Short-interval intracortical inhibition blocks long-term potentiation induced by paired associative stimulation

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Abstract

Paired associative stimulation (PAS) of the motor cortex leads to increased motor evoked potential (MEP) amplitudes in the stimulated hand muscles. We hypothesized that evoking \( \text{GABA}_A \) receptor-mediated short-interval intracortical inhibition (SICI) simultaneously with excitatory PAS would depress long-term potentiation plasticity in motor cortex. Four different PAS paradigms were tested, standard PAS (PAS25) and 3 conditioned PAS protocols (CS2-PAS25, CS2-PAS25adj and CS10-PAS25adj). A subthreshold conditioning stimulus 2 ms (CS2) or 10ms (CS10) before the test stimuli, were added to the conditioned PAS protocols. Since CS2 has inhibitory and CS10 has facilitatory effect on cortical excitability, in the CS2-PAS25adj and CS10-PAS25adj protocols, TS intensity was adjusted to produce a 1 mV MEP in the presence of CS2 or CS10 to control for the degree of corticospinal excitation. As expected, MEP amplitudes after PAS25 were higher compared to that at baseline, but importantly, MEP amplitudes did not change after PAS was induced in the presence of SICI in either the CS2-PAS25 or CS2-PAS25adj condition. Furthermore, the CS10-PAS25adj protocol showed significantly increased MEP amplitude at 60 minutes after PAS compared to baseline. These results show that SICI blocked the induction of LTP-like plasticity in the motor cortex, indicating that GABAergic circuits play an important role of in the regulation of cortical plasticity. The study demonstrates a non-invasive and non-pharmacological way to achieve focal modulation of plasticity.

Keywords: cortical plasticity, gamma-aminobutyric acid, associative plasticity, motor cortex, transcranial magnetic stimulation
Introduction

Long-term potentiation (LTP) is hypothesized to play an important role in learning and memory (Antonov et al. 2003; Iriki et al. 1989). Paired associative stimulation (PAS) is a widely used experimental paradigm to induce plasticity in the human motor cortex (Ridding and Taylor 2001; Stefan et al. 2002). This technique uses repetitive pairing of nerve stimulation and cortical transcranial magnetic stimulation (TMS) that are timed so that the peripheral input and the central stimulus arrive near-synchronously at the motor cortex. Because PAS shares several features of spike timing plasticity, such as associativity, input specificity, and cooperativity (Bear and Malenka 1994; Stefan et al. 2000), it probably represents associative plasticity and LTP in the primary motor cortex (M1). Reduction in cortical excitability is achieved when the peripheral nerve stimulation precedes the cortical stimulus (Wolters et al. 2003). These studies suggest that PAS can induce LTP- and long-term depression (LTD)-like phenomena and further indicate that temporal Hebbian rules are involved in the induction of cortical plasticity.

Cortical inhibition is critical for the regulation of neuronal excitability and plasticity (Chen et al. 2002). Studies using hippocampal slices revealed that the activation of GABA_A receptors blocked LTP induction (Evans and Viola-McCabe 1996). In humans, increased inhibition induced by administration of lorazepam, a positive allosteric modulator of the GABA_A receptor, reduced practice-dependent LTP-like plasticity in the motor cortex (Butefisch et al. 2000; Ziemann et al. 2001). Diazepam, another benzodiazepine, produced a non-significant trend towards reduction in PAS-induced LTP plasticity (Heidegger et al. 2010). The GABA_B receptor agonist, baclofen, also decreased PAS-induced LTP plasticity in the human motor cortex (McDonnell et al. 2007). However, modulation of PAS using brain stimulation has not been examined. Abnormal associative LTP-like plasticity measured by PAS may play a critical role in the pathophysiology of neurological and psychiatric disorders, such as primary focal hand
dystonia (Quartarone et al. 2006), Parkinson’s disease (Morgante et al. 2006), Costello syndrome (Dileone et al. 2010) and schizophrenia (Frantseva et al. 2007; Stephan et al. 2006).

