Conditioning intensity dependent interaction between short-latency interhemispheric inhibition and short-latency afferent inhibition

Authors

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Author contributions

All the authors were involved in the conception and design of the experiments. R.T. collected, analyzed and interpreted the data, and drafted the first version of the manuscript. Y.S., S.O., Y.T., and R.H. assisted in data collection. All the authors revised the manuscript critically for important intellectual content and approved the final version.

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Interaction between SIHI and SAI

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Abstract

Relation between sensory and transcallosal inputs into the motor cortex may be important in motor performance, but it has not been well studied, especially in humans. The aim of this paper is to reveal this relation by investigating the interaction between short-latency interhemispheric inhibition (SIHI) and short-latency afferent inhibition (SAI) in humans using transcranial magnetic stimulation. SIHI is the inhibition of the primary motor cortex (M1) elicited by the contralateral M1 stimulation given at around 10 ms before, and it reflects the transcallosal inhibition. SAI is the inhibition of M1 elicited by the contralateral median nerve stimulation preceding M1 stimulation by around 20 ms. In this investigation, we studied the intensity dependency of SIHI and SAI, and the interaction between SIHI and SAI in various conditioning intensities. Subjects were eleven normal volunteers. The degree of effects was evaluated by comparing motor evoked potential sizes recorded from the first dorsal interosseous muscle between a certain condition and control condition. Both the SIHI and SAI were potentiated by increment of the conditioning stimulus intensity, and saturated at 1.4 times resting motor threshold for SIHI and 3 times sensory threshold for SAI. No significant interaction was observed when either of their intensities was subthreshold for the inhibition on its own. Only when both intensities were strong enough for their inhibition, the presence of one inhibition lessened the other one. Based on these, we conclude that interneurons mediating SIHI and SAI have mutual, direct and inhibitory
interaction in a conditioning intensity dependent manner.

(249 words)

Kew words

transcranial magnetic stimulation, primary motor cortex, transcallosal inhibition, motor evoked potential, interneuron
Introduction

For purposeful movements, the primary motor cortex (M1) is modulated by a combination of many kinds of modulatory inputs, such as somatosensory, visual, auditory, cerebellar, basal ganglia inputs, and so on. Those inputs should influence M1 independently and also interactively. Somatosensory inputs to M1 are suggested to have some relations with transcallosal inputs from the contralateral M1. For example, short-term deprivation of sensory input decreased interhemispheric inhibition (IHI) (Werhahn et al. 2002). Moreover, in chronic stroke patients with unilateral hand weakness, cutaneous anesthesia of the normal hand produced transient site-specific improvement in motor performance of the paretic hand (Floel et al. 2004). These lead us to investigate the relation between sensory and transcallosal inputs at M1.

Transcranial magnetic simulation (TMS) is a non-invasive method to stimulate the human brain and to study its function. Many inhibitory circuits affecting M1 are studied by TMS. IHI represents the inhibition of one M1 by the contralateral M1 through the corpus callosum (Ferbert et al. 1992). Two phases of IHI are known, namely, short-latency IHI (SIHI) at ISI of ~10ms and long-latency IHI (LIHI) at ISI of 40–50ms, and these two IHIs are supposed to be mediated by different neuronal populations (Chen et al. 2003; Kukaswadia et al. 2005; Ni et al. 2009). We used SIHI in this study since we considered its short-acting, direct interaction with short-latency afferent inhibition (SAI). SAI represents M1 inhibition by the peripheral
somatosensory inputs at ISI of ~20ms (Tokimura et al. 2000). There are still several
other inhibitory influences from other areas.

The interactions between those inhibitory circuits have not been studied precisely even
though some of them have been studied. For instance, short-interval intracortical
inhibition (SICI) was reduced in the presence of SIHI and SIHI was reduced in the
presence of long-interval intracortical inhibition (LICI) (Daskalakis et al. 2002).

Recent papers reported the bidirectional inhibitory relation between SICI and SAI
(Alle et al. 2009), and LICI and SAI (Udupa et al. 2009).

We hypothesize that SIHI and SAI have inhibitory interaction to each other in light of
the previous studies. However, there is a concern that occlusion or saturation of the
inhibitory effects present similar results. For this purpose, we studied the stimulation
intensity dependency of the interaction between SIHI and SAI using six different
conditioning intensity combinations. Previous studies showed the effects of
conditioning stimulus (CS) intensities on IHI (Ni et al. 2009), CS intensities on SAI
(Fischer and Orth 2011), and the effects of test stimulus (TS) and CS intensities on SAI
(Ni et al. 2011). We chose CS intensities for SIHI and SAI to induce both weak and
saturate inhibition in the experiments.

