Effects of short latency afferent inhibition on short interval intracortical inhibition

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Abstract

Peripheral nerve stimulation inhibits the motor cortex and the process has been termed short latency afferent inhibition (SAI) at interstimulus intervals (ISI) of ~20 ms. The objective of the present study is to test how SAI interacts with short interval intracortical inhibition (SICI) under different stimulation conditions. We studied 20 healthy volunteers. Surface electromyogram was recorded from the first dorsal interosseous muscle. Using paired and triple pulse paradigms, how SAI interacts with SICI under these different conditions was investigated. The effects of different conditioning stimulus intensities (0.6 to 0.9 active motor threshold), SAI latencies (23 and 25ms) and ISI (2 and 3ms) for SICI were examined in rest and active conditions. SAI had inhibitory interactions with SICI at different CS intensities for rest or active SICI, at SAI latencies 23 and 25ms. This interaction occurs at weak CS intensities for SICI when there was no inhibition and SICI became facilitatory in the presence of SAI. This can be explained by SICI inhibiting SAI and not by saturation of inhibition. The interaction between SAI and SICI was greater for SICI at ISI of 3ms than 2ms, suggesting that different circuits may be activated at these ISIs. We conclude that SAI and SICI have inhibitory interactions which are influenced factors such as ISI and muscle activities, which should be considered in design and interpretation of cortical interaction studies.
INTRODUCTION

There are complex interactions between various intracortical inhibitory and facilitatory circuits in the motor cortex and these interactions can be studied using transcranial magnetic stimulation (TMS) (Chen 2004; Ni et al. 2011). Understanding of these interactions will help to improve our knowledge of motor cortical physiology and how they are altered in neurological diseases. Inhibition the primary motor cortex (M1) from peripheral nerve stimulation (Classen et al. 2000; Tokimura et al. 2000) depends on the interstimulus intervals (ISI) between the sensory stimulus and TMS to M1. The motor cortex is inhibited by median nerve stimulation (MNS) at the wrist at ISI of ~20 ms (Tokimura et al. 2000) and has been termed short latency afferent inhibition (SAI) (Sailer et al. 2003). Previous studies (Stefan et al. 2002b; Alle et al. 2009a) showed that SAI interacts with short interval intracortical inhibition (SICI). One study (Stefan et al. 2002) observed that MNS at three times sensory threshold applied 25 ms prior to TMS reduced SICI elicited by delivering conditioning stimulus (CS) 3 ms prior to test stimulus (TS), measured in the abductor pollicis brevis (APB, median innervated) muscle. Another study (Alle et al. 2009) showed that ulnar nerve stimulation at three times sensory threshold applied at the latency of the N₂₀ somatosensory evoked potential +2 ms and +4.1 ms prior to TMS reduced SICI measured in the abductor digiti minimi (ADM, ulnar innervated) muscle under voluntary contraction.

No previous studies investigated the interactions between SAI and SICI at the ISI that produced the strongest SAI (N₂₀ + 3 ms) (Tokimura et al. 2000) in the resting state, which may be considered the baseline condition for these studies. Moreover, studies that demonstrated abnormal SAI in neurological disorders such as Alzheimer’s disease (AD)
(Di Lazzaro et al. 2002b), Parkinson’s disease (PD) (Sailer et al. 2003) and stroke (Di Lazzaro et al. 2012) were performed under these conditions. The aim of the present study is to examine how SAI, mediated by cholinergic circuits, interacts with γ-amino butyric acid type A (GABA$_A$) mediated SICI. We studied SAI and SICI at different test stimulus intensities, investigated the interactions between these two circuits at different CS intensities and ISI for SICI, at different ISI for SAI and the effects of rest versus voluntary contraction. We hypothesize that 1) SAI is mediated by circuits distinct from SICI based on different findings for these circuits in diseases. 2) SAI and SICI have inhibitory interactions and their interactions are strongly influenced by stimulation conditions such as CS intensities, ISI for both SAI and SICI, and the state of voluntary contraction.

**METHODS**

**Subjects**

We studied 20 right-handed healthy volunteers [2 groups of 10 each; age (and gender) of group 1: 33.5 ±8.9 years (6 men and 4 women) for experiments 1 and 2; age (and gender) of group 2: 37.5 ± 4.8 years (8 men and 2 women) for experiments 3 to 6, mean ± SD]. Some of the results from Group 1 subjects have been reported (Udupa et al. 2009). Edinburgh handedness inventory (Oldfield 1971) was used to evaluate handedness and all the subjects were right handed. Written informed consent was obtained from all subjects. The protocol was approved by the University Health Network Research Ethics Board in accordance with the Declaration of Helsinki on the use of human subjects in experiments.

**Median nerve stimulation**
MNS was applied at the right wrist by a Digitimer DS7A constant current stimulator (Digitimer, Welwyn Garden City, UK, pulse width 0.2 ms) with standard bar electrodes. The cathode was positioned proximally. Stimulus intensity was adjusted to produce a slight thumb twitch (Abbruzzese et al. 2001), which was 2.68±0.65 times the perceptual threshold.

**N$_{20}$ recording**

Subjects relaxed in a comfortable chair. Cup electrodes with conducting gel were placed at CPe (centroparietal contralateral) with Fz (frontal) as the reference (International 10–20 system). MNS was applied at the right wrist at 2 Hz. The latency of the N$_{20}$ component of somatosensory evoked potential was determined by averaging 200 trials. ISI between MNS and TMS in the experiments were either fixed at 25ms (SAI$_{25}$) or set at N$_{20}$ latency +3 ms (SAI$_{23}$)(Di Lazzaro et al. 2002a). N$_{20}$+3 ms (SAI$_{23}$) was found to produce maximum SAI (Tokimura et al. 2000a). SAI$_{25}$ was chosen because it is widely used in experiments for paired associative stimulation (Stefan et al. 2000).

**Electromyographic recording**

Surface electromyogram (EMG) was recorded from the right first dorsal interosseous (FDI) muscle with 9 mm diameter Ag–AgCl electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. The signal was amplified (1000×), band-pass filtered (2 Hz to 2.5 kHz, Intronix Technologies Corporation Model 2024F, Bolton, Ontario, Canada), digitized at 5 kHz by an analog-to-digital (A/D) interface (Micro1401, Cambridge Electronics Design, Cambridge, UK) and stored in a computer for off-line analysis. The EMG signal was monitored on a computer screen and via loudspeaker to provide feedback on the state of
muscle relaxation. The subjects relaxed throughout the study except during the experiment with voluntary contraction. For experiments performed at rest, trials contaminated with voluntary muscle activities were rejected.

Transcranial magnetic stimulation

TMS was performed with a 7 cm figure-of-eight coil, four Magstim 200 stimulators and three Bistim modules (Magstim Company, Dyfed, UK) arranged in pyramid system. The outputs of two each Magstim 200 stimulators were directed to two Bistim modules. The outputs of these Bistim modules were directed to the third Bistim module. The stimulating coil was connected to the third Bistim module. The area for eliciting the best motor response in the right FDI muscle was established over the left M1 (optimal position) with the coil held about 45 degrees to the mid-sagittal line (approximately perpendicular to the central sulcus). The direction of the induced current was from posterior to anterior and was optimal to activate the corticospinal neurons transynaptically (Werhahn et al. 1994; Kaneko et al. 1996). The optimal position was marked on the scalp to ensure identical placement of the coil throughout the experiment.

