

# Modulation of I-Wave Generating Pathways With Repetitive Paired-Pulse Transcranial Magnetic Stimulation: A Transcranial Magnetic Stimulation–Electroencephalography Study

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## ABSTRACT

**Objectives:** Repetitive paired-pulse transcranial magnetic stimulation (iTMS) at indirect (I) wave intervals increases motor-evoked potentials (MEPs) produced by transcranial magnetic stimulation (TMS) to primary motor cortex (M1). However, the effects of iTMS at early and late intervals on the plasticity of specific I-wave circuits remain unclear. This study therefore aimed to assess how the timing of iTMS influences intracortical excitability within early and late I-wave circuits. To investigate the cortical effects of iTMS more directly, changes due to the intervention were also assessed using combined TMS-electroencephalography (EEG).

**Material and Methods:** Eighteen young adults (aged  $24.6 \pm 4.2$  years) participated in four sessions in which iTMS targeting early (1.5-millisecond interval; iTMS<sub>1.5</sub>) or late (4.0-millisecond interval; iTMS<sub>4.0</sub>) I-waves was applied over M1. Neuroplasticity was assessed using both posterior-to-anterior (PA) and anterior-to-posterior (AP) stimulus directions to record MEPs and TMS-evoked EEG potentials (TEPs) before and after iTMS. Short-interval intracortical facilitation (SICF) at interstimulus intervals of 1.5 and 4.0 milliseconds was also used to index I-wave activity.

**Results:** MEP amplitude was increased after iTMS ( $p < 0.01$ ), and this was greater for PA responses ( $p < 0.01$ ) but not different between iTMS intervals ( $p = 0.9$ ). Irrespective of iTMS interval and coil current, SICF was facilitated after the intervention ( $p < 0.01$ ). Although the N45 produced by AP stimulation was decreased by iTMS<sub>1.5</sub> ( $p = 0.04$ ), no other changes in TEP amplitude were observed.

**Conclusions:** The timing of iTMS failed to influence which I-wave circuits were potentiated by the intervention. In contrast, decreases in the N45 suggest that the neuroplastic effects of iTMS may include disinhibition of intracortical inhibitory processes.

**Keywords:** I-wave periodicity repetitive transcranial magnetic stimulation, motor-evoked potential, primary motor cortex, transcranial magnetic stimulation–electroencephalography, transcranial magnetic stimulation–evoked potential

**Conflict of Interest:** The authors reported no conflict of interest.

## INTRODUCTION

Transcranial magnetic stimulation (TMS) is a noninvasive brain stimulation technique that can induce and measure neuroplastic

changes in primary motor cortex (M1), providing important evidence for the flexibility of M1 neurons. Neuroplasticity involves alterations to glutamatergic and gamma-aminobutyric acid (GABA) neurotransmission (Kida and Mitsushima<sup>1</sup>) and greatly facilitates

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physiological and functional recovery after brain injury, for example, after stroke (Nudo<sup>2</sup>) or traumatic brain injury (Clayton et al<sup>3</sup>). Using TMS to modulate neuroplasticity after injury therefore has the potential to provide therapeutic benefits within neurorehabilitation.

When TMS is applied to M1, it produces a complex volley of waves within corticospinal neurons that summate at the spinal cord to produce a motor-evoked potential (MEP) (Ziemann<sup>4</sup>). The earliest component of this descending volley is the D-wave, which is thought to reflect direct activation of the corticospinal axon. This is followed by a series of indirect (I)-waves that occur with a periodicity of approximately 1.5 milliseconds: these are referred to as early (I1) and late (I2 and I3) based on their recruitment order and are thought to reflect input onto the corticospinal neuron from local interneuronal networks.<sup>5</sup> Although these waves can only be directly visualized using invasive recordings from the epidural space, it is possible to assess their activity using paired-pulse TMS. For example, when two stimuli are applied over M1 with an interstimulus interval (ISI) corresponding to the I-wave periodicity, the associated MEP is facilitated relative to the response generated by a single stimulus applied in isolation. This is referred to as short-interval intracortical facilitation (SICF) and is thought to index excitability of the I-wave circuits (Ziemann<sup>4</sup>). Importantly, though, the nature of the I-wave circuits activated by TMS (both single- and paired-pulse) is influenced by the direction of cortical current induced by stimulation. In particular, the interneuronal networks recruited by a posterior-to-anterior (PA) stimulus are distinct to those generated by an anterior-to-posterior (AP) current, and these measures can therefore provide a unique neurophysiological insight into the effects of an intervention (Opie and Semmler<sup>6</sup>).