Methods
Subjects

We studied 11 right-handed healthy volunteers [2 women and 9 men; aged 37.5 ± 7.8 (SD), range: 29–49 years old], who gave their written informed consent to participate in the experiments. No participants had neurological, psychiatric, or other medical problems, or had any contra-indication to TMS. The protocol was approved by the Ethics Committee of the University of Tokyo in accordance with the ethical standards of the Declaration of Helsinki on the use of human subjects in experiments.

Recordings

Motor evoked potentials (MEPs) were recorded from the bilateral first dorsal interosseous (FDI) muscle with 9 mm diameter Ag/AgCl surface electrodes placed with a belly-tendon montage. Responses were input to an amplifier (Biotop; GE Marquette Medical Systems Japan, Japan) through filters set at 100 Hz and 3 kHz. They were then digitized with a sampling rate of 10 kHz and stored in a computer for later offline analyses (TMS bistim tester; Medical Try System, Japan).

Transcranial magnetic stimulation

Throughout the experiments described below, subjects were seated on a comfortable chair and the FDI muscles were relaxed, as confirmed by an oscilloscope monitor. For TMS stimulation, monophasic TMS pulses were delivered by magnetic stimulators
(Magstim 200; Magstim Co., Whitland, Dyfed, UK). The intervals between the trials were set at 6 ± 0.5 s. In advance, we measured the resting and active motor thresholds (RMT and AMT) for the test (left) hemisphere and RMT for the conditioning (right) hemisphere. RMT was determined as the lowest stimulator output intensity capable of eliciting MEPs of 50 μV peak-to-peak amplitude in the relaxed FDI muscle in over 5 of 10 consecutive trials. AMT was determined as the lowest stimulator output intensity to evoke MEPs of 100 μV peak-to-peak amplitude when the participant maintained a very slight contraction of FDI muscle (5-10% of the maximum voluntary contraction) in over 5 of 10 consecutive trials.

**Study design**

We first studied the effects of TS and CS intensities on the amount of SIHI (Experiment 1) and SAI (Experiment 2) respectively. Then we studied the interaction between SIHI and SAI by applying both stimuli together (Experiment 3). We applied different conditioning intensities to compare the effects of each conditioning stimulus (Figure 1).

**Experiment 1: Short-latency interhemispheric inhibition (SIHI)**

All 11 subjects participated in this experiment. TS was given over the left M1 and CS over the right M1 (CCS) preceding TS by 10 ms. For both stimuli, we used a
figure-of-eight coil (outer diameter of each wing was 9 cm) positioned over the optimum point for FDI (about 5 cm lateral to the vertex). The coil for TS was placed tangentially over the scalp and angled 45° to the parasagittal plane so that currents flowed in an anteromedial-to-posterolateral direction at the center of the coil. The coil for CS was set towards the sagittal plane so that currents flowed in a medial-to-lateral direction at the center of the coil. The intensity of TS was adjusted to elicit 0.6 mV (TS0.6mV) or 0.3 mV (TS0.3mV) peak-to-peak amplitude in the relaxed muscles on average when they were given alone, and CS was set at 1 (CCS1), 1.2 (CCS1.2), 1.4 (CCS1.4), or 1.6 (CCS1.6) times of the right M1 RMT.

Experiment 2: Short-latency afferent inhibition (SAI)

Seven among 11 subjects [1 woman and 6 men; aged 37.3 ± 7.3 (SD), range: 29–46 years old] were involved in Experiment 2. TS was TMS over the left M1, and CS was the right median nerve stimulation (MNS) at the wrist. The median nerve was stimulated with a 0.2 ms duration square-wave electric pulses (cathode proximal) preceding TS. The interstimulus interval (ISI) between CS and TS was set individually in 2 ms stepwise at the timing where the amount of SAI was maximal. ISI ranged between 18 to 22 ms and the average was 19.8 ± 1.1 ms (SD). We measured the sensory threshold (ST) for the median nerve stimulation in advance. The intensity of TS was adjusted at TS0.6mV or TS0.3mV the same as Experiment 1, and CS was set at
1.5 (MNS1.5), 3 (MNS3), or 4.5 (MNS4.5) times of the right median nerve ST.