Short-interval cortical inhibition (SICI) is a widely studied cortical inhibitory circuit and is elicited by a subthreshold conditioning stimulus (CS) followed by a suprathreshold test stimulus (TS) at interstimulus intervals (ISI) of 1–6 ms (Kujirai et al. 1993). Pharmacological studies have shown that drugs such as lorazepam that increase GABA$_A$ activity enhance SICI (Di Lazzaro et al. 2005; Ilic et al. 2002; Ziemann et al. 1996a), which suggests that it is mediated by GABA$_A$ receptors. However, SICI has not been investigated as a focal, non-pharmacological means to modulate plasticity. In the present study, we examined the effects of activating SICI during the induction of LTP-like plasticity in the motor cortex. We hypothesize that GABA$_A$ mediated cortical inhibition activated by SICI will suppress PAS-induced LTP-like plasticity.

**Materials and Methods**

**Experiment 1**

**Subjects**

We recruited 13 right-handed subjects with no history of neurological or psychiatric disorders and with normal neurological examination results. The University Health Network Research Ethics Board approved the experimental protocol and all subjects provided written informed consent. Two subjects were excluded due to high resting motor thresholds, which made it difficult to obtain adequate motor-evoked potential (MEP) amplitudes. Therefore, 11 healthy volunteers (7 men and 4 women) aged 35.2 ± 13.4 years (mean ± SD) were studied. Each subject participated in 3 stimulation sessions and each session was administered at least 1 week apart.

**EMG recording**
Surface electromyograms (EMG) were recorded from the left abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscles using bipolar Ag-AgCl electrodes. The signals were amplified 1,000 times, filtered (5–500 Hz), digitized (Cambridge Electronic Design Micro 1401), and recorded using Signal software (version 3.07). EMG was continuously monitored with visual and auditory feedback to ensure complete muscle relaxation. The stimulation parameters, such as the coil location and stimulation intensities, were optimized based on the APB responses. The FDI measurements were used to confirm the findings in the APB muscle and to determine if the similar effects also occurred in another muscle in the proximity of the APB muscle but innervated by a different nerve (ulnar nerve).

**Transcranial magnetic stimulation**

We used two Magstim 200 stimulators (Magstim Company Ltd., Whitland, Dyfed, UK) connected via a Bistim module and an 8-shaped coil (outside diameter of each loop was 9.5 cm) to apply TMS to the right M1. The TMS trigger pulses were delivered from a Micro1401 interface (Cambridge Electronics Design, Cambridge, UK) controlled by Signal Software (3.07). The subjects were seated in a comfortable position and the coil was held tangentially to the skull with the handle pointing backwards and laterally at ~45° to the sagittal plane, at the optimal scalp site to evoke MEP in the relaxed left APB muscle. The motor hot-spot was marked on a default image in Brainsight (Magstim Company Ltd, Whitland, Dyfed, UK) stereotactic image guidance system to facilitate the positioning of the TMS coils over a subject's scalp.

The experimental protocol is shown in Figure 1. Resting motor threshold (RMT) was defined as the minimum stimulator output that evoked MEPs of >50 µV in at least 5 out of 10 trials when the APB muscle was completely relaxed. Active motor threshold (AMT) was defined as the minimum intensity of stimulation output that elicited APB MEPs of at least 200 µV in 5 out of 10 consecutive trials while the participants maintained a force level of approximately 20% of their maximum contraction. MEP amplitude was measured as the average of 10 trials in which TMS stimulation generated an EMG
response of at least 1 mV peak-to-peak amplitude at the baseline. At each time point (Fig. 1), RMT, AMT, and MEP amplitudes were measured first.