Study design

We studied the interactions between SAI and SICI in five experiments with two groups of subjects (group I: experiments 1 and 2; group II: experiments 3-6). In Experiment 1, we tested the effect of three different TS intensities on SAI and SICI. In Experiments 2-6, we studied the SAI-SICI interactions at specific parameters specified below. Each trial consisted of one or more CS (MNS or TMS) followed by a suprathreshold TS. The CS elicited by TMS was used to elicit SICI (Kujirai et al. 1993b). The test conditions are shown in Table 1.
**Experiment 1: Effects of different test stimulus intensities on SICI and SAI**

The TS intensity was labeled according to test MEP amplitudes it produced. The minimum stimulus intensity that produced MEPs of > 1mV peak-to-peak amplitude in at least five out of 10 trials was named TS1mV. Stimulus intensities of TS0.2mV and TS2mV were defined in a similar way. We tested different TS intensities while keeping the CS the same. Each run consisted of three different conditions: TS alone, CS2 (conditioning stimulus given 2ms prior to the test stimulus)–TS and MNS23 (median nerve stimulation given 23 ms prior to test to generate SAI23)–TS. The test conditions were delivered in random order and repeated 10 times. The inter-trial intervals were 6s. The three different TS intensities were studied in separate runs. In each run, long interval intracortical inhibition and intracortical facilitation were also studied but the results will not be reported here as they unrelated to the present study.

**Experiment 2: Interactions between SAI and SICI (SAI23, SICI with CS2 and 0.8 RMT at rest)**

MNS was applied at three times the sensory threshold at N20+3 ms prior to TMS. SICI was elicited with CS at 80% resting motor threshold (RMT, defined as the minimum stimulator output that induced MEPs of > 50 µV in at least 5 out of 10 consecutive trials with FDI muscle at rest) and 2 ms prior to TS in the relaxed state, and were named CS2. These parameters were chosen to investigate the interaction between SAI and SICI at their optimal activation (Kujirai et al. 1993; Stefan et al. 2002). Seven test conditions (A to G, Table 1) were delivered in random order and repeated 10 times. TS intensity was either TS1mV or TS1mV_{MNS23}. TS1mV_{MNS23} refers to TS intensity adjusted to produce 1mV MEPs in the presence of MNS23. Conditions A–C gave SICI (B/A) and SAI (C/A)
for a 1mV test MEP. Similarly, SICI (E/D) and SAI (F/D) with an adjusted TS intensity (TS1mVMNS23) were tested in states D to G. Condition G assessed the interactions between SAI and SICI. The experiment was designed to compare SICI in the presence of SAI (G/F) to SICI matched for test MEP amplitude (B/A) or TS intensity (E/D). We also compared SAI in the presence of SICI (G/E) to SAI alone with TS intensity of 1 mV (not matched for MEP amplitude) (C/A) and matched for TS intensity (F/D).

**Experiment 3-6: Interactions between SAI and SICI under different experimental conditions**

In Experiments 3-6, we tested how the interactions between SAI on SICI are affected by different CS intensities and ISI (2 or 3ms, CS2 or CS3) for SICI, different ISI for SAI and the effects of voluntary contraction. AMT was defined as the minimum stimulator output that induced MEPs of > 200 µV in at least 5 out of 10 consecutive trials when FDI muscle contracted at 20% maximum voluntary contraction (MVC). MNS preceded the TS by N20+3 ms (SAI23) or 25 ms (SAI25).

**Experiment 3: Effects of different conditioning stimulus intensities on SAI-SICI interactions**

We studied the effects of four different CS intensities (60, 70, 80, 90% AMT) on SAI-SICI interactions. The conditions were identical to Experiment 2 (Table 1) except the intensities of CS to generate SICI were 60 to 90% of AMT. Since SICI may be contaminated with short-interval intracortical facilitation (SICF)(Peurala et al. 2008), condition H consisting of TS1mVMNS23 followed by S2, 2 ms later at the identical intensity (0.6 to 0.9 times AMT) used for eliciting SICI, was added. SICF was calculated
as (H/D). Condition G assessed the interactions between SAI and SICI. We tested the four CS intensities in four separate runs.

Experiment 4: Effects of SICI at 3 ms ISI on SAI-SICI interactions

Since SICI and SICF changes with ISIs, we studied the effect of SICI elicited at 3 ms ISI on SAI-SICI interactions. The conditions were identical to Experiment 3 (Table 1) except CS for SICI was delivered with 3 ms ISI and CS intensities were 70% or 90% of AMT.

Experiment 5: Effects of SAI at 25 ms on SAI-SICI interactions

We studied the effects of SAI elicited by MNS at 25 ms before TMS (SAI25) and SICI elicited at 2 or 3 ms ISI on SAI-SICI interactions. The ISI of 25 ms was chosen because it is commonly used in studies of paired associative stimulations to induce plasticity in motor cortex (Stefan et al. 2000; Stefan et al. 2002; Elahi et al. 2012). The conditions were the same as in Experiment 3 except MNS was given at 25 ms prior to TMS. We tested two CS intensities (0.7 and 0.9 AMT) with ISI (2 and 3 ms) for SICI in four separate runs.

Experiment 6: Effects of voluntary contraction on SAI-SICI interactions

The conditions were identical to experiment 4 except the subjects voluntarily contracted the FDI muscle at 20% of MVC. We tested two CS intensities (0.7 and 0.9 AMT) with ISI of 2 and 3 ms for SICI in four separate runs.

Data analysis

MEP amplitudes were measured peak-to-peak. SICI and SAI were expressed as the ratio of the conditioned (with preceding CS) to the unconditioned (TS alone) MEP amplitudes as outlined above. Depending on the ISI, SICI2 and SICI3 were defined for SICI of 2 and 3 ms respectively. Ratio more than 1 indicates facilitation and less than 1 indicates
inhibition. Values were expressed as mean ± standard deviation (SD). For Experiment 1, the effects of different test MEP amplitudes on SICI and SAI were tested by repeated measures analysis of variance (ANOVA) and Fischer’s protected least significance difference (PLSD) post-hoc test. Statview software was used for statistical analysis. For Experiment 2-6, the effects of SAI on SICI were determined by repeated measures ANOVA by comparing SICI for TS 1mV (B/A), SICI for a TS1mV_{MNS23} (E/D) and SICI in the presence of SAI (G/F) (termed “SICI condition”) as the repeated measures. Similarly, the effects of SICI on SAI were examined by repeated measures ANOVA with test conditions of SAI for TS1mV (C/A), SAI for TS1mV_{MNS23} (G/D) and SAI in the presence of SICI (G/E) as repeated measures (termed “SAI condition”). Post-hoc test of Fischer’s PLSD was used to examine which condition was different from others if ANOVA showed significant main effect. Correlation analyses were used to determine whether the triple-pulse induced changes in SAI and SICI were related to SAI, SICI or both. Pearson’s correlation coefficient was used to examine how “SICI in the presence of SAI (G/F)” or “SAI in the presence of SICI (G/E)” is related to the strength of SICI (E/D) and SAI (F/D). Bartlett’s test was used to test for sphericity. The threshold for significance was set at P < 0.05.

