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# Involvement of different neuronal components in the induction of cortical plasticity with associative stimulation

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ABSTRACT

*Background:* Paired associative stimulation (PAS), with stimulus interval of 21.5 or 25 ms, using transcranial magnetic stimulation in the posterior-anterior (PA) current direction, produces a long-term-potentiation-like effect. Stimulation with PA directed current generates both early and late indirect (I)-waves while that in anterior-posterior (AP) current predominantly elicits late I-waves. Short interval intracortical inhibition (SICI) inhibits late I-waves but not early I-waves.
*Objective:* To investigate how cortical inhibition modulates the effects of PAS.
*Methods:* PAS at stimulus interval of 21.5 ms conditioned by SICI (SICI-PAS) was compared to PAS alone with both PA and AP directed currents.
*Results:* PAS with both current directions increased cortical excitability. SICI-PAS increased cortical excitability in the PA but not the AP current direction.
*Conclusions:* Both early and late I-waves circuits can mediate cortical PAS plasticity under different conditions. Plasticity induction with the late but not the early I-wave circuits is blocked by SICI.


## 1. Introduction

Paired associative stimulation (PAS) involves repetitive pairing of sensory afferent input with direct activation of primary motor cortex (M1) by transcranial magnetic stimulation (TMS) at specific time intervals [1–5]. PAS at 21.5 or 25 ms interval produces spike-timing-dependent long-term-potentiation-like effect in M1 [6,7]. A single-pulse TMS over M1 generates multiple descending volleys in the spinal cord, classified as early and late indirect (I)-waves [8]. TMS with posterior-anterior (PA) directed current produces both early and late I-waves while stimulation in anterior-posterior (AP) current direction predominantly generates late I-waves [9]. Short interval intracortical inhibition (SICI) refers to the cortical inhibition produced by a conditioning stimulus on the motor evoked potential (MEP) generated by a test stimulus at interstimulus intervals of 1–5 ms [10,11]. SICI inhibits late but not early I-waves [12,13]. We hypothesized that cortical circuits producing both early and late I-waves could be involved in the induction of cortical plasticity with PAS. Previous studies showed that the effects of PAS with PA current at 25 ms were different from that at 21.5 ms [7] and were blocked by conditioning SICI [14]. We therefore conducted an exploratory study and predicted that the effects of PAS at 21.5 ms with AP (involving I3-wave) but not that with PA current (involving I1-wave) would be blocked by conditioning SICI (Fig. 1A).

## 2. Methods

We studied 9 right-handed healthy adults (4 females, aged 31.7 ± 3.7 years) [15]. Surface electromyograms were recorded from right abductor pollicis brevis muscle. PAS was delivered pairing median nerve stimulation with TMS (180 pairs, 0.1 Hz) [1]. PAS conditioned by SICI (SICI-PAS) was compared to PAS alone with both AP and PA directed currents (Fig. 1B and supplementary

*Abbreviations:* AP, anterior-posterior; I-wave, indirect wave; M1, primary motor cortex; MEP, motor evoked potential; PA, posterior-anterior; PAS, paired associative stimulation; SICI, short interval intracortical inhibition; TMS, transcranial magnetic stimulation.

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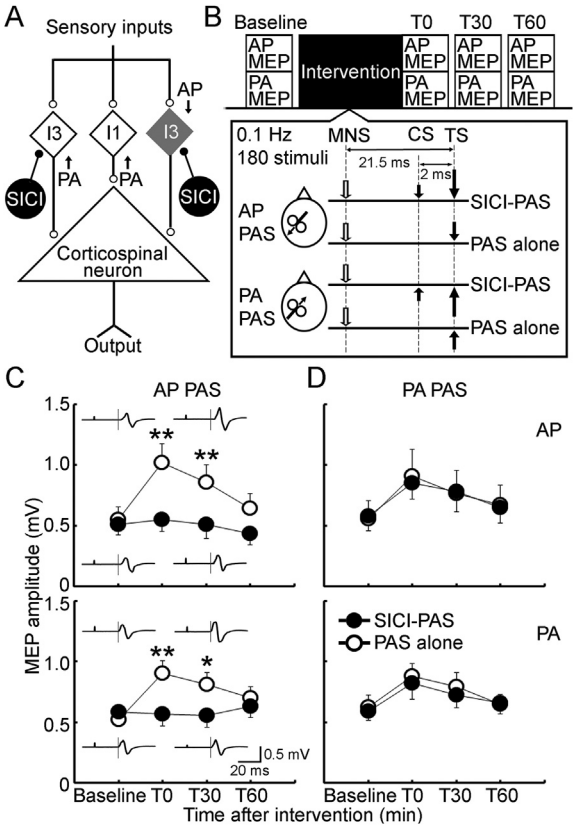


