes of maximal MEPs were compared between two level stimulation positions (maximal MEP means an MEP to supramaximal stimulation or MEP to submaximal stimulation with maximal stimulator output). The MEP amplitudes of the two level stimulation positions were compared using Wilcoxon's signed rank test; p values less than 0.05 were considered statistically significant.

3. Results

No subjects experienced any intolerable discomfort during MATS coil stimulation. No side effect was noted. Fig. 3 shows representative MEPs in a normal subject. The conventional CMCT was obtained using the MEPs to cortical and L5 level stimulation (14.0 ms). Moreover, L1 level stimulation evoked discernible MEPs. The CCCT was 10.8 ms, and the CECT 3.2 ms.

In all subjects, L1 level MATS coil stimulation evoked reproducible MEPs. The L1 level latency was longer than L5 level latency. The mean latencies and conduction times are presented in Table 1.

The correlations between each conduction time and body height are depicted in Fig. 4. Significant and positive linear relations were found between the conventional CMCT and body height ($p < 0.001$; conventional CMCT = 0.045 × body height + 7.166, $R = 0.366$), and between CECT and body height ($p = 0.001$; latency = 0.032 × body height − 2.602, $R = 0.331$). No significant correlation was found between CCCT and body height ($p = 0.298$).

The MEPs to L1 level stimulation (median: 1.0 mV, 25–75 percentiles: 0.5–1.8 mV) were significantly smaller than MEPs to L5 level stimulation were (1.3 mV, 1.0–3.5) ($p < 0.001$).

Table 1
Normal values of latencies (51 subjects, 92 sides).

	Mean ± SD (ms)
Cortical latency	26.1 ± 1.6
L1 level latency	14.0 ± 1.4
L5 level latency	11.5 ± 0.9
CCCT	12.3 ± 1.2
Conventional CMCT	14.6 ± 1.2
CECT	2.6 ± 0.9

CCCT, cortico-conus motor conduction time; CMCT, central motor conduction time; CECT, cauda equina conduction time; SD, standard deviation.

4. Discussion

In all subjects, L1 level MATS coil stimulation elicited discernible MEPs to measure onset latency. It enabled us to obtain CCCTs. The CCCT is more suitable for evaluating the corticospinal function for leg muscles than the conventional CMCT because no cauda equina conduction component contributes to CCCT. Another superior point of this stimulation method is the evaluation of conduction through the cauda equina using CECT. The authors have earlier reported some utility of this stimulation method for evaluating cauda equina conduction in patients with peripheral neuropathy (Matsumoto et al., 2010).

In this study, the CECT was found to be 2.6 ± 0.9 ms, which is similar to previously reported values obtained using an 8-shaped coil (2.3 or 2.6 ms) (Maccabee et al., 1996; Maegaki et al., 1997). Therefore, L1 level MATS coil stimulation does activate the cauda equina at the root exit site from the conus medullaris, as described in previous reports (Maccabee et al., 1996; Maegaki et al., 1997; Matsumoto et al., 2009b), namely at the conus medullaris level. Therefore, the latency difference between cortical and L1 level stimulation was designated as the cortico-conus motor conduction time (CCCT).

Regarding the relation between each conduction time and body height, the conventional CMCT and CECT had significant correlation with body height, but the CCCT did not. These results are not completely consistent with those of previous reports (Chu, 1989; Ugawa et al., 1989a; Claus, 1990; Furby et al., 1992). Previous reports have described that the conventional CMCT for lower extremities is significantly affected by the body height (Chu, 1989; Furby et al., 1992), according with our results. On the other hand, the correlation between the CCCT and body height is contro-

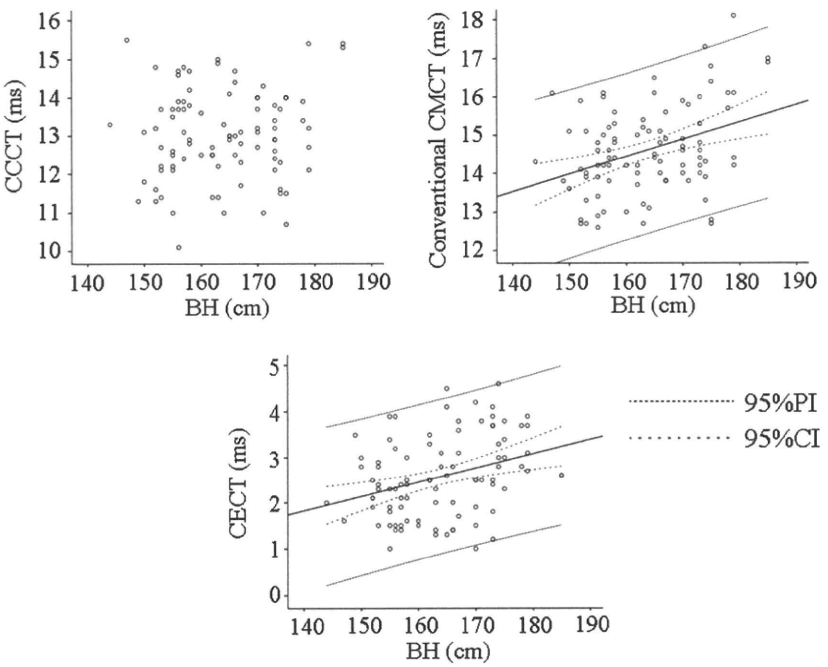

Fig. 4. Relation between each conduction time and body height. The CCCT is not significantly correlated with body height ($p = 0.298$). A significant and positive linear relation was found between the conventional CMCT and body height ($p < 0.001$; conventional CMCT = 0.045 × body height + 7.166, $R = 0.366$). Similarly, a significant correlation was found for CECT ($p = 0.001$; latency = 0.032 × body height − 2.602, $R = 0.331$). PI, prediction interval; CI, confidence interval.

versial. Ugawa et al. reported that the cortical–L1 conduction time measured using high-voltage electrical stimulation was not significantly correlated with body height (Ugawa et al., 1989b). In contrast, Claus reported that the cortical–L1 conduction time measured using transcranial magnetic stimulation and high-voltage electrical stimulation had a significant correlation with body height (Claus, 1990). The results in this study were similar to that in the former report. One plausible explanation of this discrepancy might be the difference in the body height of subjects. The average (range) of body height in the paper of Ugawa et al. was about 163 (151–178) cm and that in Claus was about 173 (156–191) cm. The body height in this study was almost same (164 cm) as that in the paper of Ugawa et al. The difference in body height seems to be due to the difference between Japanese and European peoples. Whatever the difference, this study demonstrates that the CCCT is relatively independent of body height compared to the conventional CMCT and CECT.