RESULTS

The latencies of the N_{20} component of the median nerve somatosensory evoked potential were 19.4 ± 1.1 ms (n=20). RMT were 50.7 ± 4.6 % and AMT were 42.2 ± 3.4% of the stimulator output. Bartlett’s test for sphericity was not significant for the data presented below.
**Experiment 1: Effects of different test stimulus intensities on SICI and SAI interactions**

The findings for the effects of different test stimulus intensity on SAI has been published in our earlier report (Udupa et al. 2009). The TS intensities used were 60.1 ± 8.3% of stimulator output for TS0.2mV, 67.5 ± 11.4% for TS1mV condition and 79.6 ± 13.2% for TS2mV. The amplitudes for test MEP alone were 0.21 ± 0.05 mV for TS0.2mV, 1.11 ± 0.35 mV for TS1mV and 2.24 ± 0.77 mV for TS2mV condition. The results are shown in Figure 1A. SICI showed little inhibition at TS0.2 mV and it increased with TS intensity. Repeated measures ANOVA showed no significant effect of the type of inhibition (SAI and SICI) or test stimulus intensities but there was a significant interaction (F=11.96; p<0.001) between type of inhibition and test stimulus intensity. To explore the significant interaction effect, we performed separate ANOVA for SICI and SAI. ANOVA for SICI showed a significant effect of TS intensity (F=5.62; p=0.02). Post-hoc testing showed less SICI at TS0.2mV compared to TS1mV (p=0.02), and to TS2mV (p=0.009). On the other hand, effect of test MEP amplitude on SAI was not significant, although there was a trend for less SAI with higher test MEP amplitude. The different responses for SICI and SAI to the increasing TS intensities suggest that different neuronal populations are involved in these two circuits.

**Experiment 2: Effect of SAI on SICI (SAI_{23}, CS 0.8 RMT, rest)**

The data from one subject was excluded from the analysis as the MEP amplitude in the presence of MNS_{23} (condition F, Table 1) could not be matched with that of TS 1mV alone (condition A). TS intensities used were 67.9 ± 9.9% of stimulator output for eliciting target MEPs of 1mV (A) and 75.6 ± 12.0% for MEPs of 1mV_{MNS_{23}} (D). RMT
was 50.7±4.6 (80% RMT=40.6±3.7=~95% of AMT used in experiment 3-6). The MEP amplitudes were 1.05 ± 0.46mV for the 1mV test MEP (A), 1.86 ± 0.65mV for 1mV_{MNS23} (D) and 1.24 ± 0.53mV for the MNS_{23}–1mV_{MNS23} test pulse combination (F). Thus, the amplitudes for the 1mV test MEP (A) and the MNS_{23}–1mV_{MNS23} test MEP (F) were matched. Fig 1B and 1C show the group results and results from one subject are shown in Fig 2. SAI had no significant effect on the SICI conditions although SICI appears to be slightly reduced in the presence of SAI (F=1.89; P=0.18; Fig 1B). There was no significant effect of SAI conditions (F=1.04; p=0.38; Fig 1C). Thus, the effects of SAI and SICI appear to be additive.

**Experiment 3: Effects of different conditioning stimulus intensities on SICI and SAI interactions**

The TS intensities used were 58.6 ± 4.7 % of stimulator output for the TS1mV condition and 65.1 ± 4.5 % for TS1mV_{MNS}. The amplitudes for TS alone were 0.99 ± 0.26 mV for TS1mV (Condition A), 3.31±1.31 for TS1mV_{MNS} (condition D) and 1.11 ± 0.18 mV for TS1mV_{MNS} with MNS (condition F). Thus, conditions A and F were matched for MEP amplitude. The effects of different CS intensities (60 to 90% AMT) on SAI-SICI interactions are shown in Figure 3A. Repeated measures ANOVA showed significant main effects of CS intensity (F=16.66; p<0.001) and “SICI conditions” (F=5.29; p=0.02) on SICI. Post-hoc tests showed significantly less inhibition (higher values) for SICI in the presence of SAI to both SICI matched for amplitude and intensity (p<0.01). The CS intensity x “SICI condition” interaction (F=0.22, p=0.97) was not significant.

We also compared the effects of different SICI-CS intensities on SAI and SAI in the presence of SICI. Repeated measures ANOVA showed significant main effects of SAI
conditions (F=3.86; p=0.04). Post-hoc testing showed significantly less SAI in the presence of SICI to the SAI matched for TS intensity (p=0.01)(Fig 3B) but not to SAI matched (see below) for MEP amplitude and no significant effects of CS intensity or the CS intensity x “SAI conditions” interaction. It should be noted that the MEP amplitudes of conditions E (SICI with test intensity TS1mV<sub>MNS23</sub>) were 2.9±1.7 mV (0.6 AMT), 2.2 ± 1.5 mV (0.7 AMT), 0.95 ± 0.5 mV (0.8 AMT) and 0.8 ± 0.6 mV (0.9 AMT). Thus, the analysis of SAI in the presence of SICI was matched for MEP amplitudes at CS of 0.8 and 0.9 AMT which had MEP amplitudes of around 1mV but not at the lower CS intensities. However, when we compared SAI in the presence of SICI (G/E) and SAI matched for intensity (F/D) and amplitude (C/A) for these two intensities (0.8 and 0.9 AMT), still there was only significant main effect of SAI conditions (F=3.58, p=0.05). It is interesting to note that in Fig 3B, only presence of SICI column contains SICI (the other two columns are SAI only and are identical conditions across different CS intensities for SICI). Increasing SICI leads to greater interaction between SICI-SAI, further suggesting that there is a “dose dependent” effect of SICI on this interaction. However, experiment 2 which used higher SICI, did not show any significant interaction (Fig 1C) but MEP amplitudes of conditions E (SICI with test intensity TS1mV<sub>MNS23</sub>) was 0.63±0.54 mV and was not matched to 1 mV.

For SICF, there was no significant difference between the MEP amplitudes for conditions D (test alone) and H (paired pulse to activate SICF) for the CS intensities studied (the ratio of H/D was around 1, showing no facilitation, Fig 3C), indicating that no SICF was elicited in our experimental protocol.