The balance and interactions between inhibitory and facilitatory circuits determine the final output from the M1 (Hallett 2007). We therefore tested several well established intracortical inhibitory and facilitatory circuits to explore the possible changes in these networks following each intervention. SICI mostly represent GABA_\text{A} mediated inhibition (Jung et al. 2004; Ziemann et al. 1996a), intracortical facilitation (ICF) reflects glutamatergic activities (Schwenkreis et al. 1999; Ziemann et al. 1998) and short latency afferent inhibition (SAI) is related to cholinergic (Di Lazzaro et al. 2000) neural networks.

SICI and ICF were evoked with a subthreshold conditioning stimulus (CS) followed by a test stimulus (TS). CS intensity was 90% AMT. ISI of 2 ms was used for SICI and 10 ms for ICF. Short latency afferent inhibition (SAI) was studied using a conditioning-test protocol described by Tokimura et al. (2000) (Tokimura et al. 2000). The left median nerve was stimulated through bipolar electrodes at the wrist (cathode proximal) using a 200 μs square wave pulse at 3 times the sensory threshold. The ISI between median nerve stimulation and TMS was set to the latency of the N20 somatosensory evoked potential plus 2 ms. The N20 somatosensory evoked potential was evoked by stimulation of the left median nerve and was recorded with the active electrode which was placed 3 cm posterior to C4 (according to the International 10–20 EEG system) and referenced to Fz. Two hundred responses were averaged to identify the N20 peak. SICI, ICF, and SAI were measured in the same experimental session with 10 trial for each ISI and 10 trials of TS alone, delivered in a random order. The TS generated a 1-mV MEP amplitude in the left APB muscle at baseline, and the TS intensities were adjusted at each time point after PAS to produce similar test MEP amplitudes.

Cortical silent period (CSP) was measured at the end of each assessment. CSP was defined as the time between MEP onset and return of voluntary EMG activity (Daskalakis et al. 2003) and was assessed.
Paired associative stimulation (PAS)

PAS was achieved by repetitively pairing stimulation of the left median nerve and TMS of the right M1. The median nerve was stimulated at 3 times sensory threshold and TMS intensity was adjusted to produce MEPs with 1 mV peak-to-peak amplitude. The ISI was 25 ms, which was optimal for inducing a sustained increase in motor cortex excitability through PAS (Stefan et al. 2000). One hundred and eighty paired stimuli were delivered at 0.1 Hz during a 30 min period. We refer to this paradigm as PAS25.

PAS in the presence of SICI

We combined median nerve stimulation and SICI in two different experimental paradigms. In the first paradigm, the parameters were the same as PAS25 except that a CS at 90% AMT was delivered 2 ms (CS2) before the TS (23 ms after median nerve stimulation). We refer to this paradigm as CS2-PAS25. Because CS2 reduces the MEP amplitude evoked by the TS, in the second paradigm, we increased the TS intensity to produce 1mV MEPs in the presence of SICI induced by CS2. This paradigm is designed to match the MEP amplitudes produced by the CS2-adjusted TS pulse combination to that produced by TS alone in PAS25 condition (CS2-PAS25adj). Corticospinal excitability and cortical inhibition were measured before and at different times after PAS, as indicated in Figure 1. These 3 PAS paradigms were performed in separate sessions at least 1 week apart and in random order.

MEP amplitudes during PAS

To examine the changes in MEP amplitudes during PAS, MEPs were recorded during the PAS protocols and the amplitudes of every 10 MEPs were averaged.
Experiment 2

PAS in the presence of ICF

Four new subjects and two subjects from the Experiment 1 (3 men and 3 women, aged 39.5 ± 9 years) participated. A subthreshold conditioning stimuli 10 ms before the test stimuli have been shown to cause ICF (Berardelli et al. 2008; Ziemann et al. 1996c). This protocol was designed to condition PAS with a CS 10 ms before the TS to test whether the effects seen with PAS in the presence of SICI is specific to the interstimulus interval used. The TS intensity was adjusted to produce 1mV amplitude in the presence of CS10, which was set at 90% AMT. This protocol is referred to as CS10-PAS25adj. The pre and post PAS measurements are the same as Experiment 1.