Experiment 3: Interaction between SIHI and SAI

All 11 subjects participated in this experiment. The TS and CS were the same as described in Experiments 1 and 2. TS intensity was set at TS0.6mV. We used six different CS intensity combinations: namely CCS1+MNS1.5, CCS1.2+MNS1.5, CCS1.4+MNS1.5, CCS1+MNS3, CCS1.2+MNS3, and CCS1.4+MNS3 (Figure 1). For each combination, we applied eight different configurations (Table 1). In configurations 3A to 3D, the effects of SIHI and SAI on TS0.6mV were assessed, whereas in configurations 3E to 3H, TS intensity was decreased in order to adjust the MEP size for later analysis. In 3E and 3F, we adjusted the TS intensity (0.6mV_{MNS}) which can induce similar MEP size to conditioned MEP by MNS alone in 3C. In 3G and 3H, we adjusted the TS intensity (0.6mV_{CCS}) which can induce similar MEP size to conditioned MEP by CCS alone in 3B. Configuration 3A to 3D, 3E and 3F, 3G and 3H were done separately because of the difference in TS intensities.

Data analysis

In each experiment, 15 conditioned trials for each condition were randomly intermixed with 15 unconditioned trials in which TS was delivered alone. The ratio of the mean peak-to-peak amplitude of conditioned MEPs to that of unconditioned MEPs (MEP
size ratio) was calculated for each condition in each subject. To ensure that the experiments were not affected by the difference of test MEP size between conditions, we applied one-way repeated measures analysis of variance (ANOVA) using condition as a within-subject factor in each experiment.

Experiments 1 and 2

We used two-way repeated measures ANOVA using TS and CS both as a within-subject factor. Post hoc analysis with Bonferroni correction for multiple comparisons was applied between the CS intensities afterwards. When necessary, Greenhouse-Geisser correction was used to correct for non-sphericity, which is mentioned in the Results section if used.

Experiment 3

To evaluate the effect of SAI on SIHI, we aimed to compare 'SIHI without SAI' and 'SIHI with SAI'. 'SIHI without SAI' means normal SIHI and the values were calculated from the MEP size ratio by configuration 3F (CCS) /3E (TS 0.6mV_{MNS}). 'SIHI with SAI' used both conditioning stimuli (CCS and MNS) and the values were calculated from the MEP size ratio by 3D (CCS+MNS) /3C (MNS) because we needed to compare the SIHI values when calculating the effect of SAI on SIHI. The reason for using adjusted TS (3E) was to equalize the MEP size of the denominators. First, adjusted test MEP amplitudes (3C and 3E) for each condition were compared using paired $t$-tests. Then we applied two-way repeated measures ANOVA using '6
conditions' and 'existence of MNS (without SAI or with SAI)' as a within-subject factor.

Post hoc analysis with Bonferroni correction for multiple comparisons was applied to compare the effect of SAI within the same condition group afterwards.

Vice versa, to evaluate the effect of SIHI on SAI, we aimed to compare 'SAI without SIHI' and 'SAI with SIHI'. 'SAI without SIHI' means normal SAI and the values were calculated from the MEP size ratio by configuration 3H (MNS) /3G (TS 0.6mV_{CCS}). 'SAI with SIHI' used both conditioning stimuli and the values were calculated from the MEP size ratio by 3D (CCS+MNS) /3B (CCS) because we needed to compare the SAI values when calculating the effect of SIHI on SAI. We compared the adjusted test MEP amplitudes (3B and 3G) for each condition using paired \( t \)-tests. We then applied two-way repeated measures ANOVA using '6 conditions' and 'existence of CCS (without SIHI or with SIHI)' as a within-subject factor. Post hoc analysis with Bonferroni correction for multiple comparisons was applied to compare the effect of SIHI within the same condition group afterwards.

**Correlation analysis**

To evaluate the correlation between the strength of interaction and the amount of SIHI or SAI, we performed multiple regression analyses. We calculated the strength of the interaction as the difference of the MEP size ratio. For the effect of SAI on SIHI, \( \Delta \text{SIHI} = (3D/3C) - (3F/3E) \) was used, and for the effect of SIHI on SAI, \( \Delta \text{SAI} = (3D/3B) - (3H/3G) \) was used. Criterion variables were \( \Delta \text{SIHI} \) or \( \Delta \text{SAI} \), where
explanatory variables were 3F/3E for SIHI and 3H/3G for SAI.

Statistical analyses were performed using PASW Statistics 18.0.0 (IBM Corporation, NY, USA). P-value less than 0.05 were judged as significant.

Results

The average left M1 RMT was 48.3 ± 8.8% (SD) of maximum stimulus output (MSO), left M1 AMT was 32.6 ± 6.6%MSO, and right M1 RMT was 55.4 ± 10.1%MSO.