Although discrete application of paired-stimuli can index I-wave excitability, applying the same stimulus pairs repeatedly over a 15-minute period instead produces a robust increase in MEPs and SICF. This is referred to as I-wave periodicity repetitive TMS (iTMS) and is thought to induce long-term potentiation-like changes in M1.<sup>7–9</sup> Interestingly, previous work has suggested that modifying the ISI used during iTMS can determine which I-wave circuits are influenced by the intervention.<sup>8</sup> For example, short ISIs of 1.5 milliseconds would influence the I1-wave circuitry, whereas longer ISIs of 4 to 5 milliseconds would influence the I3-wave circuitry. This is important because the early and late I-wave circuits have unique physiological and functional relevance,<sup>10,11</sup> and an ability to target them selectively has important implications for the clinical application of brain stimulation interventions. For example, we have previously shown that the late I-waves have decreased amplitude and delayed timing in older adults and that these changes contribute to age-related deficits in neuroplasticity induction and fine motor function.<sup>7,12</sup> Selective modulation of these circuits may therefore allow improved motor learning and performance in older populations. However, it remains unclear whether modulating the timing of iTMS conveys an ability to selectively target activity within specific I-wave circuits.

Therefore, the aim of this research was to investigate how iTMS applied with short and longer ISIs influences the excitability of early and late I-wave circuits. This was achieved by applying iTMS with ISIs of 1.5 milliseconds ( $iTMS_{1.5}$ , corresponding to the I1-wave) and 4 milliseconds ( $iTMS_{4.0}$ , corresponding to the I2–3 wave) in separate sessions. To characterize changes within a broader range of I-wave generating populations, MEPs and SICF were recorded using both PA and AP current directions (Opie and Semmler<sup>6</sup>). Furthermore, effects of the intervention were also assessed by using

electroencephalography (EEG) to record the TMS-evoked EEG potential (TEP). In contrast to the MEP, TEPs are less influenced by spinal excitability and can index activity within the discrete intracortical populations activated by TMS (Tremblay et al<sup>13</sup>). We hypothesized that early I-wave function would be modified by  $iTMS_{1.5}$ , whereas late I-wave function would be modified by  $iTMS_{4.0}$ .

## MATERIALS AND METHODS

### Participants

Eighteen healthy young adults (seven men and 11 women; mean age  $\pm$  SD = 24.6  $\pm$  4.2 years; age range = 19–35 years) were recruited from the university and wider community to participate in this study. All participants were right-handed, free of neurological and psychiatric disorders (assessed through self-report), and not taking any drugs that influence the central nervous system. Contraindications to TMS were assessed using the TMS adult safety screen.<sup>14</sup> A nominal payment of \$15 per hour was offered to compensate for time and cost of participation. Written informed consent was provided before inclusion, and this study was conducted in accordance with the Declaration of Helsinki. All experimental procedures were approved by the University of Adelaide Human Research Ethics Committee (approval number: H-026-2008).

### Experimental Arrangement

Each participant visited our laboratory for four experimental sessions that were approximately 2.5 hours long, held at the same time of day and separated by at least one week. Each session involved recording MEPs and TEPs before and after application of iTMS at either early or late intervals. Although iTMS was always applied using a PA current, pre- and post-iTMS measures were recorded with PA and AP current in separate sessions (Fig. 1). The order of the sessions was randomized within a participant. For the duration of each session, participants sat in a comfortable chair with their right hand pronated on a table and were instructed to keep their eyes open and remain relaxed. Surface electromyography (EMG) was recorded from the right first dorsal interosseous (FDI) muscle through disposable Ag/AgCl electrodes in a belly–tendon montage, with another Ag/AgCl electrode placed over the right ulnar styloid as an earth. EMG data were sampled at 2 kHz using a CED1401 interface (Cambridge Electronic Design, Cambridge, UK), amplified (1000 $\times$ ) and band-pass filtered (20–1000 Hz) by a CED1902 signal conditioner (Cambridge Electronic Design). Line noise was removed using a Humbug mains eliminator (Quest Scientific, North Vancouver, Canada), and recordings were stored on a computer for offline analysis.

	TMS settings	Pre	iTMS	Post
PA session	<ul style="list-style-type: none"> <li>• RMT</li> <li>• TS<sub>0.5–1.0</sub> mV</li> </ul>	MEP	<ul style="list-style-type: none"> <li>• iTMS<sub>1.5 ms</sub> (PA)</li> <li>• iTMS<sub>4.0 ms</sub> (PA)</li> </ul>	MEP
OR		<ul style="list-style-type: none"> <li>• TS</li> <li>• SICF<sub>1.5</sub></li> <li>• SICF<sub>4.0</sub></li> </ul>		<ul style="list-style-type: none"> <li>• TS</li> <li>• SICF<sub>1.5</sub></li> <li>• SICF<sub>4.0</sub></li> </ul>
AP session		TEP		TEP
		<ul style="list-style-type: none"> <li>• Control</li> <li>• TS</li> </ul>		<ul style="list-style-type: none"> <li>• TS</li> </ul>

Time

**Figure 1.** Experimental protocol. Four experimental sessions were performed involving iTMS sessions ( $iTMS_{1.5}$  and  $iTMS_{4.0}$ ) with a PA orientation and cortical assessments (both MEPs and TEPs) with PA and AP orientations separated by at least one week.

