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Threshold tracking primary motor cortex inhibition: The influence of current direction

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Running Head: Threshold tracking and current direction

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Abstract

Paired-pulse transcranial magnetic stimulation (TMS) can be used to probe inhibitory activity in primary motor cortex (M1). Recruitment of descending volleys with TMS depends on the induced current direction in M1. Anterior-posterior (AP) stimulation preferentially activates late indirect- (I-) waves that are most susceptible to paired-pulse TMS. Threshold tracking TMS can assess intracortical inhibition, however previous studies have only used a current direction that preferentially recruits early I-waves (posterior-anterior, PA). Our objective was to examine intracortical inhibition with threshold tracking TMS designed to preferentially recruit early versus late I-waves with PA and AP stimulation respectively. Electromyographic recordings were obtained from the right first dorsal interosseous muscle of 15 participants (21–50 years). Motor evoked potentials elicited by TMS over left M1 were recorded for PA, AP and lateromedial (LM) induced currents, with I-wave recruitment calculated as the onset latency difference between PA-LM and AP-LM. Short- and long-interval intracortical inhibition (SICI and LICI), across a range of conditioning stimulus intensities (65-110% active motor threshold) and interstimulus intervals (100-260 ms), were assessed with threshold tracking TMS (target motor evoked potential = 200 μV) for PA and AP stimulation. SICI and LICI were greater for AP compared with PA current direction using threshold tracking. Additionally, the efficacy of late I-wave recruitment was associated with the extent of SICI for AP but not PA stimulation, and was not associated with LICI. These findings indicate that threshold tracking with an AP induced current provides a more sensitive measure of M1 intracortical inhibition than PA.

Key words: transcranial magnetic stimulation; intracortical inhibition; threshold tracking; current direction; I-waves

Introduction

Inhibitory networks in primary motor cortex (M1) permits the fine-tuning of descending commands required for dexterous manual activity. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter within M1 (Hendry *et al.*, 1987) and can be assessed using paired-pulse transcranial magnetic stimulation (TMS). When the stimulus pair is comprised of a subthreshold conditioning stimulus and a suprathreshold test stimulus at short interstimulus intervals between 1-6 ms (Kujirai *et al.*, 1993) motor evoked potentials (MEPs) in electromyography (EMG) are suppressed (inhibited) relative to those derived from the test stimulus alone. (Ziemann *et al.*, 1996; Werhahn *et al.*, 1999; Ilic *et al.*, 2002). This is known as short-interval intracortical inhibition (SICI). SICI is mediated by $GABA_A$ receptors and is modulated temporally and spatially in human motor cortex to permit selective muscle activation during functional tasks (Stinear & Byblow, 2003; Zoghi *et al.*, 2003).

Threshold tracking has been used to assess SICI to reduce limitations that may underestimate the true value of inhibition with traditional SICI, such as a ceiling/floor effect when inhibition approaches 100%. Both SICI and long-interval intracortical inhibition (LICI) values typically peak around 20% during threshold tracking (Vucic *et al.*, 2006; Menon *et al.*, 2015). To date, previous threshold tracking studies have only used a posterior-anterior (PA) induced current in the brain. PA stimulation preferentially activates early indirect volleys (I1-wave), arising from trans-synaptic activation of corticospinal neurons by intracortical circuits (Di Lazzaro *et al.*, 2012). Interestingly, the conditioning stimulus from paired-pulse TMS that gives rise to SICI and LICI suppresses later I-waves (I2 and I3) much more so than early I-waves (Nakamura *et al.*, 1997; Di Lazzaro *et al.*, 1998b; 2002; 2010; Hanajima *et al.*, 1998). Furthermore, individuals who are unable to effectively recruit late I-waves are less susceptible to plasticity-inducing protocols, such as rTMS and transcranial direct current stimulation (Hamada *et al.*, 2013; Wiethoff *et al.*, 2014). Preferential activation of circuits

responsible for late I-waves is achieved by applying TMS over M1 with an anterior-posterior (AP) induced current in the brain (Day *et al.*, 1989; Kaneko *et al.*, 1996; Nakamura *et al.*, 1996; Wilson *et al.*, 1996; Sakai *et al.*, 1997). Traditional SICI has shown greater inhibition with AP compared to PA stimulation (Sale *et al.*, 2016). This indicates that an AP induced current is likely to provide a more sensitive measure of SICI than conventional PA. However, it remains unknown how an AP current affects SICI assessed with threshold tracking.

The aim of this study was to examine intracortical inhibition with threshold tracking TMS designed to preferentially recruit early versus late I-waves by comparing PA and AP stimulation. We hypothesized that greater inhibition and a lower threshold for inhibition would be realized for AP compared to PA stimulation for both SICI and LICI. We also hypothesized that the efficacy of late I-wave recruitment would be positively associated with the intracortical inhibition threshold found with AP stimulation.

Materials and Methods

Eighteen participants with no known history of peripheral or neurological impairment were recruited. Participants completed a TMS safety screening questionnaire that was screened by a neurologist before participation and gave written informed consent. The study was approved by the University of Auckland Human Participants Ethics Committee.