Test MEP amplitudes did not differ significantly between any conditions in every experiments ($p_s > 0.56$, Table 2). In experiment 3, adjusted test MEP amplitudes in each condition did not differ significantly between ‘SIHI without SAI (3E: TS0.6mV_MNS)’ and ‘SIHI with SAI (3C: TS0.6mV+MNS)’ ($p_s > 0.60$, Table 2), and between ‘SAI without SIHI (3G: TS0.6mV_CCS)’ and ‘SAI with SIHI (3B: TS0.6mV+CCS)’ ($p_s > 0.67$, Table 2). In experiment 1 and 3, the amplitude of MEPs contralateral to the target muscle increased as the CCS intensity increased ($p_s < 0.01$, data not shown).

Experiment 1: Effect of TS and CS intensities on SIHI

Figure 2 shows the effect of TS and CS intensities on SIHI. There was a significant effect of CS intensity on the amount of SIHI [$F (3,30) = 26.3, p < 0.001$, partial $\eta^2 =$}
There was a tendency of the effect of TS intensity \( F(1,10) = 3.9, p = 0.08, \) partial \( \eta^2 = 0.28 \), and no significant two-factor interaction between TS and CS intensities \( F(3,30) = 0.09, p = 0.96, \) partial \( \eta^2 = 0.01 \). Post hoc analyses revealed significant differences between CCS1 and CCS1.2 \( (p = 0.006) \), CCS1.2 and CCS1.4 \( (p = 0.02) \), but no significant differences between CCS1.4 and CCS1.6 \( (p = 1.00) \). The stimulus intensities of the TS were 67.9 ± 4.4%MSO (equivalent to 140.9%RMT) for TS0.6mV and 61.8 ± 4.6%MSO (127.2%RMT) for TS0.3mV, respectively.

**Experiment 2: Effect of TS and CS intensities on SAI**

Figure 3 shows the effect of TS and CS intensities on SAI. There was a significant effect of CS intensity on the amount of SAI \( F(2,12) = 28.9, p < 0.001, \) partial \( \eta^2 = 0.83 \). There was no significant effect of TS intensity \( F(1,6) = 0.03, p = 0.86, \) partial \( \eta^2 = 0.01 \), and no significant two-factor interaction between TS and CS intensities \( F(2,12) = 0.11, p = 0.76, \) partial \( \eta^2 = 0.02 \). Greenhouse-Geisser correction was only applied for interaction between TS and CS. Post hoc analyses revealed significant differences between MNS1.5 and MNS3 \( (p = 0.003) \), but no significant differences between MNS3 and MNS4.5 \( (p = 0.85) \). The stimulus intensities of the TS were 67.3 ± 4.7%MSO (139.2%RMT) for TS0.6mV and 63.3 ± 4.4%MSO (130.7%RMT) for TS0.3mV, respectively.
Experiment 3:

Effect of SAI on SIHI

Representative waveforms of one subject (condition CCS1.4+MNS3) are shown in Figure 4. ‘SIHI with SAI’ is the MEP size ratio of (c) / (b) (=configuration 3D/3C), whereas ‘SIHI without SAI’ (normal SIHI) is that of (e) / (d) (=configuration 3F/3E), with MEP size equalized between (b) and (d). The averaged data for each condition are shown in Figure 5. There was a significant effect of condition on the amount of SIHI $[F(5,50) = 10.5, p < 0.001, \eta^2 = 0.51]$ and a tendency of the effect of SAI $[F(1,10) = 4.7, p = 0.055, \eta^2 = 0.32]$. Two-factor interaction between condition and SAI was significant $[F(5,50) = 2.9, p = 0.023, \eta^2 = 0.23]$. Post hoc analyses revealed significant effect of SAI on SIHI at two conditions: CCS1.2+MNS3 and CCS1.4+MNS3 (Table 3A).

Effect of SIHI on SAI

The averaged data for each condition are shown in Figure 6. There was a significant effect of condition on the amount of SAI $[F(5,50) = 5.9, p = 0.01, \eta^2 = 0.37]$ and a tendency of the effect of SIHI $[F(1,10) = 4.4, p = 0.063, \eta^2 = 0.31]$. Two-factor interaction between condition and SIHI was not significant $[F(5,50) = 1.1, p = 0.39, \eta^2 = 0.10]$. Post hoc analyses revealed significant effect of SIHI on SAI at two conditions: CCS1.2+MNS3 and CCS1.4+MNS3 (Table 3B).

Correlation analysis
For the effect of SAI on SIHI, multiple regression analysis revealed a significant effect of the model \[ F(2,63) = 28.4, p < 0.001 \]. Standardized partial regression coefficient values were -0.68 for SIHI \( p < 0.001 \) and -0.04 for SAI \( p = 0.71 \). Multiple correlation coefficient was 0.69 and the coefficient of determination was 0.46. For the effect of SIHI on SAI, multiple regression analysis revealed a significant effect of the model \[ F(2,63) = 12.9, p < 0.001 \]. Standardized partial regression coefficient values were -0.28 for SIHI \( p = 0.01 \) and -0.42 for SAI \( p < 0.001 \). Multiple correlation coefficient was 0.54 and the coefficient of determination was 0.29. The scatter chart of each element is shown in Figure 7.