Experiment 4: Effects of ISI for SICI (SICI<sub>2</sub> vs SICI<sub>3</sub>) on SAI-SICI interactions
We tested the SAI-SICI interactions at ISI of 3ms for SICI and compared the results to Experiment 3, which used 2ms ISI for SICI. We selected 3ms ISI because it may be contaminated by SICF (Peurala et al. 2008). The amplitudes for test MEP alone were 1.10 ± 0.30 mV for TS1mV (condition A) and 2.73±0.9 mV for TS1mVMNS (condition D) and 1.19 ± 0.21 mV for TS1mVMNS with MNS (condition F). Thus conditions A and F were matched for MEP amplitude. Repeated measures ANOVA showed significant main effects of CS intensity (0.7 and 0.9 AMT; F=28.5, p<0.001; stronger SICI for CS of 0.9 AMT), SICI conditions (F=11.89; p<0.001; post-hoc tests showed SICI in the presence of SAI was significantly less than SICI matched for amplitude and intensity, p<0.001) and a trend for ISI (2 and 3 ms; F=4.59, p=0.06, stronger SICI) on SICI (Fig 4A). The ISI x SICI condition interaction was significant (F=6.13; p<0.01). Fig 4A shows that the interaction was due to much more prominent reduction of SICI and turning to facilitation in the presence of SAI at SICI3 compared to SICI2. The other interactions between ISI, CS intensity or SICI conditions were not significant.

For the effects of SICI on SAI (Fig 5A), the main effect of SAI conditions was significant (F=11.58; p<0.001). Post-hoc testing for SAI conditions showed that SAI in the presence of SICI was significantly less than SAI matched for amplitude (p<0.01) and intensity (p<0.001). The effect of ISI (F=14.67, p=0.004) and the interaction between ISI and SAI conditions (F=9.05; p<0.002) were significant indicating more inhibition of SAI in the presence of SICI3 compared to SICI2. There were no main effects for SICI-CS intensities (0.7 & 0.9 AMT) and no significant interactions between intensities and either ISI or SAI conditions (Fig 5 A & B).

**Experiment 5: Effect of SAI latency (SAI23 vs SAI25) on SAI-SICI interactions**
There was a trend for stronger SAI$_{23}$ (0.48±0.36) than SAI$_{25}$ (0.77±0.35; $t=2.16$; $p=0.06$). We tested SAI-SICI interaction at SAI$_{25}$ at two SICI CS intensities (0.7 and 0.9 AMT), two CS-ISI (for SICI$_2$ and SICI$_3$) and compared the results to Experiments 3 and 4, which tested SAI$_{23}$. The amplitudes for test MEP alone were 0.99 ± 0.12 mV for TS1mV (condition A), 2.32± 1.04 mV for TS1mV$_{MNS}$ (condition D) and 1.13 ± 0.17 mV for TS1mV$_{MNS}$ with MNS (condition F). Thus conditions A and F are matched for MEP amplitude.

For SAI$_{25}$ (Fig 4B), three way repeated measures ANOVA (CS intensities, SICI conditions and CS-ISIs) showed significant main effects of ISI (F=26.58; $p=0.0006$, less SICI for SICI$_3$), CS intensity (F=5.63; $p=0.04$; less SICI with 0.7 AMT) and SICI conditions (F=7.32, $p=0.005$; SICI in the presence of SAI is less than SICI matched for intensity) but there was no significant interactions among these factors. For SICI$_2$, (Fig 4 A and B, first 2 sets of columns) we evaluated the effects of MNS latencies (SAI$_{23}$ and SAI$_{25}$) by computing 3-way ANOVA (SICI CS intensities: 0.7 and 0.9; SICI conditions and MNS latencies as factors) and found main effects of CS intensity (F=18.71, $p=0.002$; less SICI with 0.7 AMT) and SICI conditions (F=4.65, $p=0.02$; SICI in the presence of SAI is less than SICI matched for amplitude and intensity) but no effects of MNS latency and no interaction among these factors. Similarly, for SICI$_3$ (Fig 4 A and B, last 2 sets of columns), 3-way ANOVA showed main effect of CS intensity (F=19.54, $p=0.002$; less SICI with 0.7 AMT) and SICI conditions (F=19.73, $p<0.001$; SICI in the presence of SAI is less than SICI matched for amplitude and intensity) and a trend for interaction between SAI latency and SICI condition.
(F=3.36, p=0.06; greater disinhibition of SICI in the presence of SAI23 than SAI25) but no main effect of MNS latency and no interaction among other factors.

**Experiment 6: Effects of voluntary contraction on SAI-SICI interactions**

The results are shown in Figure 6A for SICI2 and in Figure 6B for SICI3. Voluntary contraction reduced SICI (0.99 ±0.58; t=4.4; p=0.002 for SICI2, 0.7 AMT; 1.51±0.81; t=2.6, p=0.03 for SICI2, 0.9 AMT; Fig 6A) compared to the resting state (0.62 ±0.48 for 0.7 AMT and 0.37±0.19 for 0.9 AMT for SICI2). Reduction of SICI with voluntary contraction was not observed with CS3, especially at 0.7 AMT (Figure 6B). The TS intensities used were 50.5 ± 3.6 % of stimulator output for the TS1mV condition and 55.7 ± 4.1 % for TS1mV_{MNS}. The amplitudes for test MEP alone were 0.88 ± 0.13 mV for TS1mV (condition A), 3.07±0.97 mV for TS1mV_{MNS} (condition D) and 1.00 ± 0.25 mV for TS1mV_{MNS} with MNS (condition F). Thus, conditions A and F were matched for MEP amplitude. The state of muscle activity (resting vs. voluntary contraction), ISI for SICI (2 and 3ms) and intensities of SICI conditioning stimulus (0.7 and 0.9 AMT) and SICI conditions were analyzed as within-subject factors. Four-way repeated measures ANOVA showed significant main effects of activity (F=12.43; p=0.007; less SICI with contraction), SICI-ISI (F= 8.26; p=0.02; stronger SICI2), SICI conditions (F=18.79; p=0.0001; SICI in the presence of SAI is significantly lesser than SICI matched for intensity) but no significant main effect of CS intensity on SICI. There were significant interactions between activity and SICI conditions (F=3.67; p=0.046, likely due to smaller difference in SICI in the presence of SAI compared to SICI 1 mV in the active than the rest condition), activity and ISI (F=20.71; p=0.001, greater reduction of SICI from rest to active with CS2 than CS3), activity and CS intensity [F=14.16; p<0.005, greater reduction
in SICI from rest to active for higher (0.9 AMT) than lower (0.7 AMT) intensity] and activity x ISI x SICI conditions (F=4.10; p=0.034).

To further understand the significant interactions, the two different ISI for SICI were analyzed separately. For SICI2 (Fig. 6A), there were significant main effects of muscle activity (F=37.60, p=0.0002; reduced SICI in the active condition) and SICI conditions (F=7.05; p=0.006) on the extent of SICI but no significant main effects of CS intensity. There were significant muscle activity x CS intensity (F=28.34, p=0.0005) interaction and Fig 6A shows that muscle activity produced greater reduction in SICI for CS of 0.9 AMT compared to 0.7 AMT. The muscle activity x SICI condition (F=6.54; p=0.007) interaction was also significant and this appears to be due to relatively greater reduction in SICI from the rest to the active state in the TS1mV condition and smaller difference in SICI in the presence of SAI compared to SICI 1 mV in the active than the rest condition.