The target MEP amplitude required for threshold tracking could not be evoked using AP stimulation in three participants. Therefore, data were analyzed for 15 participants (3 females, 12 males; 27±8 years; range 21–50 years). All participants were right-handed as assessed by a short version of the Edinburgh Handedness Inventory (Veale, 2014), with a median Laterality Quotient (LQ) of 0.82 (range 0.5-1.0).

Electromyography recordings

Surface EMG was recorded from the right (dominant) first dorsal interosseous (FDI) muscle using 10-mm-diameter Ag-AgCl surface electrodes (Ambu Blue Sensor Paediatric NS, Ballerup, Denmark) placed ~2 cm apart in a belly-tendon montage. A ground electrode (3M Canada) was placed on the dorsum of the hand. The EMG signals were amplified, bandpass-filtered (10 – 1000 Hz) and digitized at 10 kHz with a CED interface system (MICRO1401mkII, Cambridge Electronic Design Ltd, UK) and recorded onto a computer for offline analysis using Signal Software (Version 5.03, Cambridge Electronic Design Ltd, UK).

Experimental setup

During testing participants were seated comfortably and their right shoulder was abducted ~45º with the forearm pronated and palm facing down. Throughout the experiment the participant was required to remain at rest, or maintain a voluntary contraction of ~10% of maximal voluntary contraction (MVC) by performing index finger abduction. Visual feedback of FDI EMG was displayed on a computer monitor to assist in maintaining a steady contraction. At the start of the experiment participants performed 2-3 brief MVCs for 3-5 s, separated by 30 s, with the index finger into abduction while holding a precision grip. During the experiment, 5.4±1.5% of trials were beyond ±2SD of the mean EMG, measured 100 ms before the stimulus artefact, and excluded from further analysis (Cirillo *et al.*, 2015).

Transcranial magnetic stimulation

A MagPro X100+option stimulator (Magventure A/S, Denmark) connected to a figure-of-eight coil (MC-B70, outer wing diameter 97 mm) was used to deliver focal TMS with a monophasic current waveform (pulse width 70 µs from onset to peak). Descending volleys were preferentially activated via direct, or early or late I-waves by altering current flow through the motor cortex (Day *et al.*, 1989; Werhahn *et al.*, 1994; Sakai *et al.*, 1997; Di

Lazzaro *et al.*, 1998a; 2001; Hamada *et al.*, 2013). Specifically, posterior-anterior (PA, coil handle ~45° to midline) preferentially elicits early I-waves, whereas anterior-posterior (AP, coil handle same as PA, but current reversed) preferentially elicits late I-waves. Lateromedial (LM, coil handle 90° from midline) was used to preferentially elicit D-waves. Each current direction was tested in a single block of trials, with the order of currents randomized and counterbalanced. The coil was placed at the optimal scalp position for eliciting a MEP in the contralateral FDI muscle for each induced current with the optimal positions marked on the scalp. TMS was delivered at 0.2 Hz. The optimal coil position was continually monitored throughout the experiment.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity required to elicit a MEP in the relaxed FDI of at least 50 μV in amplitude in four out of eight consecutive trials. RMT was determined for PA and AP current directions. Active motor threshold (AMT) was defined as the minimum stimulus intensity required to elicit a MEP in the FDI muscle of at least 200 μV in amplitude in four out of eight consecutive trials during a low-level voluntary precision grip contraction (10% of FDI MVC). AMT was determined for LM, PA, and AP current directions. Both RMT and AMT are expressed relative to maximum stimulator output (MSO).

Threshold tracking involved eliciting a target MEP amplitude of 200 μV (±20%), which represents the middle portion of the linear relationship between the logarithm of the MEP amplitude and the stimulus (Fisher *et al.*, 2002). Similar to RMT and AMT, a threshold tracking target (TTT) was defined as the minimum stimulus intensity required to elicit a MEP in the relaxed FDI of at least 160 μV in amplitude in four out of eight consecutive trials. The TTT was determined before and after each paired-pulse measure. In the presence of a conditioning stimulus (CS) the test stimulus (TS) intensity must be increased to reach the target amplitude (Fisher *et al.*, 2002; Figure 1).

Dependent Measures

Long-interval intracortical inhibition (LICI)

Long-interval intracortical inhibition (LICI) was investigated by using a suprathreshold CS of 130% RMT_{PA} and RMT_{AP} at interstimulus intervals (ISIs) of 100, 160, 180, 200, 220, 240, and 260 ms. The TS was defined as the minimum intensity required to elicit a MEP amplitude within or above the TTT (>160 μV) in two out of three consecutive trials. The ISIs were delivered sequentially starting from 100 ms, with the initial TS intensity set to the TS from the preceding ISI. The stimulus intensity was increased or decreased in 1-2% increments until the TTT was found.