The relative independence of the CCCT from the body height might be mainly explained by the disproportion between growths in length of the spinal cord and the vertebral column (Kunitomo, 1918; Vettivel, 1991). The spinal cord length does not elongate proportionally to body height, although the cauda equina elongates concomitantly with the spine growth proportionally to body height. Large variability of the conduction velocity of the corticospinal tracts between subjects might also explain the lack of significant relation between CCCT and body height. Indeed, the conduction velocity in awake human estimated by Ugawa et al. (1995) ranged from 62.0 to 79.0 m/s, and that in anesthetized human by Fujiki et al., (1996) ranged from 50.5 to 72.7 m/s (Ugawa et al., 1995; Fujiki et al., 1996).

One point of caution related to this method is the MEP amplitude. The MEPs evoked by L1 level stimulation were often smaller than those by L5 level stimulation in normal subjects, which suggests that an amplitude comparison between L1 and L5 level stimulation is not useful for evaluation of the conduction block within the cauda equina even though the latencies are good parameters for evaluation of motor conduction. Another point of caution is the difference of CCCT between target muscles. If another muscle is selected, the normal value of CCCT should be made for each target muscle.

In conclusion, we propose that the MATS coil is useful for the accurate evaluation of corticospinal tract function for leg muscles.

Acknowledgments

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Cerebellar Dysfunction in Progressive Supranuclear Palsy: A Transcranial Magnetic Stimulation Study

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Abstract: Progressive supranuclear palsy (PSP) rarely shows cerebellar signs and symptoms even though the cerebellar dentate nuclei are involved pathologically. This study evaluates cerebellar function using transcranial magnetic stimulation (TMS) to determine whether subclinical cerebellar involvement is present in PSP patients. We studied 11 patients with PSP, 11 patients with Parkinson's disease (PD), and 10 age-matched controls. Patients were examined with their usual medications and in their relative on state. Motor evoked potentials (MEPs) were recorded from the hand muscle. Cerebellar function was evaluated using suppressive effects of TMS over the cerebellum on MEPs elicited by TMS over the contralateral motor cortex, which we call cerebellar inhibition (CBI). Interstimulus intervals (ISIs) of 4 to 8 ms were used, and the time course of CBI was analyzed. The CBI was reduced in PSP patients. By contrast, the CBI was normal in PD patients in their on state. Although the CBI in their off state should be examined in future studies, the results described herein suggest that Purkinje cells or the dentato–thalamo–cortical pathway assessed by CBI is involved in PSP. Our results are compatible with the pathological findings showing severe dentate nucleus degeneration in PSP patients. © 2010 Movement Disorder Society

Key words: progressive supranuclear palsy; cerebellum; transcranial magnetic stimulation

INTRODUCTION

Progressive supranuclear palsy (PSP) is a syndrome that is typically characterized by postural instability and supranuclear gaze palsy.[1] Although a recent study reported pathologically confirmed PSP patients developing cerebellar ataxia as the initial and principal symptom,[2] clinical signs of cerebellar dysfunction are usually considered rare. In contrast, involvement of the cerebellar dentate nucleus has been well documented to be the cardinal neuropathological findings in PSP.[1] With this background, neurophysiological evaluation of cerebellar functions in PSP would be an interesting approach to clarify the presence of subclinical cerebellar dysfunctions, but such investigations have not been well documented.

Transcranial magnetic stimulation (TMS) is a noninvasive technique to stimulate the human brain. Cerebellar function can be studied using the paired-pulse paradigm; a preceding TMS over the cerebellum decreased the size of motor evoked potentials (MEPs) elicited by TMS over the contralateral primary motor cortex (M1) at interstimulus intervals (ISIs) of 5 to 7 ms.[3] The suppressive effect is likely to be derived from activation of Purkinje cells that inhibit or disfacilitate the dentato–thalamo–cortical pathway. For descriptive purposes only, in this article, we refer to this inhibition as cerebellar inhibition (CBI).[4]

This study evaluated cerebellar function using this technique in PSP patients. For comparison, we also studied patients with Parkinson's disease (PD), in which some brain structures common to PSP are involved.

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METHODS

Participants

We studied 11 patients with probable PSP according to the National Institute of Neurological Disorders and Stroke and the Society for PSP, (NINDS-SPSP) criteria,[5] 11 patients with PD according to the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria,[6] and 10 healthy right-handed healthy volunteers (Table 1). No participants had any contraindication to TMS.[7] No patients showed pyramidal signs or cerebellar ataxia. All participants gave their written informed consent. This study was approved by the Institutional Review Board. Severity of the disease was assessed using the Hoehn and Yahr staging and the Unified Parkinson's Disease Rating Scale (UPDRS) Part III (Table 1). Dopaminergic medications were expressed as levodopa (L-dopa) equivalent daily dose (LEDD) as reported elsewhere[8]: 1 mg of pergolide = 1 mg of pramipexole = 5 mg of ropinirole = 10 mg of bromocriptine = 100 mg of L-dopa.

Recording

A surface electromyogram (EMG) was recorded from the first dorsal interosseous (FDI) muscle using a belly tendon montage on the more affected side with larger summed score of items 23 to 25 of the UPDRS Part III in PD and PSP patients, and on the right side in healthy subjects. Responses input to an amplifier (Biotop; GE Marquette Medical Systems, Japan) through filters set at 100 Hz and 3 kHz were digitized and stored in a computer for later offline analyses (TMS bistim tester; Medical Try System, Japan).

TABLE 1. *Clinical features and basic electrophysiological values*

	PSP	PD	Control	*P* value
Female: male (*n*)	4: 7	4: 7	9: 1	
Age at exam[a] (yr)	72.7 ± 7.8	68.4 ± 8.7	64.0 ± 6.4	0.06
Disease duration[a] (yr)	4.5 ± 2.7	15.9 ± 9.7		0.001
Hoehn and Yahr stage (*n*)				0.06
2	0	5		
3	6	3		
4	4	3		
5	1	0		
UPDRS III[b]	27 (9–75)	22 (13–51)		0.77
Test MEP size[a] (mV)	0.52 ± 0.15	0.62 ± 0.22	0.50 ± 0.16	0.27
CMCT[a] (ms)	6.3 ± 0.38	6.5 ± 0.72	6.7 ± 0.64	0.45

[a]Values are shown as mean ± SD.
[b]Values are shown as median (range).
PSP, progressive supranuclear palsy; PD, Parkinson's disease; UPDRS, unified Parkinson's disease rating scale; MEP, motor evoked potential; CMCT, central motor conduction time.