Statistical analysis

For RMT, AMT, CSP, SICI, ICF, MEP, and SAI, values after PAS from T0 to T60 were expressed as a ratio of baseline, and were used for statistical analysis. In Experiment 1, repeated measures analyses of variance (ANOVA) with “INTERVENTION” (PAS25, CS2-PAS25, and CS2-PAS25adj) and “TIME” (T0, T20, T40 and T60) were used as the within-subject factors. Separate analyses were performed for the APB and FDI muscles because all the adjustments in stimulus intensities were based on the APB muscle and therefore the MEP amplitudes for different test conditions were not matched for the FDI muscle. Significant ANOVA main effects were further explored using Fisher’s post-hoc analyses and separate one-way ANOVAs.

Peak-to-peak MEP amplitudes during PAS were analyzed using an ANOVA with “INTERVENTION” as the between-subject factors and “TIME” as the within-subject factor. MEPs from every 10 consecutive stimuli were averaged and used for analyses. Baseline was defined as the first time bin of 10 MEPs at the beginning of each of the test interventions.
For Experiment 2, repeated measure ANOVA with the factor “Time” (5 levels, Baseline-T60) as within subject factors for APB muscle were tested. Values after intervention from T0 to T60 were expressed as a ratio of baseline. Effect of “Time” was further explored using Fisher's least significant difference and Fisher’s post hoc analysis.

P values < 0.05 were considered significant. StatView 5.0.1 (SAS Institute Inc.) software was used for analysis. Values are presented as mean ± SEM, unless specified otherwise.

**Results**

None of the subjects reported any adverse effect.

**Motor Thresholds (RMT, AMT)**

For Experiment 1, no significant change in RMT (43±1.4% at baseline) and AMT (36±1.2% at baseline) of the APB muscle was observed after any of the 3 PAS protocols. There were no significant interactions or main effects for either muscle. In Experiment 2, the RMT and AMT were 46±3.8% and 36±2.7% at baseline. Similarly, no significant change in RMT and AMT was detected.

**Experiment 1**

**SICI blocks LTP-like plasticity during PAS**

There were significant effects of “INTERVENTIONS” (p < 0.0001), “TIME” (p < 0.001), and “TIME × INTERVENTION” (p < 0.001; Fig. 2) on the MEP amplitude during the 30 min of PAS (median nerve stimulation followed by TMS). Post-hoc analysis confirmed significant differences among all 3 interventions with the highest MEP amplitudes in PAS25, followed by CS2-PASadj, and then CS2-PAS25. Figure 2 shows that the significant “TIME × INTERVENTION” is due to a marked increase in MEP amplitude during PAS25 and CS2-PAS25adj, but not during CS2-PAS25.
There was a significant effect of “Time” on MEP amplitudes for the PAS25 session ($p < 0.0001$). Post-hoc analysis showed that MEP amplitude was significantly increased compared to the first MEP (baseline) after 4 min and reached a plateau after about 16 min (Fig. 2). In the CS2-PAS25 session, there was also a significant effect of time on MEP amplitude ($p = 0.005$). Post-hoc analysis showed that MEP amplitude was mostly unchanged except for transient increases at 15 and 25 min (Fig. 2). For the CS2-PAS25adj session, there was also a significant effect of time after stimulation ($p < 0.001$). Post-hoc analysis showed an increased MEP from 20 min into the stimulation session through the end of the session ($p = 0.002$) (Fig. 2).

**SICI blocks LTP-like plasticity after PAS**

For MEP amplitudes from single pulse TMS in the APB muscle, ANOVA showed a significant effect of “INTERVENTION” ($p < 0.001$), but no significant effect for “TIME” and “TIME × INTERVENTION” interaction (Fig. 3). Post-hoc analysis confirmed that there were significantly higher MEP amplitudes for PAS25 compared to CS2-PAS25 ($p < 0.0001$) and CS-PAS25adj ($p = 0.0002$). The MEP amplitudes for CS2-PAS25 and CS-PAS25adj sessions were not significantly different from each other. One-way ANOVA for PAS25 showed a significant effect of time on MEP amplitude ($p = 0.009$). Post-hoc analysis showed higher MEP amplitudes at all time points measured up to 1 h after the PAS25 compared to baseline (T0: $p = 0.0006$, T20: $p = 0.02$, T40: $p = 0.013$, T60: $p = 0.009$). There was no significant effect of “TIME” following CS2-PAS25 and CS2-PAS25adj stimulation protocols.