Short-interval intracortical inhibition (SICI)

Short-interval intracortical inhibition (SICI) was investigated by applying a subthreshold CS 3 ms before the TS (Kujirai *et al.*, 1993; Murase *et al.*, 2015). The CS intensity was set to 65, 80, 95, and 110% of AMT_{PA} and AMT_{AP}. The TS was determined as above and the conditioning stimuli were delivered sequentially starting from 65% of AMT, with the initial TS intensity set to the TS from the preceding ISI. The stimulus intensity was increased or decreased in 1-2% increments until the TTT was found.

MEP latency

MEP latency was assessed during a low-level voluntary contraction (Wilson *et al.*, 1996; Sakai *et al.*, 1997; Hamada *et al.*, 2013). Latency onset was defined as the time point where rectified EMG signals exceeded 2 SD of the mean background EMG, measured 100 ms before the stimulus artefact. Stimulation intensities of 110% of AMT_{PA} and AMT_{AP} were used to target MEP latency from early and late I-waves respectively. A high intensity (150% of AMT_{LM}) was used for LM stimulation to increase the chances of D-wave recruitment. The MEP latency difference between PA-LM and AP-LM was used as a measure of I-wave

recruitment (Hamada *et al.*, 2013). Sixteen MEPs were recorded for each current direction and intensity. Rest periods (~30 s) were provided as needed within each block, with a maximum of eight stimuli administered between rest periods.

Data analysis

Trials that contained pre-stimulus EMG activity (root mean squared EMG >10 μV; 100 ms before stimulation) were rejected and repeated immediately. Intracortical inhibition induced by the CS (LICI and SICI) was quantified as the increase in TS intensity required to evoke the TTT:

$$INH\ (\%) = \frac{((Threshold\ of\ Conditioned\ TS) - (Threshold\ of\ TS))}{(Threshold\ of\ TS)}\ x\ 100\%$$

where positive values indicate inhibition and negative values indicate facilitation.

For LICI and SICI, the largest %INH for any ISI/CS intensity was used in correlation analyses.

Statistical analysis

Normality was tested using the Shapiro-Wilk's test and homoscedasticity of variance using the Levene's test of equality and Mauchly's test of sphericity. A one-way repeated measures ANOVA was performed to determine the effect of CURRENT DIRECTION (LM, PA, AP) on AMT and MEP latency. For LICI, a two-way repeated measures ANOVA was performed to determine the effect of CURRENT DIRECTION (PA, AP) and ISI (100, 160, 180, 200, 220, 240, 260 ms). For SICI, a two-way repeated measures ANOVA was also performed to determine the effect of CURRENT DIRECTION (PA, AP) and CS INTENSITY (65, 80, 95, 110% of AMT_{PA} or AMT_{AP}). A post-hoc Bonferroni test was used to test for significant comparisons. Additional one-sample *t*-tests (hypothesized mean = 0) were performed for LICI and SICI on each current direction separately, with a Bonferroni correction applied for multiple comparisons. A paired *t*-test was used to analyze RMT, TTT, and MEP latency

difference. A Pearson correlation analysis was used to investigate the relationship between the MEP latency difference (i.e., efficacy of I-wave recruitment; PA-LM and AP-LM) and maximum amount of inhibition (% of LICI and SICI for PA and AP stimulation). The significance level was set at $P<0.05$ and group data are presented as mean±SD in the text.

Results

Corticospinal excitability

TMS thresholds for each current direction and inferential statistics are displayed in Table 1. RMT and TTT were higher for AP stimulation compared with PA. TTT when normalized to RMT did not differ between PA and AP induced currents. AMT_{PA} was lower than AMT_{AP} and AMT_{LM} was lower than AMT_{PA} and AMT_{AP}.

MEP latency (I-wave recruitment)

Figure 2A illustrates examples of EMG traces from a representative participant showing MEPs from LM (150% AMT_{LM}), PA (110% AMT_{PA}), and AP (110% AMT_{AP}) stimulation in the active FDI muscle. Note the longer MEP latency for PA and AP compared with LM, and longer AP latency compared with PA.

For MEP latency there was a main effect of CURRENT DIRECTION ($F_{2,28}=148.2$, $P<0.001$; Figure 2B) such that PA (23.77±1.67 ms) MEP latency was longer compared with LM (22.40±1.59 ms, $P<0.001$) and AP (26.17±1.58 ms) MEP latency was longer than LM ($P<0.001$) and PA ($P<0.001$). For MEP latency differences indicative of I-wave recruitment AP-LM (3.77±0.80 ms) was greater than PA-LM as expected (1.37±1.09 ms, $P<0.001$; Figures 2C and 2D).

Effect of TMS current on LICI

There was a main effect of CURRENT DIRECTION ($F_{1,14}$=8.2, P=0.01), ISI ($F_{6,84}$=33.7, P<0.001), and CURRENT DIRECTION x ISI interaction ($F_{6,84}$=33.7, P<0.001; Figure 3A). With ISIs of 160 ms and 180 ms, inhibition was greater for AP current compared to PA (P<0.01 and P=0.01 respectively). With PA current, inhibition was present at ISIs of 100 ms (P<0.001) and 160 ms (P=0.03), whereas AP current showed inhibition was present at ISIs of 100 ms (P<0.001), 160 ms (P<0.001), 180 ms (P<0.001) and 200 ms (P<0.01).