## Transcranial Magnetic Stimulation

Monophasic TMS pulses were delivered to the hand area of the left M1 using a figure-of-eight branding iron coil connected to two Magstim 200<sup>2</sup> stimulators through a Bistim unit (Magstim, Dyfed, UK). The coil was held tangentially to the scalp at an angle of approximately 45° to the sagittal plane, at the location producing the largest stable response in the resting right FDI muscle. This position was coregistered to the MNI-ICBM152 brain template<sup>15</sup> using a Brainsight neuronavigation system (Rogue Research Inc, Montreal, Canada). Stimulation was applied at a rate of 0.2 Hz, with a 10% jitter between trials. Resting motor threshold (RMT) was defined as the minimum intensity needed to evoke MEPs ≥ 50 µV in five of ten consecutive trials during relaxation of the right FDI muscle.<sup>16</sup> Stimulus intensity is expressed as a percentage of maximum stimulator output (MSO).

### Short-interval Intracortical Facilitation

SICF involved a subthreshold conditioning stimulus set at 90% RMT after a suprathreshold test stimulus (TS) at ISIs of 1.5 (SICF<sub>1.5</sub>) and 4.0 milliseconds (SICF<sub>4.0</sub>), corresponding to the first and third SICF peaks.<sup>7,17</sup> The TS was set at the intensity required to produce an MEP of approximately 0.5 to 1 millivolts (mV) when averaged over 20 trials. SICF at each time point was assessed using a single block of 60 trials (20 each of SICF<sub>1.5</sub>, SICF<sub>4.0</sub>, and TS), the order of which was pseudorandomized.

### I-Wave Periodicity Repetitive TMS

iTMS involved 180 pairs of stimuli applied in a PA orientation every 5 seconds, resulting in a total intervention time of 15 minutes.<sup>7,9</sup> The intensity was the same for both stimuli and was adjusted so that paired stimulation produced a response amplitude of approximately 1 mV (assessed over 20 trials before the intervention). The ISIs targeting the first and third SICF peak (ie, 1.5 and 4.0 milliseconds) were applied in separate sessions (iTMS<sub>1.5</sub> and iTMS<sub>4.0</sub>). To mitigate the effects of coil heating during the intervention, ice packs were used to cool the coil before and during iTMS application. This ensured that the same coil could be used for all TMS measures.

### Electroencephalography

EEG data was recorded using a WaveGuard EEG cap (ANT Neuro, Hengelo, The Netherlands), with 62 sintered Ag/AgCl electrodes in standard 10-10 positions, connected to an eego mylab amplifier (ANT Neuro). CPz electrode was used as the reference for all recordings. Signals were filtered online (DC–0.26 × sampling frequency), digitized at 8 kHz, and stored on a computer for offline analysis. The impedance of all electrodes was constantly kept < 10 kΩ through the experiment.

TEPs were recorded in a single block of stimulation that involved 100 pulses set at an intensity of 100% RMT, and this was always applied after measurement of MEPs. In an attempt to identify the TEP regions more contaminated by indirect sensory input to the brain (irrespective of its mode of generation), a block of shoulder stimulation was also recorded before iTMS.<sup>18,19</sup> This involved application of 100 TMS pulses set at 100% RMT but with the coil held over the acromial process of the right shoulder. Although this approach cannot fully replicate the specific somatosensory input produced by TMS over the scalp, previous work has shown that it can produce multimodal vertex responses associated with sensory input to the brain, comparable with those seen in real TEP

recordings.<sup>19</sup> During both scalp and shoulder stimulation, participants listened to white noise played through earphones (in-ear canal type, sealed with rubber tips) to reduce the influence of auditory-evoked potentials. The volume of auditory masking was individually adjusted to minimize audition of the TMS click.<sup>19,20</sup>

## Data Analysis

### MEP Data

MEP data were inspected visually, and trials with muscle activity > 20 µV peak-to-peak amplitude in the 100 milliseconds before TMS were rejected (5.6% of single-pulse MEPs, 5.9% of SICF MEPs). MEP amplitude recorded in each trial was then quantified peak-to-peak and expressed in mV. For SICF, the magnitude of facilitation recorded with each ISI was quantified as a percentage of the TS MEP amplitude recorded at baseline.<sup>9,21</sup> MEP amplitudes recorded during iTMS were averaged over ten consecutive stimuli, resulting in a total of 18 blocks. All responses during iTMS were expressed relative to the mean response amplitude from the first block.