**Fig. 1. Effects of short interval intracortical inhibition on paired associative stimulation with different current directions**

(A) Hypothesis. The corticospinal neuron (triangle) receives inputs from the facilitatory interneurons (I1 and I3, labeled as rhombuses) in the primary motor cortex. Activation of these facilitatory interneurons leads to the generation of early and late I-waves. These interneurons receive facilitatory inputs from sensory afferent. TMS in PA current direction activates both I1 neurons and I3 neurons (producing early and late I-waves, labeled as white rhombuses) while that in AP current direction predominantly activates I3 neurons (labeled as grey rhombus). I3 neurons responding to PA and AP currents are different. SICI neurons (labeled as the large black circle) inhibit the late (I3 neurons) but not early I-waves. Small white circles indicate facilitatory synapses and small black circles indicate inhibitory synapses. Previous studies reported that MEP facilitation produced by PAS with interstimulus interval of 25 ms delivered in PA current direction (involving I3 neuron labeled as white rhombus on the left side) was blocked by a conditioning SICI (engagement of SICI during PAS). We hypothesize that PAS with interstimulus interval of 21.5 ms delivered in AP current direction is related to I3-wave generation and that in PA current direction is related to I1-wave generation. Therefore, MEP facilitation after PAS at 21.5 ms in AP but not in PA current direction will be blocked by a conditioning SICI. **(B)** Experimental setup. MEP amplitudes in both AP and PA current directions were measured before (baseline) and immediately, 30 min and 60 min (T0 −T60) after each interventional protocol. Interventional protocols were PAS alone and PAS conditioned by SICI (SICI-PAS) applied with either AP and PA directed currents. Arrows indicate the direction of current flowing in the brain. PAS alone was delivered pairing an MNS at wrist followed by a TS pulse at an interstimulus interval of 21.5 ms. SICI-PAS was delivered in a triple-pulse paradigm by adding a CS at 2 ms before TS. Dash lines indicate the timings of MNS, CS and TS. Note that TS intensity used in SICI-PAS protocol was adjusted to produce the same MEP size as that used in PAS alone protocol. One hundred and eighty stimuli (MNS-TS or MNS−CS−TS) were delivered in a frequency of 0.1 Hz (30 min). Four different interventional protocols were performed on four experimental days (at least 1 week apart). **(C)** and **(D)** Effects of SICI on MEP facilitation (N = 9) induced by PAS with AP **(C)** and PA directed currents **(D).** The effects of SICI-PAS (black circle) were compared to those of PAS alone (white circle). MEP amplitudes with TMS in both AP (top panels) and PA directed currents (bottom panels) were measured at baseline and at T0-T60. The abscissae indicate the time after different interventional protocols. The ordinates indicate the MEP amplitude. Examples of recordings (average of 20 trials) from one subject with PAS delivered in AP current are also shown in **(C).** The left tracings in each panel were recorded at baseline and right tracings were recorded at T0. The upper tracings in each panel represent SICI-PAS and lower tracings represent PAS alone. The dash lines indicate the MEP latency with PA directed current. Note that MEP latencies measured with AP current (tracings in the top panel) are longer than those with PA current (tracings in the bottom panel). MEP facilitation produced by PAS with AP materials) [9,16]. Four interventions (SICI-PAS vs. PAS alone × AP vs. PA current) were performed. The interstimulus interval between median nerve stimulation and test stimulus (intensity adjusted to produce ~0.5 mV MEPs both for SICI-PAS and PAS alone interventions) was 21.5 ms and that between conditioning and test stimulus was 2 ms. MEP amplitudes with both AP and PA current directions were measured before (baseline) and immediately, 30 and 60 min (T0-T60) after each intervention.