For LICI, the maximum amount of inhibition was primarily recorded at an ISI of 100 ms for both PA (13/15) and AP (12/15) induced currents (Figure 3B). Maximum LICI was greater for AP stimulation (24.5±8.3%) than PA (19.6±5.8%, P=0.04).

Effect of TMS current on SICI

There was a main effect of CURRENT DIRECTION ($F_{1,14}$=62.0, P<0.001), CS INTENSITY ($F_{3,42}$=15.8, P<0.001), and the CURRENT DIRECTION x CS INTENSITY interaction ($F_{3,42}$=6.9, P<0.01; Figure 3C). More inhibition was observed with the AP current compared with PA for CS intensities of 80% (P<0.001), 95% (P<0.01) and 110% (P<0.01) of AMT. For PA, inhibition was present for CS intensities of 80% (P<0.01) and 95% AMT_{PA} (P<0.01). For AP, inhibition was present for CS intensities of 65% (P<0.001), 80% (P<0.001), 95% (P<0.001) and 110% (P<0.001) of AMT_{AP}.

The maximum amount of SICI was noted at 80% or 95% of AMT for both PA (13/15) and AP (15/15) induced currents (Figure 3D). Maximum inhibition was greater for AP stimulation (23.5±8.6%) than PA (11.5±8.2%, P<0.001).

Correlations

For AP stimulation, the latency of MEPs for late I-wave recruitment (AP-LM) was correlated with the maximum amount of SICI (r=0.686, P<0.01; Figure 4B). The association between

the latency of MEPs and maximum LICI for AP stimulation was weak (r=0.418; Figure 4A), and below the level for statistical significance (*P*=0.12). There was no correlation between the latency of MEPs for early I-wave recruitment (PA-LM) and the maximum amount of LICI (r=0.069, *P*=0.81; Figure 4A) or SICI (r=0.233, *P*=0.31; Figure 4B).

Discussion

The present study investigated M1 intracortical inhibition using a threshold tracking procedure with PA and AP induced currents in the brain. In support of our hypotheses, both SICI and LICI were greater with AP stimulation compared with PA. Also, the extent of SICI was positively correlated with the efficacy of late I-wave recruitment for AP stimulation. These findings indicate that an AP induced current provides a more sensitive measure of SICI and LICI than PA when using the threshold tracking technique. With threshold tracking, both SICI and LICI were dependent on recruitment of late I-waves, which varied across individuals.

Current Direction and I-wave Recruitment

The present MEP latency results are in support of previous findings that TMS in a PA and AP induced current preferentially elicit early and late I-waves respectively, whereas LM can be used to elicit D-waves (Day *et al.*, 1989; Wilson *et al.*, 1996; Sakai *et al.*, 1997). Therefore, the ability to preferentially recruit early versus late-I-waves was achieved by altering the direction of current flow in the brain (M1). However, the generation of early and late I-waves remains unclear, despite several models postulated that account for both experimental properties of corticospinal volleys and recognized cortical circuits (Di Lazzaro *et al.*, 2012; Rusu *et al.*, 2014).

LICI and SICI Threshold Tracking is More Sensitive to AP than PA Current

Paired-pulse TMS used to assess intracortical inhibition in human M1 to produce measures of SICI and LICI preferentially recruit later I-waves (Nakamura *et al.*, 1997; Di Lazzaro *et al.*, 1998b; 2002; Hanajima *et al.*, 1998). SICI and LICI have been examined with threshold tracking previously using a PA current (Vucic *et al.*, 2006; Menon *et al.*, 2015), but PA stimulation leads to non-preferential recruitment of later I-waves. In the present study, we extend previous threshold tracking results by showing that the extent of inhibition for SICI and LICI is greater for AP compared with PA current. In addition, LICI was prolonged for AP compared to PA current. These findings indicate that threshold tracking with an AP induced current provides a more sensitive measure of intracortical inhibition than PA stimulation.

Greater susceptibility to intracortical inhibition using traditional paired-pulse TMS protocols has previously been shown with AP compared to PA stimulation (Zoghi *et al.*, 2003; Sale *et al.*, 2016). However, any derived measure of SICI and LICI may underestimate the true value of inhibition because of the variable D- and I-wave composition between individuals in the TS (Sanger *et al.*, 2001; Roshan *et al.*, 2003) and insensitivity due to ceiling/floor effects when inhibition approaches 100%. Threshold tracking is an alternate method used to overcome the potential limitations of traditional paired-pulse TMS protocols. Based on the main finding of the current study, future experiments could address whether an AP induced current using threshold tracking is a more sensitive measure of SICI and LICI than traditional paired-pulse TMS protocols.