Transcranial Magnetic Stimulation

For TMS over the cerebellum (conditioning stimulus, CS), a double-cone coil (110 mm mean diameter) was centered over the midpoint between the inion and the mastoid process ipsilateral to the recording side. Current in the coil was directed downward (that is, upward current was induced in the cerebellum).[3] The M1 was stimulated using a round coil (90 mm mean diameter) centered over the vertex (test stimulus, TS). Current in the coil was directed anteroposteriorly over the target M1 (posteroanterior current in the target M1). Monophasic TMS pulses were delivered using two magnetic stimulators (Magstim 200; The Magstim).

EXPERIMENTAL DESIGN

The CBI was examined as described previously.[3] We first determined the active motor threshold (AMT) for pyramidal tract activation at the brainstem with the double-cone coil centered over the inion.[9] CS was fixed at an intensity of 95% AMT and given at 4, 5, 6, 7, and 8 ms before the test stimulus. The intensity of the TS was adjusted to elicit MEPs of 0.5 mV on average when given alone. The experiment was performed with the target muscle relaxed, as confirmed by an oscilloscope monitor. Seven trials recorded for each ISI (i.e., conditioned trials) were randomly intermixed with 10 trials in which TS was delivered alone (i.e., unconditioned trials) with an intertrial interval of 10 s. When recording was contaminated by voluntary EMG, such trials were discarded from the analyses. When necessary, we briefly stopped the session to maintain the resting state of the target muscle. We also evaluated the central motor conduction time (CMCT), as described previously.[10] The patients on medications were studied when they were in the relative on state; that is, they took their medications as usual, and the experiments were performed ~2 hours after their morning or noon dose.

Data analyses

We used one-way analysis of variance (ANOVA) for comparisons of the following parameters among the groups (i.e., PSP, PD, and controls): the age at examination, test MEP size, and CMCT. Student's *t* test was used to compare the disease duration, and Mann-Whitney U test was used to compare the Hoehn and Yahr stages and UPDRS Part III scores between the

TABLE 2. *Clinical features of each patient*

Case No.	Age (yr)	Disease duration (yr)	H & Y stage	UPDRS part III	UPDRS item 20 (rest tremor)	UPDRS item 21 (postural tremor)	LEDD (mg)
PSP 1	61	5	3	19	0	0	0
PSP 2	76	7	4	40	0	1	300
PSP 3	57	1	3	29	0	0	0
PSP 4	78	4	3	9	0	0	0
PSP 5	84	4	3	9	0	0	0
PSP 6	73	3	3	14	0	0	0
PSP 7	71	7	4	45	0	0	100
PSP 8	75	2	4	45	0	0	0
PSP 9	78	2	3	20	0	0	0
PSP 10	77	5	4	27	0	0	0
PSP 11	70	10	5	75	0	0	300
PD 1	72	19	3	25	3	0	405
PD 2	52	9	2	20	0	0	750
PD 3	75	3	2	15	0	0	0
PD 4	81	10	4	36	5	2	500
PD 5	64	23	3	22	0	0	700
PD 6	70	21	4	51	7	2	515
PD 7	72	3	2	19	1	1	200
PD 8	60	35	2	15	0	0	975
PD 9	66	12	2	13	0	0	425
PD 10	79	24	4	36	0	0	350
PD 11	61	16	3	28	5	0	625

H & Y stage, Hoehn and Yahr stage; LEDD, levodopa equivalent daily dose; PD, Parkinson's disease, PSP, progressive supranuclear palsy; UPDRS, unified Parkinson's disease rating scale.

two disease groups. To evaluate the time course of CBI among groups, the ratio of the mean peak-to-peak amplitude of the conditioned MEPs to that of unconditioned MEPs was calculated for each ISI in each subject. These individual mean ratios from all subjects in each group were then averaged to produce a grand mean ratio for that group. We analyzed the CBIs in different groups using two-way repeated measures ANOVA with GROUP (PSP, PD, or control) as the between-subject factor and with ISI as the within-subject factor. Bonferroni's post hoc tests were used for additional analyses.

To further investigate the relations between CBI and other demographic or clinical features, average size ratio (ASR) was calculated for each participant by averaging the MEP ratio across ISIs of 5 to 7 ms.[11] The correlation of age with the ASR was tested for each group of the subjects using linear regression analyses. Possible relations between the CBI and disease severity in the patient groups were analyzed in two manners. First, one-way ANOVA was conducted for each patient group to analyze effect of Hoehn and Yahr stage. Second, the correlation between ASR and UPDRS part III score was investigated using linear regression analyses. Difference in the ASR between PD patients with and without tremor was studied using the Student's *t* test. Influence of the dopaminergic medication in PSP patients was assessed by comparing the mean ASR of the PSP patients with medications to that of the PSP patients without medications, using the Student's *t* test. We did not conduct such analyses for PD patients, because all but one patient was taking dopaminergic medication.

A P-value < 0.05 was considered significant. Data were analyzed using a commercial software (*SPSS* for Windows ver. 13; SPSS, Chicago, IL, USA).

RESULTS

No significant age difference was found among groups, but it tended to be higher in the PSP group. Disease duration differed significantly between PD and PSP (Table 1). Neither the amplitude of test MEP size nor CMCT differed significantly among groups (Table 1). Test stimulus intensity expressed as %maximal stimulator output (%MSO) was 55.6% ± 11.9% (mean ± standard deviation; range 38–72%) in the PSP group, 51.6% ± 17.1% (range 28–80%) in the PD group, and 55.4% ± 16.6% (range 39–90%) in the healthy controls. AMT for pyramidal tract activation at the brainstem was 54.3% ± 11.6% MSO (range 38–70%) in the PSP group, 63.2% ± 19.1% (range 38–100%) in the PD group, and 45.4% ± 13.5% (range 27–70%) in the healthy controls.

Table 2 shows clinical details of the PSP and PD patients. Three PSP patients were on dopaminergic medication.