### EEG Data

All preprocessing and subsequent analysis were performed according to previously reported procedures<sup>22,23</sup> using custom scripts on the MATLAB platform (R2019b, Mathworks), in addition to EEGLAB (v2020.0),<sup>24</sup> TESA (v1.1.1.) (Rogasch et al<sup>23</sup>) and Fieldtrip (v20200607)<sup>25</sup> toolboxes. Data were epoched from -2000 milliseconds to 2500 milliseconds around the TMS trigger, baseline corrected from -500 milliseconds to -5 milliseconds and merged into a single file (for each participant and session) including both M1 (before and after) and shoulder stimulation. Channels showing persistent, large amplitude muscle activity or noise were manually removed, and data segments associated with the large amplitude TMS artifacts were removed by cutting the data from -2 to 10 milliseconds and replacing it using cubic interpolation. The data were subsequently downsampled from 8 kHz to 500 Hz, and epochs indicating bursts of muscle activity or electrode noise were identified using the EEGLAB *pop\_rejmenu* command (default settings), before being visually inspected prior to removal. Interpolated data from -2 to 10 milliseconds were then replaced with constant amplitude data (ie, 0 seconds), and the conditions were split into two separate files (M1 and shoulder stimulation). An initial independent component analysis (ICA) was run on each condition using the FastICA algorithm,<sup>26</sup> and one to two independent components (ICs) representing the tail of the TMS-evoked muscle artifact were removed (Rogasch et al<sup>23</sup>). Constant amplitude data from -2 to 10 milliseconds were then replaced with cubic interpolation before the application of bandpass (1–100 Hz) and notch (48–52 Hz) filtering (fourth order zero-phase Butterworth filter). In order to remove any additional decay artifacts still present after the first round of ICA, the source-estimate-utilizing noise-discarding (SOUND) algorithm was then applied (five iterations, lambda = 0.1); this approach estimates and removes artifactual components within source space, and also allows missing electrodes to be estimated and replaced.<sup>27</sup> After SOUND, data around the TMS pulse were again replaced with constant amplitude data before application of a second round of ICA, and ICs associated with blinks, eye movements, electrode noise, and muscle activity were automatically identified using the TESA *compselect* function (default settings), and visually inspected before removal (Rogasch et al<sup>23</sup>). Data around the TMS

pulse were then replaced with cubic interpolation, and all channels were rereferenced to average before a final baseline correction ( $-500$  milliseconds to  $-5$  milliseconds).

### Statistical Analysis

All analysis was performed using PASW statistics software version 28 (SPSS; IBM, Armonk, NY) or Fieldtrip toolbox (EEG data only). Unless otherwise stated, data are displayed as mean  $\pm$  SEM. Normality was assessed using Kolmogorov-Smirnov tests. Significance was set at  $p < 0.05$ .

### MEP Data

Two-factor linear mixed model analysis with repeated measures ( $LMM_{RM}$ ) was used to compare baseline RMT, TS intensity, iTMS intensity, and TS MEP amplitudes between iTMS sessions ( $iTMS_{1.5}$  and  $iTMS_{4.0}$ ) and coil orientations (PA and AP). Three-factor  $LMM_{RM}$  was also used to compare baseline SICF between iTMS sessions, coil orientations, and ISIs (SICF<sub>1.5</sub> and SICF<sub>4.0</sub>). Two-factor  $LMM_{RM}$  was used to compare normalized MEP amplitudes during iTMS between iTMS sessions and blocks (B2-B18). For TS MEP amplitudes before and after iTMS, three-factor  $LMM_{RM}$  was used to compare values between iTMS sessions, coil orientations, and time points (before and after). Furthermore, four-factor  $LMM_{RM}$  was used to compare SICF between iTMS sessions, coil orientations, time points, and ISIs. For all models, participant was included as a random effect; an autoregressive<sup>1</sup> covariance structure was used; and restricted maximum likelihood estimation was applied. Each model also included single-trial MEP data. Significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction, implemented using the "Compare" sub-command in SPSS.

### TEP Data

In an attempt to identify the elements of the EEG signal that were likely to be more contaminated by sensory inputs, the TEP produced by M1 stimulation was compared with the response generated by shoulder stimulation in both spatial (ie, between electrodes at each time point) and temporal (ie, across time points within each electrode) domains using the Spearman correlation coefficient.<sup>18,19</sup> Spatial analyses were conducted from  $-50$  to  $350$  milliseconds, whereas temporal analyses were averaged over early ( $15$ – $60$  milliseconds), middle ( $60$ – $180$  milliseconds), and late ( $180$ – $280$  milliseconds) time periods.<sup>18</sup> For both measures, correlation coefficients were converted to Z-values using Fisher's

transform before group analysis.<sup>18,20</sup> Statistical significance was subsequently determined using a one-sample permutation test (derived from 10,000 permutations) assessing the hypothesis that each Z-score was greater than zero (ie, positive correlation), with the  $t_{max}$  method used to control the family-wise error rate for multiple comparisons.<sup>28</sup> The Z-values were transformed back into their original form for display.<sup>28</sup> For data within each session, TEPs were compared between pre- and post-iTMS time points using cluster-based permutation analysis. Clusters were defined as two or more neighboring electrodes, and 10,000 iterations were applied. A cluster was deemed significant if the cluster statistic exceeded  $p < 0.05$  compared with the permutation distribution. Because correlation analysis showed that TEPs were highly related to the response to shoulder stimulation from approximately 60 milliseconds after TMS (see Results), comparisons between conditions were limited to the early TEP components. This included N15 (10–15 milliseconds), P30 (20–30 milliseconds), and N45 (40–50 milliseconds).