For SICI3 (Fig. 6B), there were no significant main effect of muscle activity but significant main effects of CS intensity (F=7.06; p=0.03; stronger SICI with 0.9 AMT) and SICI conditions (F=14.75; p=0.0002). Also there was significant interaction between muscle activity and intensity (F=18.97; p=0.002), with reduction in SICI following voluntary activity being more prominent for 0.9 AMT than 0.7 AMT. There were no significant interactions between muscle activity and SICI conditions or between intensity and SICI conditions.

Voluntary contraction reduced SAI in Experiment 6 (0.94 ±0.72; t=3.13; p=0.01) compared to the resting state (0.48±0.36). We examined the effects of activity (resting and voluntary contractions), SICI-CS intensities (0.7 and 0.9*AMT) and SICI-ISI (2 and 3 ms) on SAI in the presence of SICI (SAI conditions). Four-way repeated measures
ANOVA showed significant main effects of SICI-ISI (F=19.6; p=0.002), SAI conditions (F=19.23; p=0.0001) but no significant main effects of activity and SICI-CS intensity. There was significant interaction for ISI x SAI conditions (F=3.94; p=0.03, greater SAI-SICI interactions at 3ms CS-ISI), but no other interaction effect (Fig 7).

Correlations between the interactions of SICI and SAI and magnitudes of SICI and SAI

We correlated the combined inhibitory effects of SICI and SAI to SICI and SAI alone to examine the factors that affect the interactions between these two circuits. SICI in the presence of SAI correlated with SICI (condition E/D) at 0.9AMT CS intensity (Fig 8A; R=0.67, p=0.03) whereas SAI in the presence of SICI did not correlate with SAI (condition F/D) (Fig 8B; R=0.18, p=0.61). We also compared the MEP amplitude when triple pulses are given (MNS-CS₂-TS, condition G) to the paired pulse conditions (MNS-TS, condition F and CS₂-TS, condition E) to study the interactions of these two circuits. The MEP amplitude when both inhibitory circuits are activated (MNS-CS₂-TS) correlated with the MEP of SICI (CS₂-TS) (Fig 4C; R=0.66, p=0.04) but not that of SAI (MNS-TS) (Fig 8D; R=0.32, p=0.37). These significant correlations of SICI in the presence of SAI with SICI suggesting that SICI likely inhibits rather than SAI inhibiting SICI.

We correlated the changes from SAI-SICI interaction during voluntary contraction to the individual intracortical circuit (SAI and SICI alone). SICI in the presence of SAI significantly correlated with SICI at intensities of 0.9 AMT (R=0.63; p=0.05; Fig. 8E) whereas SAI in the presence of SICI did not correlate with SAI (R=0.11; p=0.77; Fig. 8F), suggesting that effects of SICI predominates when SICI and SAI were applied together during voluntary contraction.
DISCUSSION

We examined the interactions between SAI and SICI under different conditions. Afferent stimulation that produced SAI had inhibitory interaction with SICI at different CS intensities of SICI in both rest and active conditions at SAI$_{23}$ or SAI$_{25}$. This interaction occurs at weak SICI when there is no MEP inhibition (e.g. CS of 0.6 AMT) and SICI in the presence of SAI became facilitatory. The inhibitory effect of SICI on MEP is different from that of SAI. SICI correlates with SICI in the presence of SAI but SAI does not correlate with SAI in the presence of SICI, suggesting that the effects of SICI are dominant over those for SAI in determining the outcome of SAI-SICI interaction. Interaction between SAI and SICI was greater for SICI$_3$ than SICI$_2$, even though inhibitory strength of SICI$_2$ is stronger than that of SICI$_3$.

The importance of SICI-SAI interaction

The interactions between SAI and SICI have implications for other protocols such as paired associative stimulation (PAS) that incorporated repeated pairing of median nerve stimulation and TMS around the range of ISI used for SAI (Stefan et al. 2000). However, the mechanisms of SAI and PAS are different as SAI is based on single pairing of MNS and TMS where as PAS involves repeated pairings of these two stimuli. A recent study has found involvement of cerebellar neuronal circuits in longer latency PAS (PAS25) and but not in shorter latency PAS21.5 (Hamada et al. 2012). Further, SAI was found to be abnormal in PD (Sailer et al. 2003) and AD (Di Lazzaro et al. 2002) but is normal in frontotemporal dementia (Di Lazzaro et al. 2006), and may be used to predict treatment response in AD (Di Lazzaro et al. 2005a) and prognosis in stroke patients (Di Lazzaro et al. 2012). SAI was reduced by intravenous administration of the scopolamine, a
cholinergic muscarinic (M1) receptor antagonist (Di Lazzaro et al., 2000) but is also decreased by certain benzodiazepines that potentiate GABA transmission (Di Lazzaro et al. 2007). Dopaminergic drugs decrease SAI (Sailer et al. 2003) in Parkinson’s disease (Sailer et al. 2007). Based on these findings, it was suggested that SAI may reflect the functions of central cholinergic circuits. Recordings of corticospinal waves in the cervical spinal cord showed that SAI is due to cortical inhibition (Tokimura et al. 2000). It has been postulated that SAI is mediated by afferent inputs from thalamus directly to M1 or via a short relay through the primary sensory cortex. Therefore, inhibitory or facilitatory circuits in the M1 may affect SAI. Hence, it is important to understand the effects of SAI on SICI, which is considered as one of the main intracortical inhibitory circuits.

**Different neuronal circuits mediate SICI and SAI**

Experiment 1 showed that SICI increased with higher test MEP amplitude while, SAI showed no significant change. The findings for SICI are in agreement with previous studies (Sanger et al. 2001; Sailer et al. 2002), though SAI has not been studied in this manner. Since changes in test stimulus intensities had different effects for the different types of cortical inhibition and facilitation, different sets of neuronal circuits likely mediate SICI and SAI (Chen 2004). Furthermore, SAI was influenced by benzodiazepines that enhance GABA<sub>A</sub> mediated inhibition. Lorazepam markedly reduced SAI, whereas diazepam had no significant effect. In contrast, both drugs increase SICI, suggesting that diazepam and lorazepam may have similar affinity to the GABA receptor subtypes that mediate SICI, while they show different affinity to the ones involved in SAI (Di Lazzaro et al. 2005b; Di Lazzaro et al. 2007). Inhibition of SAI by lorazepam could
be explained by GABA<sub>A</sub> receptor mediated reduction of acetylcholine release (Di Lazzaro et al., 2005b). Pharmacological studies showed that SICI is likely mediated by GABA<sub>A</sub> receptors (Ziemann et al. 1996; Di Lazzaro et al. 2000; Werhahn et al. 1994; Muller-Dahlhaus et al. 2008). Normal SAI in PD is decreased by dopaminergic drugs (Sailer et al. 2003) whereas SICI is reduced in PD and is partially normalized by dopaminergic medications (Ridding and Rothwell 1995; Ridding et al. 1995a; Sailer et al. 2007; Chu et al. 2009; Ni et al. 2013). Taken together, our findings and previous studies suggest that SAI and SICI are mediated by different neuronal circuits involving different neurotransmitters with possible interaction between these circuits and neurotransmitter systems. The study of the interactions between SAI and SICI circuits improves the understanding of motor cortical networks and potentially provide further cues on the pathophysiology of neurological and psychiatric disorders.