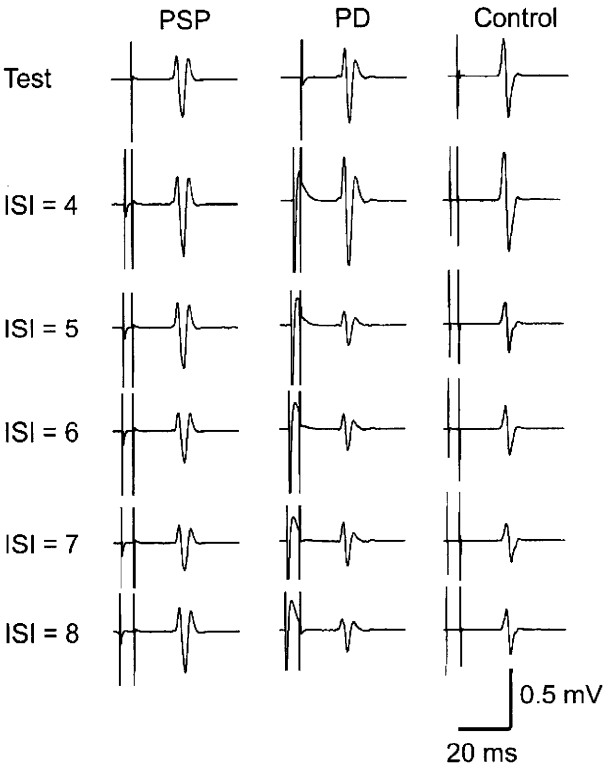

FIG. 1. Representative responses from a single subject of each group. Traces show averaged motor evoked potentials (MEPs) from one PSP patient, one PD patient, and one healthy volunteer. The top row shows unconditioned responses (averaged over 10 trials). The lower rows demonstrate conditioned responses for each ISI (averaged over seven trials). In a PSP patient, the suppression was reduced at ISIs of 6 and 7 ms. No suppression was present at ISIs of 5 and 8 ms. In contrast, MEPs were inhibited at ISIs of 5 to 8 ms in a PD patient and a healthy volunteer.

Figure 1 presents representative responses in a single participant from each group. In a healthy volunteer, the conditioned response at an ISI of 4 ms was similar in size to the unconditioned response. In contrast, the conditioned MEPs were smaller than the unconditioned MEP at ISIs of 5 to 8 ms. Similar results were obtained in a PD patient, indicating normal CBI. In a PSP patient, in contrast, the inhibition at ISIs of 5 to 8 ms was reduced. The mean time courses of CBI are depicted in Figure 2. They also demonstrated reduced CBI in PSP patients. The statistical comparisons disclosed an effect of GROUP (F (2,29) = 7.703, P = 0.002) and an effect of ISI (F (4,116) = 7.206, P < 0.001). Post hoc analysis revealed that PSP showed reduced CBI than PD (P = 0.005) or controls (P = 0.008), but no significant difference between PD and controls. Furthermore, a significant difference between PSP and PD was found at ISIs of 5, 6, and 7 ms (P = 0.011, 0.029, and 0.005, respectively). No significant difference was found between PD and controls at any ISIs.

We found no significant correlation between the CBI assessed by ASR and age in any of the groups (R^2 = 0.034, P = 0.61 in the control group, R^2 = 0.012, P = 0.75 in the PSP group, and R^2 = 0.001, P = 0.90 in the PD group). One-way ANOVA with regard to the Hoehn and Yahr stages revealed significant main effect of disease severity on CBI in the PSP group (P = 0.007, Fig. 3A), but not in the PD group (P = 0.70, Fig. 3B). Furthermore, we found a significant correlation between the ASR and UPDRS part III score in the PSP group (R^2 = 0.76, P < 0.001, Fig. 3C), but not in the PD group (R^2 = 0.006, P = 0.81, Fig. 3D). We found normal CBI in PD patients, irrespective of the presence or absence of tremor. ASR was 0.66 ± 0.08 in PD patients with tremor; and 0.60 ± 0.16 in those without (P = 0.49). CBI was abnormally reduced (ASR 0.90) in one PSP patient showing tremor (patient No. PSP 2 in Table 2). The ASR of PSP patients with medication was 1.14 ± 0.29, and that of PSP without

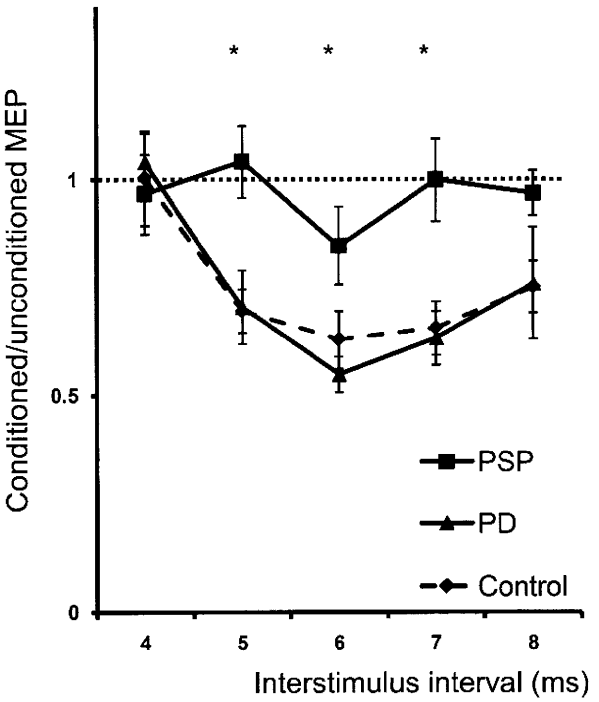

FIG. 2. Mean time courses of CBI in each group. The averaged time courses of CBI showed decreased CBI in a PSP group (rectangles) and normal CBI at ISIs of 5 to 8 ms in a PD group (triangles). A control group is shown by diamonds. The abscissa denotes ISI. The ordinate shows the MEP size ratio. Error bars represent SE. * indicates statistical significance between PSP and PD (P < 0.05 with Bonferroni's correction).