Paired-pulse TMS protocols have demonstrated that LICI may be followed by a period of facilitation (Cash *et al.*, 2010; 2011; Caux-Dedeystere *et al.*, 2014; 2015), which may result from a transitory period of late cortical disinhibition (LCD). However, the presence of LCD is not consistent in resting muscle (Caux-Dedeystere *et al.*, 2015). Using

threshold tracking, we show that the conditioned MEP returns to baseline with paired-pulse TMS at longer ISIs, and that no LCD was evident after the period of LICI in the resting FDI for both PA and AP current directions. Future studies may choose to maximize the possibility of detecting the presence of LCD by voluntarily contracting the target or adjacent muscles (Caux-Dedeystere *et al.*, 2014; 2015). Currently it remains unclear whether LCD is differentially sensitive to AP or PA stimulation.

Intracortical Inhibition is Dependent on the Efficacy of Late I-wave Recruitment

Another novel finding in the present study was that threshold tracking SICI was positively associated with late I-wave recruitment for AP stimulation only. No association was seen for early I-wave recruitment (PA-LM latency difference) with SICI and LICI. This is likely explained by PA stimulation preferentially recruiting early I-waves (Day *et al.*, 1989; Wilson *et al.*, 1996; Sakai *et al.*, 1997; Di Lazzaro *et al.*, 1998a; Hanajima *et al.*, 1998; Hamada *et al.*, 2013) that are not affected by paired-pulse TMS protocols of SICI and LICI (Nakamura *et al.*, 1997; Di Lazzaro *et al.*, 1998b; 2002; Hanajima *et al.*, 1998). While some late I-waves are also recruited by PA current, the composition of late I-waves with PA current is severely limited during threshold tracking because the stimulation strength is quite low relative to traditional paired-pulse TMS. In the present study there was a weak association between LICI with AP stimulation and late I-wave recruitment. This trend was likely due to the suppression of late I-waves at ISIs of 100 and 150 ms (Nakamura *et al.*, 1997; Di Lazzaro *et al.*, 2002), mediated by $GABA_B$ receptors in the cortex at these long ISIs (McDonnell *et al.*, 2006). However, since the CS is suprathreshold for LICI spinal mechanisms may also influence the net inhibition (McNeil *et al.*, 2011). Conversely SICI, known to be $GABA_A$ receptor mediated (Ziemann *et al.*, 1996; Werhahn *et al.*, 1999; Ilic *et al.*, 2002), provides a more purely intracortical mechanism which appeared to be contingent on the ability for TMS to recruit late I-waves in the present study.

Differentiation of early and late I-wave circuits varies between individuals and has been associated with the effectiveness of specific TMS-induced plasticity protocols that preferentially target late I-waves. For example, theta burst stimulation is more effective for individuals with a longer MEP latency difference between LM and AP current directions (Hamada *et al.*, 2013). This finding is indirectly supported by the demonstration of an association between the extent of SICI and corticospinal excitability following paired associative stimulation (Murase *et al.*, 2015). Therefore, individuals with greater SICI are likely to elicit MEPs that depend more on late I-wave recruitment. The positive correlation between SICI with AP current direction and efficacy of late I-wave recruitment (AP-LM latency difference) supports this idea.

In summary, the extent of SICI and LICI in M1 using a threshold tracking TMS procedure were greater for AP compared with PA current direction. Furthermore, the efficacy of late I-wave recruitment was associated with the extent of SICI for AP stimulation. These findings indicate that threshold tracking with AP induced current provides a more sensitive measure of intracortical inhibition by preferentially recruiting neural elements that are more susceptible to paired-pulse TMS, particularly SICI. This may have implications for assessment of intracortical inhibitory function diagnostically, where small changes in inhibition are not apparent or highly variable with conventional PA stimulation or traditional paired-pulse protocols.

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Author Contributions

J.C. and W.D.B. were responsible for experimental design and manuscript preparation. J.C. was responsible for data collection and analysis. All authors provided important intellectual input to the study and approved the submitted version of the manuscript.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Abbreviations

RMT, resting motor threshold; AMT, active motor threshold; TTT, threshold tracking target; FDI, first dorsal interosseous muscle; GABA, gamma aminobutyric acid; LICI, long-interval intracortical inhibition; SICI, short-interval intracortical inhibition; CS, conditioning stimulus; TS, test stimulus; M1, primary motor cortex; MEP, motor evoked potential; MSO, maximum stimulator output; MVC, maximum voluntary contraction; TMS, transcranial magnetic stimulation; LM, lateromedial; PA, posterior-anterior; AP, anterior-posterior

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Table 1. Group TMS thresholds for different current directions

	TMS Current Direction			
	LM	PA	AP	P-value
RMT (% MSO)		45.5 ± 5.8	63.4 ± 10.7	<0.001
TTT (% MSO)		49.6 ± 6.4	69.4 ± 12.3	<0.001
TTT (% RMT)		109.1 ± 3.4	109.5 ± 3.8	0.73
AMT (% MSO)	44.8 ± 9.9	36.5 ± 5.2	52.6 ± 9.8	<0.001

Values are mean ± SD. *LM*, lateromedial; *PA*, posterior-anterior; *AP*, anterior-posterior; *RMT*, FDI resting motor threshold; *TTT*, FDI threshold tracking target; *AMT*, FDI active motor threshold; *MSO*, maximum stimulator output.