**Discussion**

In the present study, we studied the interaction between SIHI and SAI. In their relation, the presence of one inhibition lessened the other one when the CS intensity was strong enough to elicit their own effect for both of them.

**Conditioning intensity dependency of SIHI and SAI**

For SIHI, CS is needed to be suprathreshold, where the higher CS elicited deeper inhibition and it was saturated at 1.4 RMT. Stronger CS induced greater SIHI similarly to a previous (Ni et al. 2009). The larger MEPs to higher TS tended to be less inhibited,
but the difference was statistically insignificant. These findings of TS effects also agree
with previous studies (Ferbert et al. 1992; Daskalakis et al. 2002; Kukaswadia et al. 2005). Experiment 2 showed that the higher conditioning stimulus intensity evoked
deeper SAI and saturated at 3 ST. This finding is consistent with previous studies (Fischer and Orth 2011; Ni et al. 2011). TS intensity range we used had no effect on SAI.

**Interaction between SIHI and SAI**

The presence of SIHI lessened SAI effect under the conditions of CCS1.2+MNS3 and CCS1.4+MNS3. The reverse reductive interaction was true under the same two conditions. These two CS intensities were strong enough to elicit definite inhibitory effects. In the other all conditions, the conditioning stimuli must be not strong enough for inducing a significant interaction.

There was a bidirectional inhibitory interaction between SIHI and SAI. This interaction was produced only when CS was strong enough to evoke sufficient inhibition. The most plausible explanation for this interaction is that the interneurons for SIHI and SAI have a direct, mutual, suppressive interaction. Similar direct inhibitory interaction is suggested in previous studies of other interactions, such as SAI and LICI (Udupa et al. 2009), SAI and SICI (Alle et al. 2009), and long-interval interhemispheric inhibition and LICI (Udupa et al. 2010). One new point of our results is that the interaction
between SIHI and SAI was CS intensity dependent. This was reinforced by the
correlation analysis, which showed significant correlation between the amount of SIHI
and the effect of SAI on SIHI. Also, there was a significant correlation between the
effect of SIHI on SAI and either the amount of SIHI or SAI. Lack of significant
correlation between the amount of SAI and the effect of SAI on SIHI suggests a
weaker effect of SAI on the interaction than SIHI. This may suggest a dominancy of
SIHI in the interaction between SIHI and SAI.

Another possible explanation might be occlusion or saturation of inhibitory effect. If
the inhibition produced by one system is almost maximal, the other inhibitory system
may not add more inhibition when the two inhibitory interneurons are merged to the
common neuron somewhere in the cortical circuit. The fact that the interaction was
seen only when the CS intensities were strong enough supports this idea. This could
explain why interaction was not observed in other conditions, because the total
inhibitory effect did not reach the maximum. Under this hypothesis, if the CS intensity
was weaker such as CCS1.2+MNS3, the interaction should have been weaker than the
strong condition such as CCS1.4+MNS3. Our result showed that the interaction was
similar between the two conditions. This indicates that the interaction between SIHI
and SAI is not likely explained only by occlusion or saturation, although they might
partly contribute to the interaction.

The physiological meaning of the present mutual suppressive interaction between SIHI
and SAI, the two inhibitory circuits, may be the escape from excessive inhibition of M1. Concerning SIHI and SAI, mediating SICI and LICI, all interactions that have been studied show inhibitory effects; SIHI reduces SICI, SICI reduces SAI, SAI reduces SIHI, and SAI reduces LICI, LICI reduces SIHI, SIHI reduces SAI (Daskalakis et al. 2002; Alle et al. 2009; Udupa et al. 2009). This suggests a universal machine to avoid excessive inhibition, rather than a one-way interaction for a specific circuit. Although we suppose this mutual suppressive interaction, unfortunately we do not know its precise mechanism. Further investigation is needed to pursue the mechanism and the physiological meaning of this interaction.

There are several limitations in the present study. One is LIHI. LIHI is supposed to be mediated by different neuronal populations from those for SIHI. SIHI and LIHI had different interactions with the long latency afferent inhibition (Kukaswadia et al. 2005). This indicates that SIHI and LIHI may interact with SAI differently. Further interaction study between LIHI and SAI may help us to interpret the intracortical circuits.