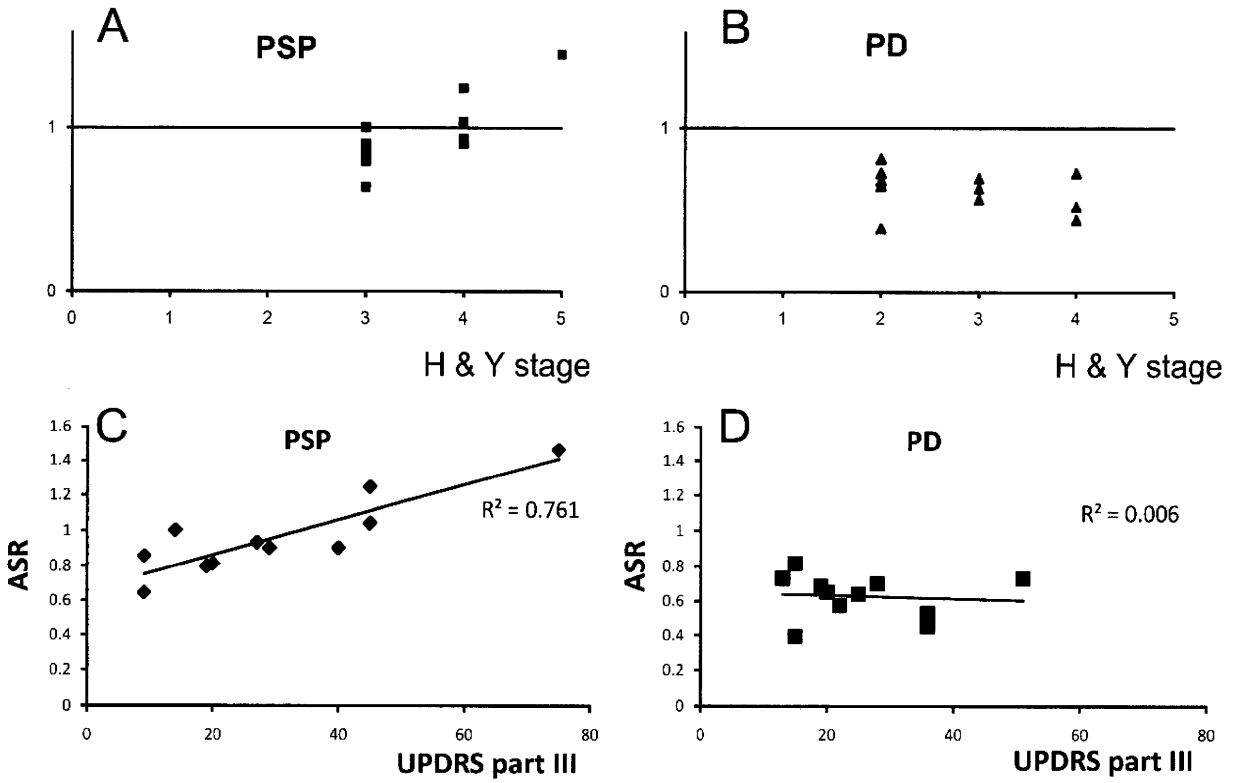

FIG. 3. Correlations between the degree of CBI and disease severity. **(A,B)** The degree of CBI expressed as average size ratio (ASR) was plotted against Hoehn and Yahr stage (H & Y stage) for each patient for each disease group. In the PD group (B), ASR is similar among the H & Y stages of 2 to 4. In contrast, PSP patients with higher H & Y stage showed more decreased CBI, that is, larger ASR (A). **(C,D)** For each patient, ASR was plotted against UPDRS part III total score. In PSP patients (C), there is a significant correlation between ASR and UPDRS part III total score ($R^2 = 0.76$, $P < 0.001$). PD patients (D) did not show any significant correlation ($R^2 = 0.006$, $P = 0.81$).

medication was 0.89 ± 0.19. They did not significantly differ from each other ($P = 0.14$).

DISCUSSION

The results showed that CBI was significantly reduced in PSP patients, although it was normal in PD patients. TMS over the cerebellum has been proposed to activate Purkinje cells that inhibit the dentate nucleus, which in turn engenders suppression of contralateral M1[3]. Consequently, the present results suggest that Purkinje cells or the dentato–thalamo–cortical pathway is involved in PSP patients, although no clinical cerebellar sign was observed.

Our results are consistent with previous pathological and radiological findings of PSP. Pathologically, the cerebellar dentate nucleus and superior cerebellar peduncle (SCP), which connects the cerebellar dentate nucleus with the thalamus, are severely involved in PSP.[1,2,12] A study using magnetic resonance imaging also demonstrated atrophy of the SCP quantitatively.[13] It is also consistent with a recent report that 3 of 22 pathologically confirmed PSP patients showed cerebellar ataxia as an initial and cardinal symptom.[2]

Our present results also agree with the following issues. It has been proposed recently that PSP should be divided into several subtypes[14]: Richardson's syndrome (RS) is the classical type, as reported in the original article. PSP-parkinsonism (PSP-P) resembles idiopathic PD in some respects such as asymmetric symptoms at onset, presence of tremor, lack of supranuclear gaze palsy at an early stage, and moderate responsiveness to L-dopa. Dentate nucleus degeneration was severer in RS than in PSP-P.[15] Because our patients were all classified as RS based on the clinical criteria, the significant reduction in CBI revealed in this study is compatible with such pathological findings. Whether the CBI of PSP-P is different from that of RS warrants further investigation.

Can dysfunction of neural systems other than the cerebellum or some other confounding factors account for the present findings? Four possibilities might be

discussed. First, given that PSP patients sometimes show severe corticospinal tract degeneration and frontal lobe degeneration,[16] dysfunction of the corticospinal tract or M1 might be responsible for our findings. However, the lack of pyramidal sign in the PSP patients suggested that this possibility is less likely. Second, some other changes in the motor cortex excitability, which might be revealed by investigations of motor threshold, short-interval intracortical inhibition, or intracortical facilitation, could possibly have an influence on the present results. Third, dopaminergic medications which may affect motor cortex excitability[17] might be responsible for the present findings. However, the PSP patients showed reduced CBI irrespective of medication. Thus, this leads us to conjecture that dopaminergic drugs had no significant influence on the degree of CBI in PSP. Considering the fact that PD patients took more dopaminergic medications, however, we cannot exclude a possibility that PD patients without medication or in their off state may have abnormal CBI. Finally, there was a trend for difference in age among groups. But, this factor is again unlikely to explain the reduced CBI in PSP patients because we did not find any significant correlation between CBI and age. These issues raised above should be addressed in more detail in future studies because our sample size may be too small to draw any firm conclusions.

CBI was reduced in PSP, but none of our patients showed cerebellar symptoms and signs. Why do PSP patients rarely show limb ataxia even though cerebellar structures are involved? A plausible explanation is that other symptoms of PSP such as akinesia or rigidity would mask clinical cerebellar signs. Indeed, cerebellar dysfunction is sometimes masked by parkinsonian symptoms.[11]

In the present study, PSP patients tended to be clinically severer than PD patients. Can disease severity affect the results? First, in PD patients, we did not find any relation between CBI and disease severity; patients with different Hoehn and Yahr stages showed similar ASR (Fig. 3B), and UPDRS part III did not correlate with ASR (Fig. 3D). In contrast, more advanced PSP patients showed larger ASR, that is, more abnormal CBI (Fig. 3A,C). These results suggest that neural structure which is affected in PSP but not in PD can explain the reduced CBI shown here. One of the candidates for such neural structures may be the cerebellum. Further studies, however, are needed to confirm the possible relation between CBI and disease severity in PSP.