Figure Legends

Figure 1. Tracking threshold. Traces depict average MEPs from an individual participant. A suprathreshold conditioning stimulus (preceding the test stimulus at interstimulus intervals between 100 and 260 ms) was used for LICI, whereas a subthreshold conditioning stimulus (preceding the test stimulus at an interstimulus interval of 3 ms) was used for SICI. (A) TMS intensity required to elicit a fixed MEP amplitude (200 μV) to the single-pulse test stimulus (threshold tracking target, TTT). (B) Paired-pulse protocol of LICI where the conditioning stimulus was delivered 100 ms before the test stimulus. (C) Paired-pulse protocol of SICI where the conditioning stimulus was delivered 3 ms before the test stimulus. Threshold tracking requires an increase in the test stimulus intensity to evoke the target response in the presence of conditioning (grey traces in B and C).

Figure 2. I-wave recruitment. (A) MEP onset latency tested in the FDI muscle during a low-level isometric voluntary contraction (~10% of MVC) in an individual participant. LM stimulation (top) was set to 150% AMT, whereas PA (middle) and AP (bottom) were set to 110% AMT. Traces show the average of 16 MEPs. Blue arrows indicate current flow in the underlying motor cortex, black arrows indicate stimulus (TMS), and vertical dashed lines indicate the MEP onset. (B) Group data showing LM (red bar), PA (blue bar) and AP (grey bar) MEP onset latencies (n=15). (C) Individual participant responses for latency difference between PA-LM (early I-wave recruitment) and AP-LM (late I-wave recruitment). (D) Group data showing PA-LM (blue bar) and AP-LM (grey bar) latency difference (n=15). MEP onset latency was longer for PA and AP compared with LM, and AP was longer than PA. AP-LM latency difference was greater than PA-LM for all participants. Error bars indicate SEs. *$P<0.05$.

Figure 3. Intracortical inhibition. (A) LICI tested in the resting FDI muscle for PA (circles) and AP (triangles) induced currents. LICI was greater for AP stimulation compared with PA at ISIs of 160 and 180 ms. (B) Group data showing the maximum LICI for PA and AP induced currents over the ISIs tested. Maximum inhibition was greater for AP stimulation compared with PA. (C) SICI tested in the resting FDI muscle for PA (circles) and AP (triangles) induced currents. SICI was greater for AP stimulation compared with PA at CS intensities of 80%, 95% and 110% AMT. (D) Group data showing the maximum SICI for PA and AP induced currents. Maximum inhibition was greater for AP stimulation compared with PA. Error bars indicate SEs. Filled symbol denotes statistical significance compared with baseline ($P<0.05$). *$P<0.05$ compared with PA.

Figure 4. Correlations between I-wave recruitment with intracortical inhibition. Correlation analyses between maximum LICI and MEP onset latency for PA (blue circles) and AP (grey triangles) induced currents (A), and between maximum SICI and MEP onset latency (B).

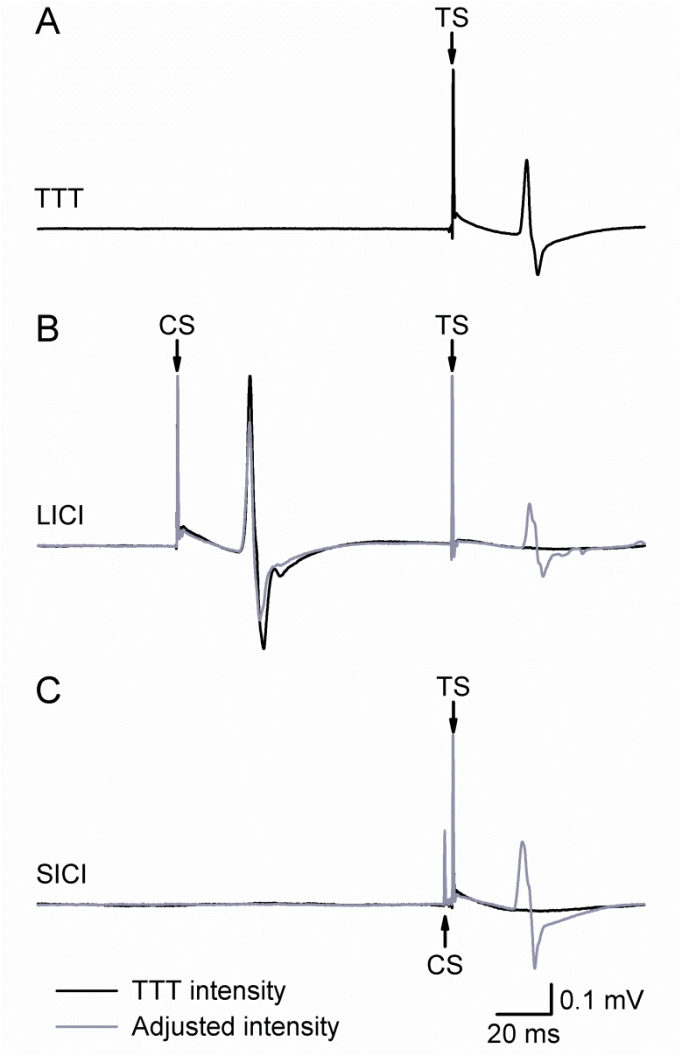
A
TS
TTT
B
CS
TS
LICI
C
TS
SICI
CS
TTT intensity
Adjusted intensity
0.1 mV
20 ms