## RESULTS

All 18 participants completed the sessions involving PA stimulation, but three participants had high stimulation thresholds that precluded collection of data with an AP orientation. Consequently, all measures for AP stimulation included data from 15 participants. No adverse events were reported. Baseline stimulus characteristics are compared between sessions and current directions in Table 1. Comparisons of RMT and TS intensity between coil orientations showed that stimulus intensities were all higher during AP stimulation (RMT:  $F_{(1,26,17)} = 82.98$ ,  $p < 0.01$ ; TS:  $F_{(1,19,14)} = 103.76$ ,  $p < 0.01$ ), but this was not different between iTMS sessions (RMT:  $F_{(1,46,1)} = 0.95$ ,  $p = 0.34$ ; TS:  $F_{(1,45,83)} = 1.65$ ,  $p = 0.21$ ), and there was no interaction between factors (RMT:  $F_{(1,40,44)} = 2.12$ ,  $p = 0.15$ ; TS:  $F_{(1,38,30)} = 0.39$ ,  $p = 0.54$ ). Baseline TS MEP amplitudes showed no differences between iTMS sessions ( $F_{(1,432,52)} = 0.001$ ,  $p = 0.98$ ) or coil orientations ( $F_{(1,441,17)} = 1.41$ ,  $p = 0.24$ ), and no interaction between factors ( $F_{(1,480,74)} = 0.29$ ,  $p = 0.59$ ). Comparisons of iTMS intensity showed higher intensities during the  $iTMS_{4.0}$  sessions ( $F_{(1,18,656)} = 5.35$ ,  $p = 0.03$ ) and during the PA sessions ( $F_{(1,22,100)} = 22.77$ ,  $p < 0.01$ ), but no interaction between factors ( $F_{(1,42,173)} = 0.59$ ,  $p = 0.45$ ). Comparisons of baseline SICF between ISIs showed that SICF<sub>1.5</sub> resulted in greater facilitation than did SICF<sub>4.0</sub> ( $F_{(1,570,74)} = 260.36$ ,  $p < 0.01$ ). However, this was not different between iTMS sessions ( $F_{(1,540,95)} = 1.48$ ,  $p = 0.22$ ) or coil

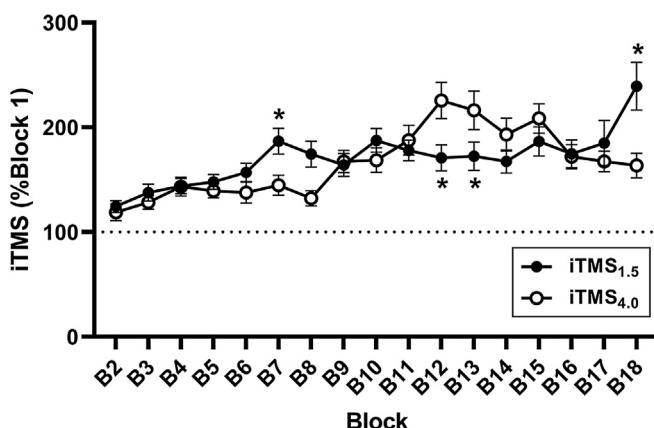
**Table 1.** TMS Intensities and MEP Amplitudes at Baseline.

TMS measurement	$iTMS_{1.5}$		$iTMS_{4.0}$	
	PA	AP	PA	AP
RMT (%MSO)	$56.6 \pm 1.3$	$69.1 \pm 1.7^*$	$57.1 \pm 1.4$	$67.1 \pm 1.8^*$
MEP <sub>0.5-1mV</sub> intensity (%MSO)	$67.0 \pm 1.7$	$81.0 \pm 1.7^*$	$66.5 \pm 1.8$	$79.4 \pm 2.0^*$
iTMS intensity (%MSO)	$57.2 \pm 1.3$	$55.8 \pm 1.3^*$	$62.1 \pm 1.2^{\dagger}$	$59.3 \pm 1.3^*,\dagger$
MEP <sub>0.5-1mV</sub> amplitude (mV)	$0.74 \pm 0.04$	$0.69 \pm 0.04$	$0.71 \pm 0.04$	$0.71 \pm 0.04$
SICF <sub>1.5</sub> (%Test)	$254.9 \pm 11.0$	$281.9 \pm 14.6$	$304.7 \pm 16.5$	$286.3 \pm 13.0$
SICF <sub>4.0</sub> (%Test)	$124.1 \pm 6.1^{\#}$	$109.9 \pm 6.9^{\#}$	$117.0 \pm 6.4^{\#}$	$116.7 \pm 6.8^{\#}$

\* $p < 0.05$  compared with PA stimulation.

<sup>†</sup> $p < 0.05$  compared with  $iTMS_{1.5}$ .

<sup>#</sup> $p < 0.05$  compared with SICF<sub>1.5</sub>.