Effects of SAI on SICI at different CS intensities

Since we found no significant interaction between SAI and SICI at 80% RMT (~95% AMT)(Experiment 2), we extended our study to different CS intensities (0.6 to 0.9 times AMT) for SICI. The strength of SICI varied from almost no SICI at 0.6 AMT (Fig 3A) to maximum SICI at about 0.9 AMT. We found inhibitory interactions between SAI and SICI for CS intensities from 0.6 to 0.9 AMT. An important observation is that at lower CS intensities of 0.6 and 0.7 AMT that produced SICI, SAI-SICI still showed inhibitory interactions (Fig. 3A and 8A). This effect can not be explained by saturation of inhibition because of weak SICI (only ~10% inhibition at 0.6 AMT, Fig 3A) and interaction resulted in facilitatory response (ratio >1). Further, this effect is similar to the interaction between LICI and SICI in which weak LICI that did not produce MEP inhibition still
disinhibits SICI (Sanger et al. 2001a). Thus, the SICI circuits responsible for MEP inhibition are likely distinct from those mediating disinhibition of SAI. The reason for this difference is not known but one possible explanation is at lower CS intensities (such as 0.6AMT) are subthreshold to cortical inhibitory neurons but they activate the more superficial (easily excitable segment) inhibitory dendritic branch of these neurons inhibiting neurons that mediate SAI thus ultimately producing disinhibition. These GABA_A mediated interneuron interactions could be explained by the axo-axonic interactions of the circuits involved (Ren et al. 2007).

There are several possible reasons why we found significant interaction between SAI and SICI in Experiments 3 to 6 but not in Experiment 2, which showed a non-significant trend (Fig. 1 B & C). The inhibitory interaction between SAI and SICI occurs at lower CS intensities for SICI whereas the inhibitory interaction was not present when the CS intensity used in Experiment 2 (0.8 RMT equivalent ~0.95 AMT). Higher CS intensities might activate other circuits, such as SICF. Since increasing CS intensities produced a U-shaped curve on the MEP amplitude ratio (Chen et al. 1998; Peurala et al. 2008), SICI produced by 0.8RMT (~0.95 AMT; 60% inhibition, Fig 1B) is weaker than 0.9AMT (70% inhibition, Fig 3A). This in turn might have reduced interaction with SAI. Moreover, different sets of subjects were tested in Experiments 2 and 3 and different subjects may show considerable differences in the strength of cortical circuits tested (Boroojerdi et al. 2000).

For the effects of SAI in the presence of SICI (Fig 3B), SAI-SICI interaction was prominent at higher intensities of SICI (0.8 and 0.9). Thus increasing SICI resulted in increased SICI-SAI interaction, indicating the dose-dependency effect of SICI.
Effect of different ISI for SICI on the SICI-SAI interactions

We found a trend for stronger SICI$_2$ than SICI$_3$, consistent with earlier reports (Kujirai et al. 1993; Vucic et al. 2009). SICI$_3$ used by Stefan et al. (Stefan et al. 2002b) may be contaminated by SICF whereas SICI$_2$ is less likely to be affected (Peurala et al. 2008). Hence, we studied the influence of both ISI on SAI-SICI interactions and also tested SICF. Since we found no SICF at the CS intensities and ISI studied, SICF is unlikely to explain our results. Another study (Alle et al. 2009) showed inhibitory SAI-SICI interactions for different CS-SICI ISI (1, 1.5, 2.1, 2.7 and 3ms) with no significant difference between them, but it was performed in the active condition and the resting state was not studied. We found that CS-ISI influences SAI-SICI interactions at rest as demonstrated by a significant ISI x SICI conditions interaction (Fig 4 & 5) with SICI$_3$ produced greater inhibitory interaction with SAI than SICI$_2$. This cannot be explained by different strengths of inhibition for SICI$_2$ and SICI$_3$ as SICI$_2$ was stronger at 0.7 AMT but SICI$_3$ was stronger at 0.9 AMT, but at both CS intensities the SAI-SICI interaction was greater for SICI$_3$. There may be subtle differences in cortical circuits activated by SICI$_2$ and SICI$_3$.

Effect of SAI latency on SAI-SICI interactions

We found that afferent inhibition produced by SAI$_{23}$ is stronger than SAI$_{25}$, consistent with previous studies (Tokimura et al. 2000). There were interactions between SICI and SAI at both latencies (SAI$_{23}$ and SAI$_{25}$), and our results for SAI$_{25}$ are similar to a previous study (Stefan et al. 2002a) which investigated the effects of SAI$_{25}$ with median nerve stimulation at three times the sensory threshold on the SICI$_3$ at CS intensity of 70% RMT. It was observed that SAI$_{25}$ reduced SICI$_3$ measured in the APB muscle (median
We observed a trend for interaction between SAI latency and SICI conditions (at CS$^3$) suggesting that the interaction between SICI and SAI was greater at SAI$^{23}$ than SAI$^{25}$. This may be explained by the finding that there is more SAI to inhibit in SAI$^{23}$ than SAI$^{25}$. Since SAI$^{25}$ is known to involve cerebellar circuits with repeated pairing through PAS$^{25}$ (Hamada et al. 2012), the neuronal circuits mediating these two latencies of SAI are likely different. Further studies with different latencies of MNS and the neuronal pathways involved may provide further insights on the effects of afferent inhibition latencies.

**Effects of voluntary contraction on SAI-SICI interactions**

Voluntary contraction reduced both SICI and SAI. The reduction of SICI with voluntary contraction is consistent with previous reports (Ridding et al. 1995b; Coxon et al. 2006; Ni et al. 2007) and is partially due to the activation of the SICF (Ortu et al. 2008). However, our finding of decreased SAI with voluntary activity has not been reported.

Previous studies (Alle et al. 2009; Stefan et al. 2002) did not compare SICI-SAI interaction between the rest and active states. Voluntary contraction reduced the inhibitory SAI-SICI interaction as demonstrated by a significant interaction effect of voluntary contraction and SICI conditions in Experiment 6. This may be due to reduced SAI and SICI with voluntary contraction, leading to reduction in their interaction as there was less SAI or SICI to inhibit. Studies have found that graded reduction in the inhibitory effects of different levels of voluntary contraction on SICI to maintain the precision level (Kouchtir-Devanne et al. 2012). Thus, reduction of inhibitory interaction during voluntary contraction in our study indicates the role of voluntary activity on interactions of inhibitory circuits to achieve better precision of the desired task. Further, it
would be interesting to study the contribution at different levels of voluntary contraction (10-100%) on the SAI-SICI interactions. Interestingly, the interaction between SICI conditions and activity was not significant for SICI 3 whereas it was significant for SICI 2, indicating the voluntary activity had greater effect on SAI-SICI interaction for SICI 2 than SICI 3, which explains the significant 3-way activity x ISI x SICI condition interaction. This may be because there was greater reduction of SICI 2 with muscle contraction than SICI 3, leading to better preserved inhibitory interaction between SICI and SAI with voluntary contraction for SICI 3. Thus, we conclude that voluntary activity decreases both SAI and SICI circuits and also reduces their inhibitory interaction, but more so for SICI 2 than SICI 3.