A shortcoming of our study is that the diagnosis was based solely on clinical findings and was not confirmed pathologically. The clinical criteria we used, however, can diagnose PSP or PD with a high positive predictive value.[5] Another issue relates to the technical procedure. Although we have monitored participant's voluntary EMG activity using oscilloscope and discarded the trials contaminated by voluntary EMG, we did not record the degree of EMG activity quantitatively. Then, some small difference in the muscle state may explain the present results. In future studies, this point may need to be controlled more quantitatively.

In conclusion, although the PSP patients showed no clinical cerebellar signs, the results described herein suggest that Purkinje cells or the dentato–thalamo–cortical pathway assessed by CBI is involved in PSP. Our results are compatible with the pathological findings showing severe dentate nucleus degeneration in PSP patients.

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

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Shirota Y, Hamada M, Terrao 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 named 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% 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 are 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) (Orth 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.

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2008), 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 200^2; 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 response of ≥50 μV in the relaxed FDI in ≥5 of 10 consecutive trials (Rossini et al. 1994). The AMT was defined as the lowest intensity that evoked a small response (>100 μV) when the participant maintained a slight contraction of the right FDI (5–10% of the maximum voluntary contraction) observing an oscilloscope monitor, in >5 of 10 consecutive trials. The experiments were performed separately on several days, and RMT and AMT were determined every experimental day.

Paired- and triple-pulse stimulation procedures

Paired- or triple-pulse stimuli were delivered using two or three magnetic stimulators (Magstim 200^2; Magstim) connected with a specially designed combining module (Magstim). This device com-

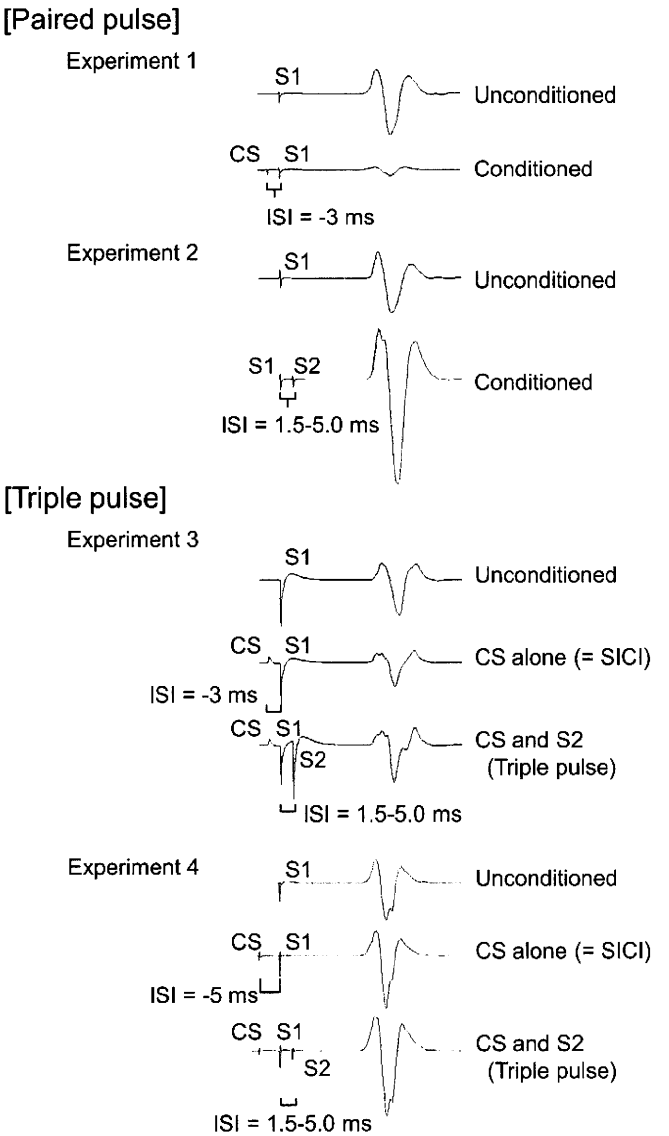

FIG. 1. Experimental procedures. The experimental design is exhibited schematically. In the 1st 2 experiments using paired-pulse stimulation (i.e., *experiments 1* and *2*), conditioned responses are compared with unconditioned ones. In *experiment 1*, conditioning stimulus (CS) is followed by the 1st 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.

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 SICI 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)*

				Motor Thresholds		Stimulus Intensities		
	CS	S1	S2	RMT	AMT	CS	S1	S2
Experiment 1	80% AMT	$S1_{0.5mV}$	—	55.7 ± 3.79	37.0 ± 1.73	30.7 ± 1.55	73.5 ± 4.84	—
	100% AMT	$S1_{0.5mV}$	—	55.4 ± 3.57	37.2 ± 1.80	37.2 ± 1.80	74.5 ± 5.08	—
	120% AMT	$S1_{0.5mV}$	—	56.7 ± 2.09	37.3 ± 2.09	44.8 ± 2.49	74.4 ± 5.00	—
Experiment 2	—	$S1_{0.5mV}$	120% AMT	56.6 ± 3.67	37.2 ± 2.32	—	74.1 ± 4.62	44.5 ± 2.73
	—	$S1_{0.5mV}$	140% AMT	54.9 ± 3.52	37.1 ± 2.40	—	73.2 ± 5.28	51.9 ± 3.26
	—	$S1_{0.2mV}$	120% AMT	53.4 ± 3.74	36.1 ± 1.90	—	66.3 ± 5.30	43.4 ± 2.29
	—	$S1_{0.2mV}$	140% AMT	55.4 ± 3.77	37.0 ± 2.31	—	67.6 ± 5.50	51.9 ± 3.13

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.