**Figure 2.** Corticospinal excitability changes during iTMS. iTMS<sub>1.5</sub> (black circles) and iTMS<sub>4.0</sub> (white circles) are averaged over ten consecutive MEP trials, and then, blocks 2 to 18 were normalized by the first block. \* $p < 0.05$  compared with iTMS<sub>4.0</sub>. B, block.

orientations ( $F_{(1,543.02)} = 0.77, P = 0.38$ ), and there was no interaction between factors (all  $p > 0.18$ ).

#### Corticospinal Excitability During iTMS

Figure 2 shows changes in MEP amplitude expressed as percentages relative to the first iTMS block. No difference was found between iTMS sessions ( $F_{(1,2116.46)} = 0.41, p = 0.52$ ). However, values varied over blocks ( $F_{(16,3207.57)} = 4.87, p < 0.01$ ), with post hoc comparisons showing increased amplitudes during blocks 10 to 15, 17, and 18 relative to block 2 (all  $p < 0.03$ ). Furthermore, there was an interaction between factors ( $F_{(16,3204.25)} = 1.90, p = 0.02$ ), with post hoc comparisons showing differences between iTMS<sub>1.5</sub> and iTMS<sub>4.0</sub> at B7, B12, B13, and B18 (all  $p < 0.05$ ). Post hoc comparisons also showed increased amplitudes during block 18 relative to block 2 in iTMS<sub>1.5</sub> ( $p < 0.01$ ) and during blocks 12 to 15 relative to block 2 in iTMS<sub>4.0</sub> (all  $p < 0.02$ ).

#### Changes in Corticospinal and Intracortical Excitability After iTMS

TS MEP amplitudes before and after iTMS are shown in Figure 3a,b. MEP amplitudes were not different between iTMS sessions ( $F_{(1,635.68)} = 0.02, p = 0.89$ ). However, responses were larger with PA stimulation ( $F_{(1,627.7)} = 13.81, p < 0.01$ ) and at the post-iTMS time point ( $F_{(1,649.23)} = 46.86, p < 0.01$ ), and there was an interaction between coil orientation and time point ( $F_{(1,642.07)} = 4.16, p = 0.04$ ). Post hoc analysis showed that although MEPs were increased after iTMS for both coil orientations ( $p < 0.01$ ), post-iTMS responses were larger for PA than for AP stimulation ( $p < 0.01$ ). No other interactions between factors were found (all  $p > 0.44$ ).

SICF before and after iTMS is shown in Figure 3c,d. Although SICF was not different between coil orientations ( $F_{(1,991.59)} = 3.63, p = 0.06$ ), it was increased after iTMS ( $F_{(1,1017.89)} = 27.3, p < 0.01$ ) and within the iTMS<sub>4.0</sub> session ( $F_{(1,989.45)} = 7.5, p < 0.01$ ), and was greater for SICF<sub>1.5</sub> ( $F_{(1,1090.98)} = 449.61, p < 0.01$ ). Furthermore, there was an interaction between iTMS session and ISI ( $F_{(1,1072.22)} = 4.97, p = 0.03$ ). Post hoc analysis showed that for each iTMS session, the magnitude of SICF<sub>1.5</sub> was larger than the magnitude of SICF<sub>4.0</sub> ( $p < 0.01$ ). Furthermore, when comparing SICF between iTMS sessions, SICF<sub>1.5</sub> during the iTMS<sub>4.0</sub> session was greater than during the iTMS<sub>1.5</sub> session ( $p < 0.01$ ). No other interactions between factors were found (all  $p > 0.13$ ).

#### TEPs Preprocessing and Correlation Analysis

The average number of channels, epochs, and ICs removed during each step of the preprocessing pipeline is shown in Table 2. Figures 4 and 5 show grand average TEP waveforms elicited by M1 and shoulder stimulation, whereas Figure 6 shows correlation coefficients resulting from comparisons between M1 and shoulder stimulation in both spatial (Fig. 6a-d) and temporal (Fig. 6e,f) domains. For both current directions, spatial correlations identified significant relationships between these conditions that began at approximately 60 milliseconds after TMS. In support of this, results of the temporal correlations suggested that the two signals were largely unrelated within the Early period but became highly correlated across the scalp in Mid and Late periods. These results suggest that although the Early TEP response was likely to be less contaminated by sensory inputs, signals within the Mid and Late periods were likely to be heavily contaminated. Consequently, all statistical analyses of TEP amplitude were limited to the Early period (Fig. 7).