Figure 1

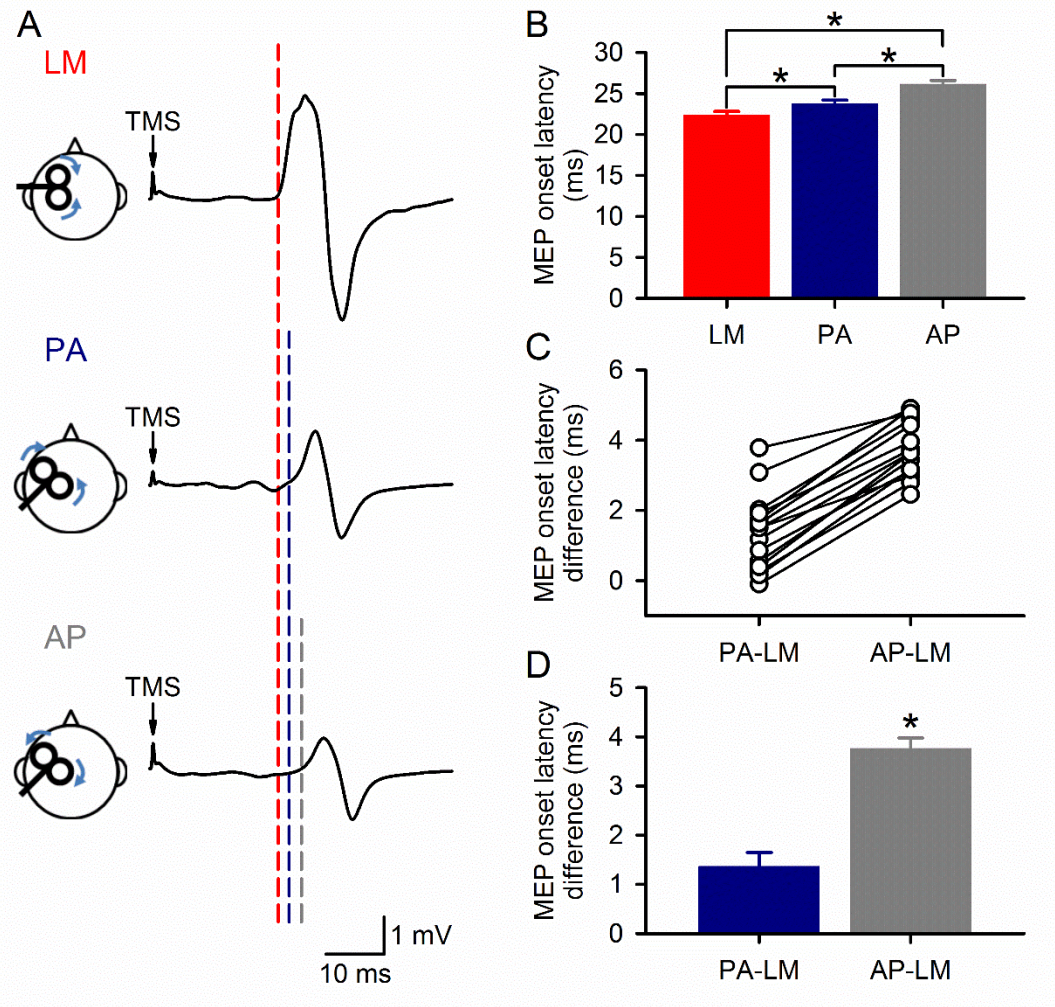
A
LM
TMS
PA
TMS
AP
TMS
1 mV
10 ms
B
MEP onset latency (ms)
30
25
20
15
10
5
0
*
*
*
LM
PA
AP
C
MEP onset latency difference (ms)
6
4
2
0
PA-LM
AP-LM
D
MEP onset latency difference (ms)
5
4
3
2
1
0
*
PA-LM
AP-LM