Another limitation is the hemispheric difference. We used the dominant (left) hemisphere for the experiment. IHI comparing both sides of the hemisphere has been reported. A few papers reported that the dominant hemisphere inhibited the non-dominant hemisphere through corpus callosum more than the other way round (Netz et al. 1995; Bäumer et al. 2007), whereas others reported no difference between the hemispheres (Salerno and Georgesco et al. 1996; De Gennaro et al. 2004; Nelson et
al. 2009). Based on these, we need to study the interaction between IHI and SAI on the non-dominant hemisphere in understanding the hemispheric asymmetry in the near future.

In conclusion, SIHI and SAI interacted only when both conditioning intensities were strong enough and the presence of one inhibition lessened the other one. Interneurons mediating SIHI and SAI have mutual, direct and inhibitory interaction in a conditioning intensity dependent manner.

(3500 words)
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Fischer M, Orth M. Short-latency sensory afferent inhibition: conditioning stimulus intensity, recording site, and effects of 1 Hz repetitive TMS. Brain Stimul 2011;4:202-209.


Figure Captions

**Figure 1.** Study design and conditioning parameters of the experiments. Test stimulus (TS) intensity is shown as the average amplitude of the motor evoked potential by transcranial magnetic stimulation (TMS) on left motor cortex (M1). Conditioning stimulus (CS) intensity to the contralateral (right) hemisphere (CCS) is based on the resting motor threshold (RMT). CS intensity to the contralateral (right) median nerve (MNS) is based on the sensory threshold (ST).

**Figure 2.** Effect of test and conditioning stimulus (TS and CS) intensities on short-latency interhemispheric inhibition (SIHI). CS intensity had a significant effect on SIHI, whereas TS intensity had no significant effect. The vertical axis shows the motor evoked potential (MEP) size ratio. Error bars show the standard errors. RMT; resting motor threshold for right motor cortex. * $p < 0.05$, ** $p < 0.01$

**Figure 3.** Effect of test and conditioning stimulus (TS and CS) intensities on short-latency afferent inhibition (SAI). CS intensity had a significant effect on SAI, whereas TS intensity had no significant effect. The vertical axis shows the motor evoked potential (MEP) size ratio. Error bars show the standard errors. ST; sensory threshold for right median nerve stimulation. ** $p < 0.01$
Figure 4. Representative motor evoked potential (MEP) waveforms recorded from right first dorsal interosseous muscle of one subject in experiment 3. Effect of short-latency afferent inhibition (SAI) on short-latency interhemispheric inhibition (SIHI) is shown. (a) Test stimulus (TS) alone to evoke 0.6 mV MEP (Configuration 3A); (b) MNS was applied with TS0.6mV (Configuration 3C); (c) CCS and MNS were both applied with TS0.6mV (Configuration 3D); (d) TS0.6mV MNS (TS which evoke MEP amplitude equivalent to the MEP evoked by TS0.6mV with MNS (=b)) alone (Configuration 3E); (e) CCS was applied with TS0.6mV MNS (Configuration 3F). Conditioning stimulus (CS) were 1.4 resting motor threshold for CCS and 3 sensory threshold for MNS. The value for ‘SIHI with SAI’ calculated from the MEP size ratio by (c) / (b) was larger than the value for ‘SIHI without SAI’ calculated from the MEP size ratio by (e) / (d). The MEP size of the denominators (b and d) were equalized by adjusting the TS.

Figure 5. Effect of short-latency afferent inhibition (SAI) on short-latency interhemispheric inhibition (SIHI). Overall analysis showed a tendency of the effect of SAI on SIHI. Post hoc analyses revealed significant effect of SAI at Condition CCS1.2+MNS3 and CCS1.4+MNS3. The vertical axis shows the motor evoked potential (MEP) size ratio. Error bars show the standard errors. * p < 0.05
Figure 6. Effect of short-latency interhemispheric inhibition (SIHI) on short-latency afferent inhibition (SAI). Overall analysis showed a tendency of the effect of SIHI on SAI. Post hoc analyses revealed significant effect of SIHI at Condition CCS1.2+MNS3 and CCS1.4+MNS3. The vertical axis shows the motor evoked potential (MEP) size ratio. Error bars show the standard errors. * $p < 0.05$

Figure 7. Correlation between the strength of interaction and the amount of short-latency afferent inhibition (SIHI) or short-latency afferent inhibition (SAI). The trend indicated that stronger SIHI or SAI had more interaction between SIHI and SAI. Filled triangles show SIHI with full line drawn as the linear regression line. Circles show SAI with dashed line drawn as the linear regression line. The horizontal axis shows the motor evoked potential (MEP) size ratio of SIHI or SAI. The vertical axis shows the change of MEP size ratio from ‘SIHI without SAI’ to ‘SIHI with SAI’ ($\Delta$ SIHI, left), or ‘SAI without SIHI’ to ‘SAI with SIHI’ ($\Delta$SAI, right).
Table 1. Study design for Experiment 3