**SICI inhibiting SAI**

Based on the significant correlations of SICI in the presence of SAI to SICI and not the other way round, it is likely that the effects of SICI are dominant over those for SAI in determining the outcome of SAI-SICI interaction. This is consistent with a previous study (Alle et al. 2009) that proposed a neuronal model that the interneurons mediating SICI and SAI inhibit each other with predominant inhibition of SICI on SAI based on the finding of correlation between the strength of SICI and SICI in the presence of SAI in the active condition. We found similar correlations in both active and rest conditions (Figures 8A to 8F) as SICI correlated with SICI in the presence of SAI (Fig 8A, 8C and 8E) but SAI did not correlated with SAI in the presence of SICI (Fig 8B, 8D and 8F) during rest and voluntary contraction. This suggests that inhibitory interaction between SICI and SAI is predominately due to SICI inhibiting SAI. Also, the apparent facilitation for SICI in the presence of SAI when there was little or no SICI (rest 0.6 AMT and SICI during
voluntary activity) can be explained by inhibition of SAI by SICI but not by SAI inhibiting SICI as there is no SICI to inhibit. However, we also observed SAI turning into facilitation in the presence of SICI3 (Fig 5 and 7), suggesting that is also inhibition of SICI by SAI.

**Conclusions**

We found CS intensity for SICI, ISI for SICI, MNS latency and activity status of the target muscle all influence the interaction between SICI and SAI and these factors interact in a complex manner. SICI and SAI have inhibitory interactions. It is predominately due to SICI inhibiting SAI but mechanisms of SICI mediated MEP inhibition is likely different from that of SAI inhibition. SAI likely also inhibits SICI. The interaction increases with the strength of SICI and is more prominent with SICI3 than SICI2. It is decreased with voluntary contraction and this decrease is more pronounced with SICI2 than SICI3. Studying such interactions in neurological disorders such as AD and PD, which are known to involve one or both of these circuits, may provide further insights into the pathophysiology of these disorders. This may be particularly relevant in PD since presynaptic inhibition (the interaction between long-interval intracortical inhibition and SICI) is impaired in PD (Chu et al. 2009).
Author Contributions

All the authors were involved in the conception and design of the study. KU collected, analyzed and interpreted the data, and drafted the first version of the manuscript. CG assisted in data collection. All the authors revised the manuscript critically for important intellectual content.

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References


Figure captions

Figure 1. A) Effects of different test stimulus intensities on SICI and SAI in Experiment 1. The y-axis shows the ratio of the conditioned (CS followed by TS) to the unconditioned (TS alone) MEP amplitude. Values < 1 represent inhibition. Error bars represent standard errors of the mean. Filled columns represent target MEP amplitudes of 0.2mV, hatched columns target MEP amplitudes of 1mV and open columns target MEP amplitudes of 2mV. Significant differences as shown by repeated-measures ANOVA and post hoc testing are indicated by asterisks (*: p<0.05; **: p<0.01). B) Interactions between SICI and SAI at CS intensity of 0.8RMT and SICI2. (Experiment 2): The y-axis shows SICI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. SICI in the presence of SAI (hatched column) was compared with the SICI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV MNS23, open column). SICI was not significantly different in the presence of SAI. C) Interactions between SAI and SICI at CS intensity of 0.8RMT and SICI2. (Experiment 2): SAI in the presence of SICI (hatched column) was compared with the SAI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV MNS23, open column). SAI was not significantly different in the presence of SICI.

Figure 2. Effects of SAI on SICI in a representative subject. Each trace represents averaged MEPs from 10 trials. (A) The test stimulus (TS) alone. TS was set to produce MEP of about 1 mV (state A in Table 1); (B) TS1mV MNS23 alone. TS was adjusted to
produce test MEP of 1 mV in the presence median nerve stimulation (MNS$_{23}$) preceding the TS by ~23 ms (state D) (C) short latency afferent inhibition (SAI) induced by MNS$_{23}$ preceding the TS 1mV$_{MNS23}$ by ~23 ms (state F). The MEP amplitude matched was similar to the MEP amplitude in condition A. (D) Short-interval intracortical inhibition (SICI) elicited by a subthreshold conditioning stimulus (CS$_2$) that precedes the TS1mV$_{MNS23}$ by 2 ms (state E). (E) Combined SAI and SICI (MNS$_{23}$–CS$_2$–TS1mV$_{MNS23}$ combination, condition G). The inhibition is greater compared to (C) and (D) demonstrating, that there is an additive effect of the two inhibitory pulses.

**Figure 3.** Effects of different CS intensities on SICI, SAI and SICF. A) Interactions between SICI and SAI at different CS intensities (0.6 to 0.9*AMT) for SICI. The y-axis shows SICI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. SICI in the presence of SAI was compared with the SICI alone matched for test MEP amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV$_{MNS23}$, open column). SICI was significantly decreased in the presence of SAI for all CS intensities for SICI. B) Interactions between SICI and SAI at different CS intensities (0.6 to 0.9 AMT) for SAI. The y-axis shows SAI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition. Error bars represent standard errors of the mean. SAI in the presence of SICI was compared with the SAI alone matched for test MEP amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV$_{MNS23}$, open column). SAI was reduced in the presence of SICI for all CS intensities studied. C) Effect of different CS intensities (0.6 to 0.9*AMT) on SICF. The y-axis shows SICF as
the ratio of the conditioned versus the unconditioned MEP (H/D). Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. Facilitation was not observed for all the CS intensities studied.

**Figure 4.** Interactions between SICI and SAI at different MNS latencies, ISI for SICI and CS intensities for SICI. A) SAI$_{23}$ B) SAI$_{25}$. The y-axis shows SICI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. SICI in the presence of SAI (hatched column) was compared with the SICI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV$_{MNS23}$, open column). Similar SICI-SAI interactions were observed at both MNS latencies of MNS studied at 2 and 3 ms ISI of SICI and at both CS intensities of 0.7 and 0.9 AMT.

**Figure 5A.** SAI in the presence of SICI at two different CS intensities (0.7 and 0.9*AMT) and at two different CS-ISIs (SICI$_2$ and SICI$_3$) at SAI$_{23}$. The y-axis shows SAI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. SAI in the presence of SICI (hatched column) was compared with the SAI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV$_{MNS23}$, open column). There was significant facilitation (inhibitory interaction at SICI$_3$ with 0.9*AMT).

**Figure 5 B.** SAI in the presence of SICI at two different CS intensities (0.7 and 0.9*AMT) and at two different CS-ISIs (SICI$_2$ and SICI$_3$) at SAI$_{25}$. The y-axis shows SAI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the
The groups of three columns show the results for SAI: SAI in the presence of SICI (pres SICI, hatched column) was compared with the SAI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV_MNS23, open column). There was significant facilitation (inhibitory interaction at SICI3 and SICI2 with 0.9*AMT).