TABLE 2. *Motor thresholds and stimulus intensities in the triple-pulse experiments (3 and 4)*

				Motor Thresholds		Stimulus Intensities		
	CS	S1	S2	RMT	AMT	CS	S1	S2
Experiment 3	—	$S1_{0.3mV}$	120% AMT	54.4 ± 3.56	36.5 ± 1.83	—	70.7 ± 4.33	44.0 ± 2.16
	—	$S1_{0.3mV}$	140% AMT	55.8 ± 3.12	38.3 ± 2.02	—	72.6 ± 4.67	53.5 ± 2.77
	80% AMT	$S1_{0.5mV}$	120% AMT	55.9 ± 3.94	37.4 ± 2.15	30.0 ± 1.68	74.7 ± 5.35	45.1 ± 2.49
	100% AMT	$S1_{0.5mV}$	120% AMT	54.9 ± 3.45	36.5 ± 1.41	36.5 ± 1.41	75.0 ± 5.02	43.9 ± 1.66
	120% AMT	$S1_{0.5mV}$	120% AMT	56.0 ± 3.65	36.9 ± 2.06	44.2 ± 2.48	73.8 ± 5.11	44.2 ± 2.48
	80% AMT	$S1_{0.5mV}$	140% AMT	55.4 ± 3.57	37.1 ± 2.33	29.7 ± 1.87	76.1 ± 5.44	51.8 ± 3.17
	100% AMT	$S1_{0.5mV}$	140% AMT	55.4 ± 3.37	37.1 ± 2.47	37.1 ± 2.47	77.4 ± 5.13	51.8 ± 3.41
	120% AMT	$S1_{0.5mV}$	140% AMT	56.5 ± 4.06	37.4 ± 1.70	44.9 ± 2.04	74.9 ± 5.08	52.1 ± 2.44
Experiment 4	100% AMT	$S1_{0.5mV}$	140% AMT	51.5 ± 3.02	35.1 ± 1.20	35.1 ± 1.20	67.8 ± 4.31	49.3 ± 1.68
	120% AMT	$S1_{0.5mV}$	140% AMT	52.0 ± 3.05	33.4 ± 1.64	40.0 ± 1.88	68.0 ± 4.28	46.9 ± 2.34

Motor thresholds and stimulus intensities are expressed as percentage maximal stimulator output (%MSO).

with Bonferroni correction for multiple comparisons were applied. The mean unconditioned MEP amplitude was compared with the mean conditioned MEP amplitude for each condition using a paired *t*-test. In *experiment 2,* a three-way repeated measures ANOVA was used to elucidate effects of ISI and S2 intensities on SICF. Namely, within-subject factors were S1 ($S1_{0.5mV}$ and $S1_{0.2mV}$), 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 ($S1_{0.5mV}$ and $S1_{0.2mV}$) 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. In *experiment 3,* two-way repeated measures ANOVA was applied for each experimental session using within-subject factors of condition (triple-pulse and SICF alone) and ISI. In *experiment 4,* two-way repeated measures ANOVA was applied for each experimental session using within-subject factors of condition and ISI. In *experiments 2–4,* post hoc analyses with Bonferroni's correction for multiple comparison were applied for each S1-S2 ISI (i.e., 1.5–5 ms) if interaction was significant.

Analyses were performed using software (Dr. SPSS II for Windows version 11.0; SPSS, Chicago, IL). Greenhouse–Geisser correction was used if necessary to correct for nonsphericity, and a P value <0.05 was considered significant. All the values are expressed as means ± SE.

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 $S1_{0.2mV}$ was used, SICF (especially at 1.5 and 3.0 ms) were more enhanced than those with $S1_{0.5mV}$. 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 [$F(1.53,13.78) = 7.42$, $P = 0.010$ 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 $S1_{0.5mV}$ and $S1_{0.2mV}$ 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

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

	CS	S1	S2	Test MEP Size, mV	CS-S1 MEP Size, mV
Experiment 1	80% AMT	$S1_{0.5mV}$	—	0.50 ± 0.04	0.37 ± 0.04
	100% AMT	$S1_{0.5mV}$	—	0.52 ± 0.03	0.21 ± 0.04
	120% AMT	$S1_{0.5mV}$	—	0.52 ± 0.05	0.32 ± 0.05
Experiment 2	—	$S1_{0.5mV}$	120% AMT	0.52 ± 0.03	—
	—	$S1_{0.5mV}$	140% AMT	0.47 ± 0.04	—
	—	$S1_{0.2mV}$	120% AMT	0.23 ± 0.03	—
	—	$S1_{0.2mV}$	140% AMT	0.20 ± 0.03	—

MEP, motor evoked potential.

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

	CS	S1	S2	Test MEP Size, mV	CS-S1 MEP Size, mV
Experiment 3	—	$S1_{0.3mV}$	120% AMT	0.31 ± 0.05	—
	—	$S1_{0.3mV}$	140% AMT	0.29 ± 0.04	—
	80% AMT	$S1_{0.5mV}$	120% AMT	0.49 ± 0.02	0.38 ± 0.03
	100% AMT	$S1_{0.5mV}$	120% AMT	0.48 ± 0.04	0.25 ± 0.05
	120% AMT	$S1_{0.5mV}$	120% AMT	0.51 ± 0.04	0.29 ± 0.04
	80% AMT	$S1_{0.5mV}$	140% AMT	0.50 ± 0.03	0.38 ± 0.04
	100% AMT	$S1_{0.5mV}$	140% AMT	0.53 ± 0.04	0.22 ± 0.04
	120% AMT	$S1_{0.5mV}$	140% AMT	0.47 ± 0.05	0.28 ± 0.04
Experiment 4	100% AMT	$S1_{0.5mV}$	140% AMT	0.48 ± 0.05	0.42 ± 0.04
	120% AMT	$S1_{0.5mV}$	140% AMT	0.47 ± 0.06	0.43 ± 0.11

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. 4*C*). 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 $S1_{0.3mV}$ 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 ($S1_{0.3mV}$). In contrast, the *right traces* from the triple-pulse stimulation using 120% AMT CS, $S1_{0.5mV}$, 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 $S1_{0.5mV}$ in Fig. 5), the sessions using 100% AMT CS and 120% AMT CS were not compared with SICF alone using $S1_{0.5mV}$ but with SICF alone using $S1_{0.2mV}$ or $S1_{0.3mV}$. 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. 4*F*]. 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.

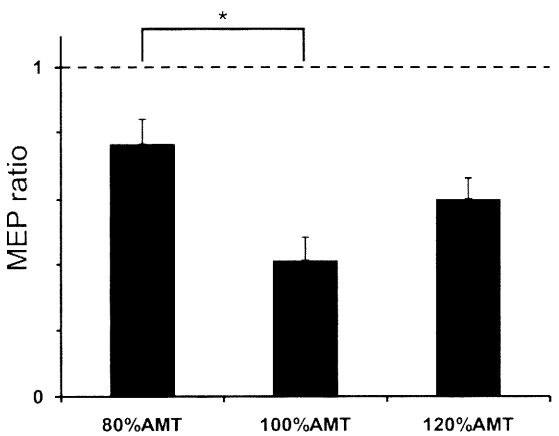

FIG. 2. Conditioning stimulus intensity dependency of SICI. Means and SEs of SICI in different CS intensities: 80% active motor threshold (AMT), 100% AMT, and 120% AMT. SICI was normalized as a ratio of conditioned motor evoked potential (MEP) to test MEP. SICI using 100% AMT CS showed the most profound inhibition. *, statistical significance ($P < 0.05$).