**Changes in intracortical inhibitory and facilitatory networks following PAS**

**SICI**

There was no significant effect of “INTERVENTION,” “TIME,” and “TIME × INTERVENTION” interaction for SICI of the APB muscle.

**SAI**
There was a significant effect of “INTERVENTION” for the APB muscle (p = 0.02), but there was no significant effect of “TIME” and “TIME × INTERVENTION” interaction (Fig. 4A). Post-hoc analysis showed SAI was significantly less after CS2-PAS25adj compared to that after CS2-PAS25 (p = 0.008; Fig. 4B). Separate one-way ANOVAs for each intervention revealed no effect of “TIME” with CS2-PAS25, CS2-PAS25adj, and PAS25.

**ICF**

There was no significant effect of “INTERVENTION,” “TIME,” and “TIME × INTERVENTION” interaction for APB muscle.

**Cortical Silent Period (CSP)**

ANOVA showed a significant effect of “TIME” (ANOVA APB p = 0.006), but there was no effect of “INTERVENTION” and “TIME × INTERVENTION” interaction (Fig. 5). Post-hoc analysis showed that CSP was increased at 40 and 60 min after interventions compared to that at baseline (T40, p = 0.002; T60, p = 0.009).

**Stimulation effects in the FDI muscle**

The intensity that generates ~1mV amplitude in APB resulted in 1.7 ± 0.3, 1.4 ± 0.3, and 1.8 ± 0.3 mV MEP amplitude in PAS25, CS2-PAS25, and CS2-PAS25adj protocols respectively at baseline. A similar pattern of changes in MEP amplitude was observed after PAS protocols in FDI muscle. ANOVA showed significant effect for “INTERVENTION” (p = 0.006) and no effect for “TIME” and “TIME × INTERVENTION” on MEP amplitude ratios after PAS. Post-hoc analysis showed higher MEP amplitude in PAS25 compared to CS2-PAS25 (p = 0.01) and CS2-PAS25adj (p = 0.01). This confirms similar time courses for MEP amplitude after PAS in both APB and FDI muscles.

**Experiment 2**
The MEP amplitude at baseline was $0.94\pm0.08$ mV. Repeated measure ANOVA showed a non-significant trend for the effect of “Time” ($p=0.09$) in the APB muscle. No significant effect of “Time” was observed for FDI muscle. Fisher’s post hoc analysis showed a significantly higher MEP amplitude at T60 compared to baseline ($p=0.01$) in APB muscle. (Fig. 6A)

One-way ANOVA showed no significant effect of “TIME” on normalized values of SICI (53.9±11% at baseline), ICF (131.2±24% at baseline), SAI (35.9±9.4% at baseline) and CSP (172±11ms at baseline) for both APB (Fig. 6B) and FDI muscle groups.

**Discussion**

MEP amplitudes significantly increased following PAS25 compared to baseline but the MEP amplitudes after CS2-PAS25 and CS2-PAS25adj were unchanged. Thus, applying SICI, which activated GABAergic interneurons, inhibited PAS-induced LTP-like plasticity after-effect in the motor cortex. This effect was specific to the conditioning-test pulse (CS2) interval that elicited SICI because the same stimuli at a different conditioning pulse interval (CS10) that did not produce SICI showed MEP facilitation after the intervention (Fig. 6). These findings demonstrate that non-invasive and non-pharmacological TMS techniques can effectively modulate cortical plasticity.