<table>
<thead>
<tr>
<th>Configuration</th>
<th>TS (left M1)</th>
<th>CCS (right M1)</th>
<th>MNS (right median nerve)</th>
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</thead>
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<tr>
<td>3A</td>
<td>0.6 mV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3B</td>
<td>0.6 mV</td>
<td>1/1.2/1.4 RMT</td>
<td>-</td>
</tr>
<tr>
<td>3C</td>
<td>0.6 mV</td>
<td>-</td>
<td>1.5/3 ST</td>
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<tr>
<td>3D</td>
<td>0.6 mV</td>
<td>1/1.2/1.4 RMT</td>
<td>1.5/3 ST</td>
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<tr>
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<td>0.6 mV_{MNS}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3F</td>
<td>0.6 mV_{MNS}</td>
<td>1/1.2/1.4 RMT</td>
<td>-</td>
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<tr>
<td>3G</td>
<td>0.6 mV_{CCS}</td>
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<td>-</td>
</tr>
<tr>
<td>3H</td>
<td>0.6 mV_{CCS}</td>
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<td>1.5/3 ST</td>
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</tbody>
</table>

In configurations A to D, the effects of short-latency interhemispheric inhibition (SIHI) and short-latency afferent inhibition (SAI) were studied with TS0.6mV, which means test stimulus (TS) intensity is set to evoke 0.6 mV motor evoked potential (MEP). In configurations 3E and 3F, TS0.6mV_{MNS} was used which means that TS is set to evoke MEP amplitude equivalent to the MEP evoked by TS0.6mV with MNS (configuration 3C). In configurations 3G and 3H, TS0.6mV_{CCS} was used which means that TS is set to evoke MEP amplitude equivalent to the MEP evoked by TS0.6mV with CCS (configuration 3B). M1, primary motor cortex; CCS, conditioning TMS to contralateral hemisphere; MNS, conditioning electrical stimulation to contralateral median nerve;
RMT, resting motor threshold for contralateral M1; ST, sensory threshold of the contralateral median nerve
Table 2. Test motor evoked potential amplitudes for each condition

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>CCS1</th>
<th>CCS1.2</th>
<th>CCS1.4</th>
<th>CCS1.6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS0.6mV</td>
<td>0.58 ± 0.04</td>
<td>0.60 ± 0.03</td>
<td>0.60 ± 0.04</td>
<td>0.59 ± 0.07</td>
<td>0.96</td>
</tr>
<tr>
<td>TS0.3mV</td>
<td>0.31 ± 0.04</td>
<td>0.29 ± 0.02</td>
<td>0.31 ± 0.03</td>
<td>0.31 ± 0.03</td>
<td>0.82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>MNS1.5</th>
<th>MNS3</th>
<th>MNS4.5</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS0.6mV</td>
<td>0.62 ± 0.03</td>
<td>0.65 ± 0.05</td>
<td>0.58 ± 0.12</td>
<td>0.83</td>
</tr>
<tr>
<td>TS0.3mV</td>
<td>0.36 ± 0.04</td>
<td>0.31 ± 0.01</td>
<td>0.33 ± 0.04</td>
<td>0.56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 3</th>
<th>CCS1+MNS1</th>
<th>CCS1.2+MN</th>
<th>CCS1.4+MN</th>
<th>CCS1+MNS3</th>
<th>CCS1.2+MN</th>
<th>CCS1.4+MN</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>.5</td>
<td>S1.5</td>
<td>S1.5</td>
<td>S3</td>
<td>S3</td>
<td>P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS0.6mV</td>
<td>0.52 ± 0.04</td>
<td>0.56 ± 0.04</td>
<td>0.60 ± 0.04</td>
<td>0.58 ± 0.04</td>
<td>0.56 ± 0.04</td>
<td>0.57 ± 0.04</td>
<td>0.66</td>
</tr>
<tr>
<td>(intensity)</td>
<td>(65.8 ± 4.3%)</td>
<td>(66.0 ± 4.5%)</td>
<td>(64.2 ± 4.8%)</td>
<td>(66.9 ± 4.2%)</td>
<td>(67.7 ± 4.7%)</td>
<td>(67.2 ± 5.0%)</td>
<td></td>
</tr>
<tr>
<td>TS0.6mV+MNS</td>
<td>0.46 ± 0.05</td>
<td>0.46 ± 0.06</td>
<td>0.52 ± 0.06</td>
<td>0.35 ± 0.04</td>
<td>0.30 ± 0.04</td>
<td>0.30 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>(intensity)</td>
<td>(63.8 ± 4.3%)</td>
<td>(62.7 ± 4.5%)</td>
<td>(65.4 ± 5.0%)</td>
<td>(63.0 ± 4.5%)</td>
<td>(63.8 ± 5.4%)</td>
<td>(61.6 ± 4.8%)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.60</td>
<td>0.88</td>
<td>0.96</td>
<td>0.83</td>
<td>0.92</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