**Figure 6 A & B.** Interactions between SICI and SAI at different CS intensities (0.6 and 0.9*AMT), latencies of CS (A: SICI2 and B: SICI3) and during voluntary contractions. The y-axis shows SICI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. The groups of three columns show the results for SICI: SICI in the presence of SAI (pres SAI, hatched column) was compared with the SICI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV_MNS23, open column). SICI was significantly changed in the presence of SAI only during voluntary contractions whereas there were no significant changes in resting states in these intensities and latencies studied. There was significant interaction effect voluntary contraction on SAI-SICI interactions in such a way that voluntary activity reduces both SAI and SICI and increasing their inhibitory interactions.

**Figure 7 A & B:** SAI in the presence of SICI at different CS intensities (0.7 and 0.9*AMT), at two different CS-ISIs (SICI2 and SICI3) and at resting and active conditions. The y-axis shows SAI as the ratio of the conditioned versus the unconditioned MEP. Ratios < 1 represent inhibition, ratios > 1 represent facilitation. Error bars represent standard errors of the mean. The groups of three columns show the results for SAI: SAI in the presence of SICI (pres SICI, hatched column) was compared with the SAI alone matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV_MNS23, open column).
matched for test stimulus amplitude (TS 1mV, filled column) and test stimulus intensity (TS1mV_MNS23, open column). There was significant facilitation of active contraction which abolished inhibitory interaction of SAI in the presence of SICI3 with 0.9*AMT CS-intensity.

**Figure 8. A)** Relationship between SICI in the presence of SAI and SICI matched for TS intensity. SICI in the presence of SAI was calculated as the ratio of state G/F whereas SICI matched for intensity was calculated as state E/D. The significant correlation indicates that subjects with stronger SICI have greater SICI in the presence of SAI, suggesting that SICI inhibits SAI. **B)** Relationship between SAI in the presence of SICI and SAI matched for intensity. SICI in the presence of SAI was calculated as the ratio of state G/E whereas SAI matched for intensity was calculated as state F/D. There was no correlation between SAI and SAI in the presence of SICI. **C)** Relationship between conditioned MEP amplitude elicited by triple pulse (in the presence of MNS23, CS2 and TS1mV_MNS23) and the conditioned MEP amplitude elicited by paired pulses (CS2 and TS1mV_MNS23). The significant correlation indicates that subjects with stronger SICI have greater SICI in the presence of SAI, suggesting that SICI inhibits SAI. **D)** Relationship between conditioned MEP amplitude during triple pulse (in the presence of MNS23, CS2 and TS1mV_MNS23) and the conditioned MEP amplitude in the presence of paired pulses (MNS23 and TS1mV_MNS23). The non-significant correlation indicates that subjects with stronger SAI have no correlation with SAI in the presence of SICI, suggesting that SAI has no inhibitory interaction with SICI. **E)** Relationship between SICI in the presence of SAI and SICI matched for intensity during voluntary contraction (experiment 6). SICI in the presence of SAI was calculated as the ratio of state G/F whereas as SICI matched for
intensity was calculated as state E/D. The significant correlation indicates that subjects
with stronger SICI have greater SICI in the presence of SAI, suggesting that SICI inhibits
SAI during voluntary contraction. F) Relationship between SAI in the presence of SICI
and SAI matched for intensity during voluntary contraction (experiment 6). SAI in the
presence of SICI was calculated as the ratio of state G/E whereas SAI matched for
intensity was calculated as state F/D. There was no correlation between SAI and SAI in
the presence of SICI.
Table 1: Pulse configurations used in Experiments 2 to 6

<table>
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<th>Condition</th>
<th>Median nerve stimulation to elicit SAI (SAI\textsubscript{23/25})</th>
<th>CS for SICI (CS\textsubscript{2/3})</th>
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Experiments to investigate the effect of SAI induced by MNS at two different latencies (23 for experiment 2, 3, 4 and 6 and 25 ms for experiment 5) on SICI at different CS\textsubscript{2} (for experiment 2, 3, 5 and 6) or CS\textsubscript{3} (for experiment 4, 5 and 6) intensities (X = 0.8 RMT in 1B; 0.6 to 0.9 AMT in 2A; 0.7 or 0.9 in experiment 3 to 6 in separate runs). In conditions A to C, the effects of SICI and SAI on 1 mV test motor evoked potential (MEP) were assessed where as in conditions D to H, test stimulus intensity was increased in order to produce 1 mV tests MEP in the presence of MNS\textsubscript{23} (1mV\textsubscript{MNS23}). [*In experiment 5 test stimulus intensity was increased in order to produce 1 mV tests MEP in the presence of MNS\textsubscript{25} (1mV\textsubscript{MNS25})]. In state H (#), second stimulus (S\textsubscript{2}) was given 2 or 3ms (separate runs depending on the CS-ISI used) after TS to check for short intracortical facilitation in
experiment 3, 4 and 6. MEP amplitude ratios of G/F, B/A and E/D were compared to examine the SICI in the presence of SAI, SICI matched for amplitude and intensity respectively. Similarly G/E, C/A and F/D were compared to examine the SAI in the presence of SICI, SAI matched for amplitude and intensity respectively. AMT: active motor threshold, CS$_2$: conditioning stimulus given 2 ms prior to TS, CS$_3$: conditioning stimulus given 3 ms prior to TS, SAI$_{25}$: short afferent inhibition produced by median nerve stimulation given 25 ms prior to test TS, SAI$_{23}$: short afferent inhibition produced by median nerve stimulation given 23 ms prior to test TS, SICI: short interval intracortical inhibition, TS: test stimulus
1. Target test MEP amplitude (mV)

**Fig. 1**

A

Ratio of conditioned/unconditioned MEP amplitude

- **SICI**
- **SAI**

B

- **TS1mV**
- **TS1mV_{MNS23}**
- **Presence of SICI**

C

- **TS1mV**
- **TS1mV_{MNS23}**
- **Presence of SAI**
Fig. 2
**Fig. 3**

(A) SICI

(B) SAI

(C) SICF

- **TS1mV**
- **TS1mV_{MNS23}**
- **Presence of SAI**

Intensity of conditioning stimulus (x AMT)

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**Legend:**
- TS1mV
- TS1mV_{MNS23}
- Presence of SAI
Fig. 4

**SAI_{23}**

- TS1mV
- TS1mV_{MNS23}
- Presence of SAI

**SAI_{25}**

- TS1mV
- TS1mV_{MNS23}
- Presence of SAI
**Fig. 5**

(A) **SAI_{23}**

- **TS1mV**
- **TS1mV_{MNS23}**
- **Presence of SICI**

(B) **SAI_{25}**

- **TS1mV**
- **TS1mV_{MNS23}**
- **Presence of SICI**
A

**SICl$_2$**

- **TS1mV**
- **TS1mV$_{MNS23}$**
- **Presence of SAI**

B

**SICl$_3$**

- **TS1mV**
- **TS1mV$_{MNS23}$**
- **Presence of SAI**

Fig. 6
Fig. 7
Fig. 8