Inhibitory interneurons play an important role in the regulation of cortical plasticity. In rat hippocampal slices (Evans and Viola-McCabe 1996), application of midazolam, a benzodiazepine, markedly inhibited LTP induction and prevented the expected increase in excitatory post synaptic potentials (EPSP) and population spikes following theta burst stimulation. This effect was reversed by bicuculline, a GABA$_A$ receptor antagonist (Evans and Viola-McCabe 1996), which indicates that benzodiazepines suppress LTP induction through enhancement of GABA$_A$ receptor-mediated inhibition.
In rat motor cortex (Hess and Donoghue 1994), bicuculline increased the ability of theta burst stimulation to induce LTP. Several pharmacological studies in humans have shown that benzodiazepines suppressed cortical plasticity (Butefisch et al. 2000; Ziemann et al. 2001). In the present study, we investigated the role of inhibitory circuits in the regulation of plasticity in motor cortex by activating SICI during facilitatory PAS25 and we found disruption of LTP-like plasticity induction. Because SICI decreased the MEP amplitude induced by TS and this may affect LTP-like plasticity induction, we included a control experiment (CS2-PAS25adj) with adjusted TS intensity to generate the same MEP amplitude as in PAS25 as a measure of post-synaptic activity. Because the induction of LTP-like plasticity was suppressed in both CS2-PAS25 and CS2-PAS25adj conditions, the finding cannot be explained by reduced postsynaptic activity in the CS2-PAS25 condition but is likely the result of activation of inhibitory circuits responsible for SICI. This effect is cannot be attributed to non-specific effects of adding the CS during PAS because the same CS at a different interstimulus interval (10 ms) resulted in LTP-like effect.

Direct recordings of corticospinal descending volleys showed that SICI reduced late I-waves (Di Lazzaro et al. 1998) whereas facilitatory PAS increased later I-waves (Di Lazzaro et al. 2009). The possibility that our results could partially be explained by different I-wave composition in the CS2-PAS25adj condition compared to PAS25 condition cannot be excluded. However, epidural recordings of corticospinal waves (Ni et al. 2011) showed that adjustment of MEP amplitude in the presence of CS2 also restored the late I-wave amplitude to that of TS alone, suggesting the PAS25 and the CS2-PAS25adj protocols in our study likely had similar late I-wave amplitudes.

Another consideration is whether repetitive 0.1 Hz paired pulse stimulation at 2 ms ISI itself depressed MEP amplitude. Further studies are needed to exclude this possibility but we considered it unlikely because 0.1 Hz single pulse TMS had no effect (Chen et al. 1997) whereas 0.2 Hz suprathreshold paired pulse TMS at 1.5 ms ISI increased in MEP amplitude (Thickbroom et al. 2006).
LTP-like plasticity is decreased in several neurological and psychiatric disorders with abnormal motor learning, such as Parkinson’s disease (Morgante et al. 2006), schizophrenia (Daskalakis et al. 2008; Frantseva et al. 2007; Stephan et al. 2006), and Huntington’s disease (Orth et al. 2010). On the other hand, exaggerated LTP-like and LTD-like plasticity in the motor cortex and a loss of topographic specificity has been observed in writer’s cramp (Quartarone et al. 2003; Quartarone et al. 2006; Weise et al. 2006). Our study shows that SICI modulates the controlling mechanism of cortical plasticity, which can potentially be useful in the treatment of conditions with abnormal plasticity and disrupted inhibition, such as dystonia.

**MEP amplitude during PAS**

EMG recording during the stimulation phase showed that at the beginning of PAS, the MEP amplitudes (with median nerve stimulation followed by TMS) were <1 mV (Fig. 2). This is likely because the median nerve stimulation 25 ms before TMS produced MEP inhibition, similar to SAI. The MEP amplitudes induced by CS2-PAS25 were smaller than those elicited after PAS25 (Fig. 2), likely due to further MEP inhibition caused by adding the CS2 pulse. In the CS2-PAS25adj condition, the TMS intensities were adjusted to produce 1 mV MEP in the presence of CS2 (without median nerve stimulation). With the addition of median nerve stimulation, the higher MEP amplitude for CS2-PAS25adj as compared to PAS25 at the beginning of PAS suggests that there exists an inhibitory interaction between SAI and SICI. These findings are consistent with those of previous studies, which showed mutual inhibitory interactions between SICI and SAI (Alle et al. 2009; Stefan et al. 2002).