| TS0.6mV+CCS | 0.45 ± 0.05 | 0.40 ± 0.04 | 0.36 ± 0.04 | 0.51 ± 0.05 | 0.40 ± 0.04 | 0.34 ± 0.04 |
| (intensity) | (63.8 ± 4.8%) | (62.9 ± 4.0%) | (62.7 ± 4.3%) | (68.0 ± 4.8%) | (63.4 ± 4.4%) | (64.5 ± 4.7%) |
| P value     | 0.81     | 0.72     | 0.67     | 0.69     | 0.93     | 0.79     |
Values (mV) are shown as means ± SE. Stimulus intensities are shown by the percentage of maximum stimulus output. TS, test TMS; CCS, conditioning TMS to contralateral hemisphere referring resting motor threshold; MNS, conditioning electrical stimulation to contralateral median nerve referring sensory threshold; TS0.6mV, TS intensity set to evoke 0.6 mV motor evoked potential (MEP); TS0.6mVMNS, TS intensity set to evoke MEP amplitude equivalent to the MEP evoked by TS0.6mV with MNS; TS0.6mVCcS, TS intensity set to evoke MEP amplitude equivalent to the MEP evoked by TS0.6mV with CCS.
Table 3. Statistical values for Experiment 3

A. Effect of short-latency afferent inhibition (SAI) on short-latency interhemispheric inhibition (SIHI)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Difference</th>
<th>$F$ (1,10)</th>
<th>$P$ value</th>
<th>Partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCS1+MNS1.5</td>
<td>0.04 ± 0.08</td>
<td>0.23</td>
<td>0.64</td>
<td>0.02</td>
</tr>
<tr>
<td>CCS1.2+MNS1.5</td>
<td>0.08 ± 0.07</td>
<td>1.66</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>CCS1.4+MNS1.5</td>
<td>0.09 ± 0.07</td>
<td>1.57</td>
<td>0.24</td>
<td>0.14</td>
</tr>
<tr>
<td>CCS1+MNS3</td>
<td>0.03 ± 0.13</td>
<td>0.04</td>
<td>0.84</td>
<td>0.004</td>
</tr>
<tr>
<td>CCS1.2+MNS3</td>
<td>0.37 ± 0.15</td>
<td>6.01</td>
<td>0.034*</td>
<td>0.38</td>
</tr>
<tr>
<td>CCS1.4+MNS3</td>
<td>0.29 ± 0.10</td>
<td>8.01</td>
<td>0.018*</td>
<td>0.45</td>
</tr>
</tbody>
</table>

B. Effect of SIHI on SAI

<table>
<thead>
<tr>
<th>Condition</th>
<th>Difference</th>
<th>$F$ (1,10)</th>
<th>$P$ value</th>
<th>Partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCS1+MNS1.5</td>
<td>0.02 ± 0.09</td>
<td>0.06</td>
<td>0.82</td>
<td>0.01</td>
</tr>
<tr>
<td>CCS1.2+MNS1.5</td>
<td>0.07 ± 0.08</td>
<td>0.98</td>
<td>0.35</td>
<td>0.09</td>
</tr>
<tr>
<td>CCS1.4+MNS1.5</td>
<td>0.10 ± 0.13</td>
<td>0.59</td>
<td>0.46</td>
<td>0.06</td>
</tr>
<tr>
<td>CCS1+MNS3</td>
<td>0.08 ± 0.06</td>
<td>1.68</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>CCS1.2+MNS3</td>
<td>0.19 ± 0.08</td>
<td>6.03</td>
<td>0.034*</td>
<td>0.38</td>
</tr>
<tr>
<td>CCS1.4+MNS3</td>
<td>0.25 ± 0.11</td>
<td>4.96</td>
<td>0.050*</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Differences of the motor evoked potential values are shown as means ± SE. CCS, conditioning TMS to contralateral hemisphere referring resting motor threshold; MNS, conditioning electrical stimulation to contralateral median nerve referring sensory threshold. * $p < 0.05$
Effect of SAI on SIHI

Effect of SIHI on SAI

ΔSIHI

ΔSAI

SAI

SIHI

MEP size ratio (SIHI or SAI)

MEP size ratio (SIHI or SAI)