#### Changes in Cortical Excitability After iTMS

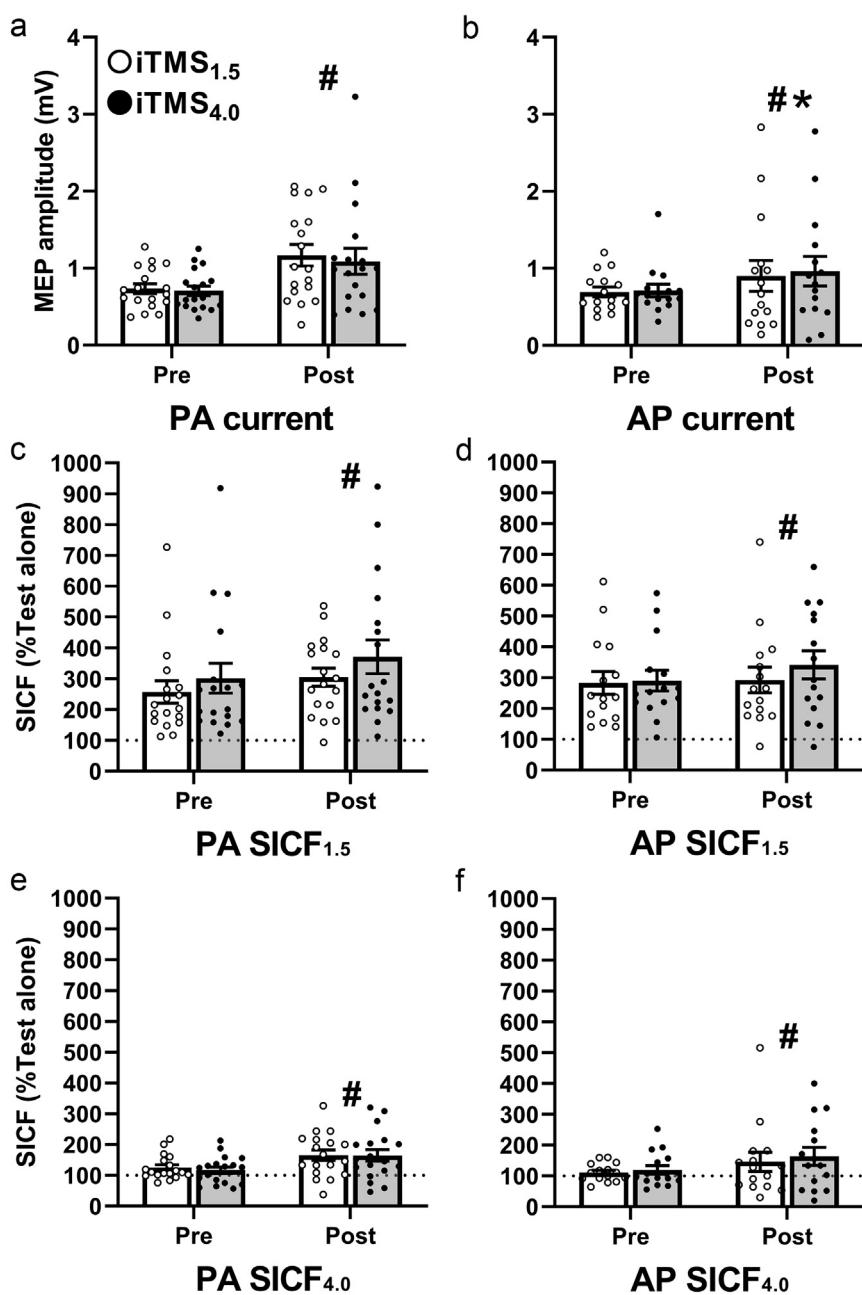
For PA sessions, there were no differences between pre- and post-iTMS TEP amplitude (all  $p > 0.06$ ). In contrast, cluster-based comparisons of the N45 generated by AP stimulation identified a positive cluster ( $p = 0.039$ ), which was associated with a decrease in amplitude after iTMS<sub>1.5</sub>. However, no differences were found for N15 and P30 (all  $p = 1$ ). Furthermore, there was no change in any of the investigated components after iTMS<sub>4.0</sub> (all  $p = 1$ ).

## DISCUSSION

The aim of this study was twofold: 1) to contrast the effects of iTMS applied with short and longer ISIs on the activity of early and late I-wave circuits and 2) to investigate the cortical response to iTMS. To achieve this, MEPs and TEPs were recorded using PA and AP current before and after iTMS<sub>1.5</sub> and iTMS<sub>4.0</sub>. This approach produced facilitation of corticospinal (MEPs) and intracortical (SICF) excitability that was comparable between iTMS intervals. In contrast, changes in the TEP were only apparent after iTMS<sub>1.5</sub> and were limited to the N45 produced by AP stimulation. Although supporting the cortical effects of iTMS, these results also suggest that we were unable to specifically target different I-wave circuits by modifying the temporal profile of iTMS.

#### Modifying iTMS ISI did not Manipulate Specific I-Wave Circuits

While previous work has investigated the effects of iTMS applied with short<sup>21,29</sup> and longer<sup>8,9</sup> ISIs, this study is the first that, to our knowledge, compares these directly. In keeping with the existing literature, we found that iTMS with both intervals produced facilitation of MEPs and SICF, indicating a neuroplastic increase in M1 excitability. However, given that previous work has suggested that modifying ISI determines which I-waves are influenced by iTMS,<sup>8</sup> we expected that the effects of iTMS would vary between ISIs. In particular, SICF is thought to provide a more specific index of excitability within different I-wave circuits,<sup>17</sup> and we therefore expected its modulation by iTMS to be ISI-dependent (eg, iTMS<sub>1.5</sub> increases SICF<sub>1.5</sub> but not SICF<sub>4.0</sub>, and vice versa). In contrast, changes to both MEPs and SICF were not different between iTMS ISIs. Consequently, our findings do not support the idea that modifying iTMS ISI allows specific targeting of different I-wave circuits.