The MEP increase during PAS25 and CS2-PAS25adj may be due to an increase in MEP amplitude or a decrease in SAI. After PAS25, there was no change in SAI (Fig. 4A) but the MEP amplitude was increased (Fig. 3), it is likely due to an increase in MEP amplitude and corticospinal excitability. In contrast, after CS2-PAS25adj, there was no increase in MEP amplitude compared to baseline (Fig. 3) but there was reduction in SAI (Fig. 4B). Therefore, the increase in MEP amplitude during CS2-PAS25adj
(Fig 2) is likely related to decrease in SAI. The finding that MEP amplitudes did not change during CS2-PAS25 is consistent with the observation that neither MEP amplitudes (Fig. 3) nor SAI changed after CS2-PAS25 (Fig. 4A).

**Intracortical inhibition and facilitation after PAS**

Similar to our findings of no overall change in SICI and ICF after PAS25, previous PAS studies did not show significant changes in SICI after excitatory PAS25 (Di Lazzaro et al. 2011; McDonnell et al. 2007; Russmann et al. 2009; Stefan et al. 2002) although a significant decrease in SICI after inhibitory PAS was observed in one study (Russmann et al. 2009). The finding that SICI was unchanged after CS2-PAS25 and CS2-PAS25adj conditions is consistent with the blockade of LTP plasticity by SICI. If SICI was diminished during these conditions, it would not be expected to block PAS induced plasticity.

We found a significant increase in CSP (Fig. 5) after PAS25 and the conditioned PAS interventions, similar to previous studies (Morgante et al. 2006; Stefan et al. 2000). Because administration of the GABA<sub>B</sub> receptor agonist, baclofen, and GABA re-uptake inhibitor, tiagabine, prolonged CSP (Siebner et al. 1998; Werhahn et al. 1999), it is probably mediated through postsynaptic GABA<sub>B</sub> receptors. CSP is thought to be partially mediated through activity in recurrent collaterals from discharging pyramidal tract neurons (Orth and Rothwell 2004). Prolonged CSP after PAS25 is probably due to facilitation of inhibitory interneurons and up-regulation of GABA<sub>B</sub> receptors in postsynaptic neurons. MEP amplitude reflects the excitability of the corticospinal system and it increased after PAS25, whereas CSP increased after all 3 interventions. Both electrophysiology and pharmacological studies suggest that multiple mechanisms are involved in generating the CSP, including the loss of voluntary drive, activation of inhibitory interneurons, activation of corticospinal recurrent collaterals, and after-hyperpolarization (Fuhr et al. 1991; Tergau et al. 1999; Ziemann et al. 1996b). The different effects of PAS interventions on MEP and CSP suggest that the same circuits do not mediate them. However, authors cannot precisely explain why CSP is increased in all 3 protocols.
SAI is decreased after the stimulation in CS2-PAS25adj. This observation could suggest decreased
SAI itself or an increased tonic inhibitory interaction on SAI from other circuits after CS2-PAS25adj. We
showed in a previous study that GABA\textsubscript{B}-mediated long-interval intracortical inhibition (LICI) and SAI
had mutual inhibitory interactions (Udupa et al. 2009). We speculate that this inhibitory interaction or
interactions with other cortical circuits may be involved, but this needs to be clarified in future studies.