## 3. Results

MEP with same amplitude (~0.5 mV) required higher TMS intensity in AP than that in PA direction. MEP latency from AP current was longer than that from PA current by ~2.5 ms (Table S1). PAS with AP current produced MEP facilitation (Fig. 1C), as tested with both AP and PA currents (analysis of variance, AP, $F_{3,24} = 18.60$, $P = 0.002$; PA, $F_{3,24} = 8.47$, $P = 0.014$). Conditioning SICI blocked this facilitation when measured with AP current ($F_{1,24} = 31.01$, $P < 0.001$). There was a trend for reduced facilitation measured with PA current ($F_{1,24} = 5.17$, $P = 0.053$). The interaction between time and interventions were significant (AP, $F_{3,24} = 11.18$, $P = 0.007$; PA, $F_{3,24} = 10.36$, $P = 0.009$). Post-hoc tests found that the reduction of MEP facilitation was significant at T0 and T30 measured with both AP and PA currents. PAS with PA current direction produced MEP facilitation when tested with AP current (AP, $F_{3,24} = 8.71$, $P = 0.013$). There was a trend for MEP facilitation when measured with PA current ($F_{3,24} = 3.81$, $P = 0.077$). Conditioning SICI did not change the MEP facilitation (main factor of intervention: AP, $F_{1,24} = 0.01$, $P = 0.935$; PA, $F_{1,24} = 0.09$, $P = 0.775$; interaction between main factors: AP, $F_{3,24} = 0.96$, $P = 0.470$; PA, $F_{3,24} = 0.09$, $P = 0.962$) (Fig. 1D). Repetitive SICI alone with both AP and PA currents did not change MEP amplitudes (Fig. S1).

## 4. Discussion

Spike-timing-dependent plasticity explains the long-term-potentiation-like effect produced by PAS [1–5,17]. We studied a longstanding question of which neuronal components mediate PAS induced cortical plasticity [7,18–20]. In addition to the well-studied I3-wave [3,14,20,21], we found the involvement of I1-wave for MEP facilitation during PAS. Interneurons for I-wave generations receive sensory inputs from the peripheral nerve stimulation with the earliest volley arriving at ~21.5 ms latency (Fig. 1A). TMS with PA current produces I1-wave. The interactions between I1-wave neurons activated by TMS and the earliest sensory volley likely mediate MEP facilitation produced by PA PAS at 21.5 ms. Since I1-wave is not affected by SICI [8,12,13,22], SICI did not block MEP facilitation. Previous studies reported that PAS induced by PA current at 25 ms was blocked by SICI [14] because PAS at 25 ms interval involves interactions between sensory volley and I3-wave neurons, and SICI inhibits the I3-wave [8,12,13,22]. We found that PAS at 21.5 ms with AP current also produced cortical plasticity and was sensitive to the conditioning SICI, likely due to activation of I3-wave neurons that are different from those mediate the I3-wave for PA PAS [8,9,23]. Although we matched MEP amplitudes for four interventions and the approach is well-established in the field [7,11,19], it might still be argued that different I-waves compositions could contribute to different degrees of MEP facilitation because AP and PA currents

directed current but not that with PA directed current was blocked by the conditioning SICI. *P < 0.05, **P < 0.01, posthoc paired *t*-test comparing SICI-PAS to PAS alone. AP = anterior-posterior, CS = conditioning stimulus, I-wave = indirect wave, MEP = motor evoked potential, MNS = median nerve stimulation, PA = posterior-anterior, PAS = paired associative stimulation, SICI = short interval intracortical inhibition, TMS = transcranial magnetic stimulation, TS = test stimulus.

had different preferences in I-wave generations [9] and SICI predominantly inhibited late I-waves [13]. Additionally, previous studies suggest that lower intensity TMS during voluntary muscle contraction preferentially activates the I1-wave with PA current and I3-wave with AP current [7,19,24]. This may lead to an expectation that cortical plasticity produced by PAS in a certain current direction will show greater effects if the same directed current is used to measure it, but we did not observe this effect. This is likely because we used relatively high TMS intensity at rest to ensure reliable activation of large population of corticospinal neurons and to avoid potential metaplastic effects caused by voluntary contraction that may interfere with PAS [25]. Importantly, our exploratory study found different modulatory effects on cortical plasticity for PAS with AP and PA currents: MEP facilitation was blocked in the SICI-PAS intervention with AP but not with PA current. The findings were consistent with different modulatory effects with AP and PA currents when intrinsic M1 interneuron circuits [24,26] and motor behavior (movement preparation) [27] were tested. However, the strength of our conclusion is limited by the exploratory nature of our study. The precise role of SICI in the regulation of PAS induced plasticity should be defined with further studies in more subjects with direct comparisons between PAS in AP and PA currents. We conclude that PAS plastic effects can be elicited through activation of neuronal components related to I1- and I3-wave generations, and they have different sensitivities to cortical inhibition.

## Conflicts of interest

All authors declare no conflict of interest. We further confirm that all subjects provided written informed consent and any aspect of the work covered in this study that has involved human subjects has been conducted with the ethical approval of the University Health Network (Toronto) Research Ethics Board.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.brs.2018.08.019.

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