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 [$F(2.2,15.7) = 4.15$, $P = 0.032$; Fig. 6*B*]. 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. 4*E*). 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. 4*F*).

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

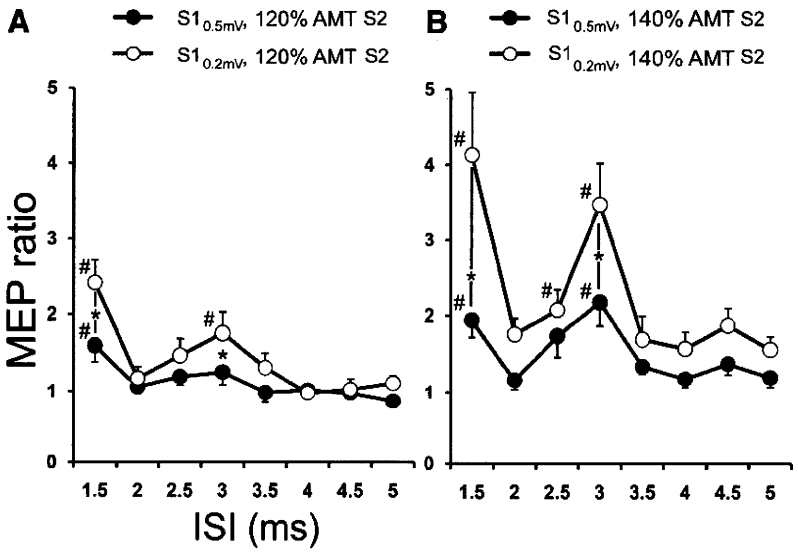

FIG. 3. Mean time courses of SICF in different S1 or S2 intensities. The degree of SICF (the ordinate) is shown as the ratio of the mean conditioned MEP size to the unconditioned MEP size for each ISI (the abscissa). The time courses using S2 of 120% AMT (*A*) are less facilitatory than those using S2 of 140% AMT (*B*). The SICF using $S1_{0.2mV}$ (○) is more facilitatory than that using $S1_{0.5mV}$ (●). Error bars represent SE. *, statistical significance between $S1_{0.5mV}$ and $S1_{0.2mV}$ ($P < 0.05$). #, significant facilitation ($P < 0.05$) of the conditioned responses compared with the unconditioned ones.

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

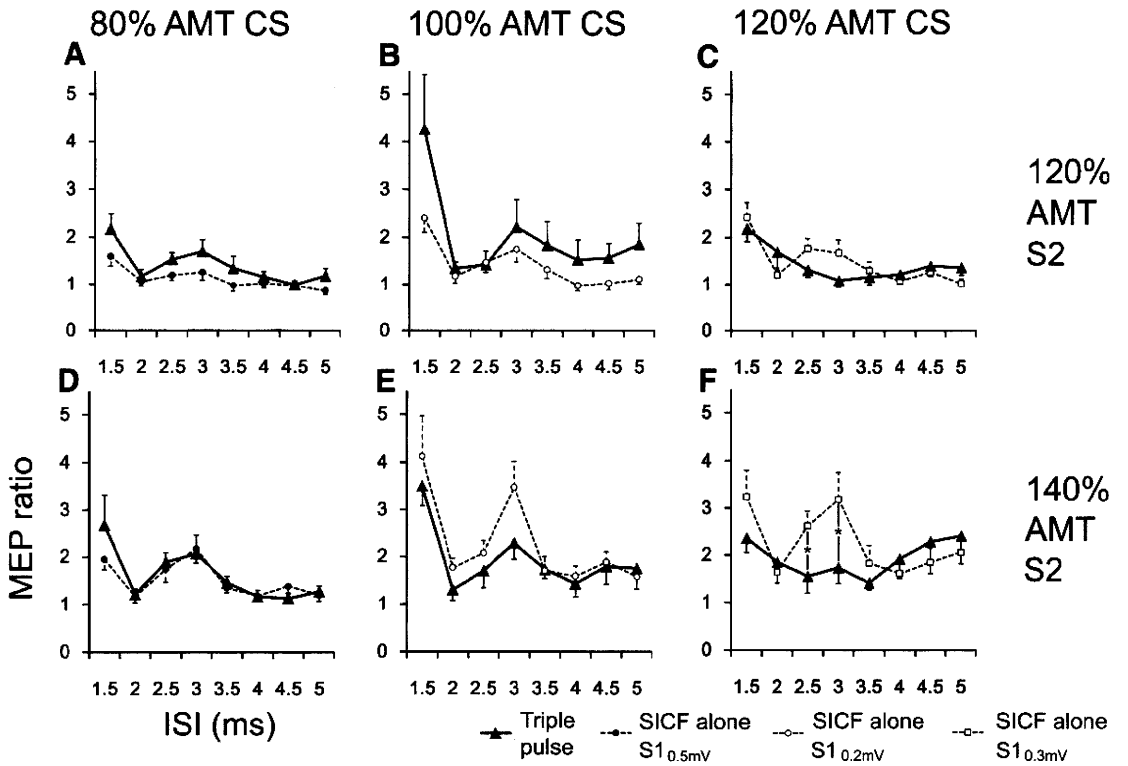

FIG. 4. Mean time courses of triple-pulse stimulation at CS-S1 ISI of −3 ms. ▲—▲, the time course of the triple-pulse stimulation in *experiment 3*. *A–C*: the results with S2 of 120% AMT; CS intensities are 80% AMT (*A*), 100% AMT (*B*), and 120% AMT (*C*). *D–F*: results with S2 of 140% AMT; CS intensities are 80% AMT (*D*), 100% AMT (*E*), and 120% AMT (*F*). ●- - -●, SICF alone using $S1_{0.5mV}$ with S2 of 120% AMT (*A*) or 140% AMT (*D*). ○- - -○, SICF alone using $S1_{0.2mV}$ with S2 of 120% AMT (*B*) or 140% AMT (*E*). □- - -□, SICF alone using $S1_{0.3mV}$ with S2 of 120% AMT (*C*) or 140% AMT (*F*). The abscissa denotes ISI. The ordinate shows the MEP size ratio. Error bars represent SE. *, statistical significance ($P < 0.05$).