**Potential implications of the present findings**

TMS techniques, such as repetitive TMS, have been used to assess and treat diseases with abnormal
cortical excitability and plasticity (Chen and Udupa 2009; Elahi and Chen 2009). Our finding that
simultaneously activating SICI during PAS suppresses LTP-like plasticity supports the hypothesis that
GABA\textsubscript{A}-mediated inhibition plays a crucial role in regulating cortical plasticity. This demonstration of a
non-pharmacological way of modulating plasticity in humans has several implications. The method can
be modified by using different ISI or stimulus intensities to examine the effects of other cortical circuits
on cortical plasticity. This may be a method to examine abnormal regulation of plasticity in neurological
and psychiatric disorders. Moreover, suppression of LTP-like mechanisms may be tested as a possible
treatment for disorders associated with increased LTP-like plasticity such as dystonia. Non-invasive and
non-pharmacological modulation of cortical plasticity may be more advantageous than pharmacological
intervention because it may have fewer side effects and it can be targeted to specific brain areas.

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Figure Legends

Figure 1. Study timeline. Abbreviations: active motor threshold (AMT), cortical silent period (CSP), intracortical facilitation (ICF), motor evoked potentials (MEP), resting motor threshold (RMT), short latency afferent inhibition (SAI), and short interval cortical inhibition (SICI). These measures were taken before the PAS protocols and immediately (T0), 20 (T20), 40 (T40), and 60 (T60) minutes after the interventions. The right top corner illustrates the combination of peripheral and TMS pulses used in each PAS protocol.

Figure 2. MEP amplitude recorded during the PAS interventions. (A) Open symbols represent significant difference compared to the first time bin for each intervention. Each point represents averaged MEP amplitudes (mV) from 10 stimuli for PAS25 (■), CS2-PAS25 (●), and CS2-PAS25adj (▲). * = p < 0.05 and ** = p < 0.001; error bars denote ± SEM

Figure 3. MEP amplitudes after PAS protocols. MEP in APB muscle is shown. MEP amplitudes were normalized to baseline (before interventions) for PAS 25 (■), CS2-PAS25 (●), and CS2-PAS25adj (▲). Values more than 1 indicate increased MEP amplitude and values less than 1 indicates decreased MEP amplitude after PAS.

Figure 4. Short latency afferent inhibition (SAI) before and after PAS interventions. (A) SAI in the APB muscle. Values above 1 indicate facilitation and values below 1 indicate inhibition. (B) The effects of the different interventions are shown as the ratio of SAI averaged for all time points after PAS normalized to the baseline SAI for the three PAS conditions tested (PAS 25, CS2-PAS25, and CS2-PAS25adj). Values above 1 indicated reduction in SAI after PAS and values less than 1 indicate an increase in SAI after PAS compared to baseline values. Error bars represent ± SEM; * = p < 0.05
Figure 5. Cortical silent period (CSP) duration before and after PAS interventions. CSP are shown in milliseconds (ms). Each bar represents 1 intervention protocol. Error bars represent ± SEM. * = < 0.05

Figure 6. Results from the CS10-PAS25adj paradigm. (A) MEP amplitudes in the APB muscle after the CS10-PAS25adj protocol. MEP amplitudes were normalized to baseline (before interventions). (B) Results for short-interval intracortical inhibition (SICI), short latency afferent inhibition (SAI), and intracortical facilitation (ICF) are shown. Values above 100% indicate facilitation and values below 100% indicate inhibition. Error bars represent ± SEM.
Figure 4

(A) SAI-ABP (% test stimuli alone) over time for different interventions:
- PAS25
- CS2-PAS25
- CS2-PAS25adj

(B) SAI ratio (APB) for different interventions:
- PAS25
- CS2-PAS25
- CS2-PAS25adj

* indicates a significant difference.
Figure 5

The bar chart shows the cortical silent period duration (ms) at different time points: Baseline, T0, T20, T40, and T60. The chart compares three groups: PAS25 (white bars), CS2-PAS25 (gray bars), and CS2-PAS25adj (black bars). The x-axis represents time, and the y-axis represents the duration. The * symbol indicates a significant difference between the groups.