Figure 2

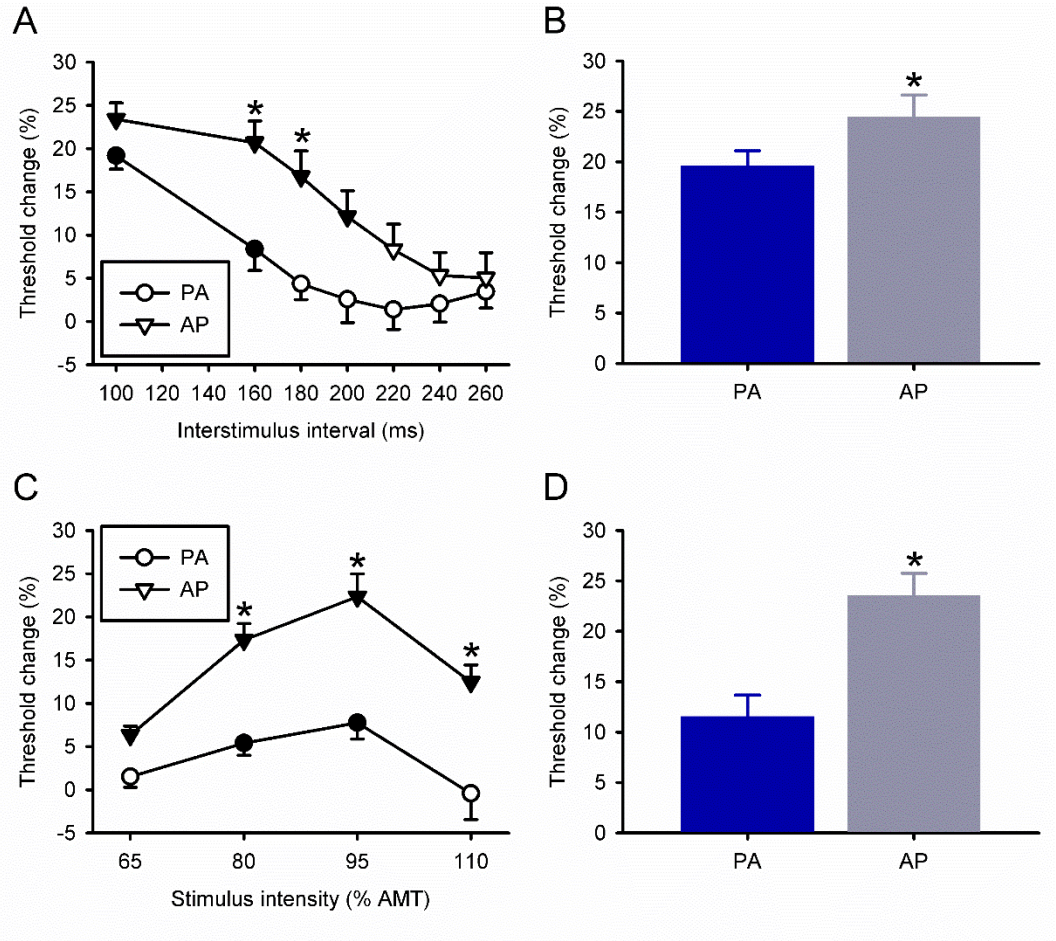
A
B
C
D
Threshold change (%)
Interstimulus interval (ms)
Stimulus intensity (% AMT)
PA
AP
100 120 140 160 180 200 220 240 260
65 80 95 110
30 25 20 15 10 5 0 -5
*

Figure 3

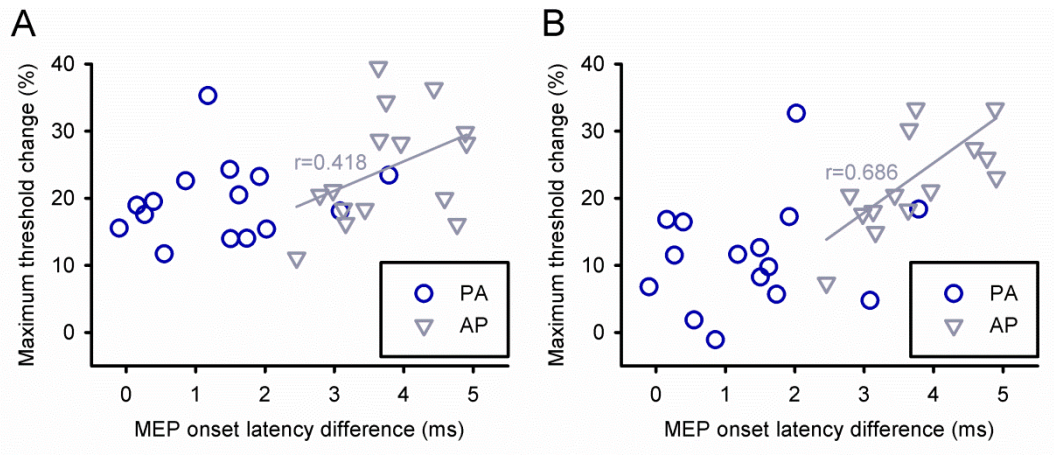
A
B
Maximum threshold change (%)
MEP onset latency difference (ms)
r=0.418
r=0.686
PA
AP
0
10
20
30
40
0
1
2
3
4
5

Figure 4