**Figure 3.** Corticospinal and intracortical excitability changes after iTMS. Top panels (a, b) represent TS MEPs with PA (a) and AP orientations (b) before and after iTMS<sub>1.5</sub> and iTMS<sub>4.0</sub>. Bottom panels (c–f) represent SICF, which was normalized to baseline TS MEP amplitudes, with interstimulus intervals of 1.5 (c, d) and 4.0 milliseconds (e, f) for PA (left panels) and AP (right panels) orientations before and after iTMS<sub>1.5</sub> and iTMS<sub>4.0</sub>. Each panel contains individual and mean values. # $p < 0.05$  compared between before and after; \* $p < 0.05$  compared with PA responses at the same time point. stim, stimulation.

Although we were unable to show the expected specificity, it is important to note that stimulus intensities within the current study differed between SICF and iTMS. In contrast, previous work reporting differential effects of iTMS on specific I-waves used the same stimulus intensity for both. An alternative explanation for our results could therefore be that the neuronal populations targeted by our intervention may have differed from the population recruited by SICF, and this may have resulted in an apparent loss of specificity in how SICF was influenced by iTMS. In particular,

disynaptic disinhibition of an inhibitory circuit (likely involving gamma-aminobutyric acid type A; GABA<sub>A</sub>) has been shown to influence I-wave excitability assessed with SICF at short and longer latencies.<sup>30</sup> Furthermore, the perithreshold intensity we applied during iTMS would be expected to recruit relatively greater proportions of low threshold inhibitory circuits than did the higher stimulus intensity used by Long et al.<sup>8</sup> Consequently, although the neuroplastic effects reported by Long et al were likely more focused on the excitatory interneuronal circuits responsible for I-

**Table 2.** Number of Channels, Epochs, and Independent Components Removed During Cleaning of TEPs.

TMS measurement	iTMS <sub>1.5</sub>		iTMS <sub>4.0</sub>	
	PA	AP	PA	AP
Channels	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.3 ± 0.2
Epochs (TS_pre)	2.9 ± 0.9	5.6 ± 2.0	3.2 ± 0.7	5.5 ± 1.5
Epochs (TS_post)	4.4 ± 1.2	4.4 ± 1.0	4.6 ± 0.9	5.1 ± 1.6
Epochs (Control)	2.6 ± 0.7	2.3 ± 0.5	3.7 ± 0.9	4.0 ± 1.6
ICA1 (TS)	2.3 ± 0.3	2.5 ± 0.5	2.2 ± 0.3	2.0 ± 0.2
ICA1 (Control)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ICA2 (TS)	5.4 ± 0.6	6.1 ± 0.6	6.9 ± 0.6	5.5 ± 0.7
ICA2 (Control)	3.7 ± 0.4	3.1 ± 0.3	3.7 ± 0.4	3.1 ± 0.3

wave generation, it is possible that the effects of our intervention involved activation of both the low threshold disinhibitory circuit and higher threshold excitatory circuits. Within this construct, activation of the disinhibitory circuit may have produced a generalized facilitation that obscured any temporally specific effects of iTMS. Although speculative, this possibility nonetheless indicates the importance of future work investigating the influence of stimulus intensity on the effects of iTMS.

To more broadly characterize interneuronal circuits that might be differentially influenced by iTMS, excitability measures were recorded using both PA and AP currents. This approach found that single-pulse MEPs recruited with PA current were more potentiated than those recruited with AP current. One explanation for this response could be that the intervention was applied with a PA current, and elements activated by PA stimulation were therefore modulated to a greater extent. Given this, it remains possible that iTMS applied with an AP current may be more selective for modifying AP circuits. Because this has not been attempted previously, it will be important to assess in future work. Nonetheless, the response within each current direction did not vary between iTMS intervals, further suggesting that modification to ISI did not influence specific I-wave circuits. A caveat to this interpretation is that stimulus conditions in the current study (ie, 0.5–1 mV response in resting muscle) were unlikely to have produced isolated recruitment of early (PA current) or late (AP current) I-waves.<sup>6</sup> Consequently, our measures may not have been sensitive enough to identify subtle effects within different intracortical elements. Future work implementing more sensitive indices of I-wave recruitment (ie, low intensity stimulation in active muscle) after iTMS will therefore be an interesting topic of investigation.

### TEP Measures of Cortical Excitability are Modulated by iTMS

Correlation analyses comparing TEP amplitude with the peripherally evoked potential generated by shoulder stimulation suggested that responses were highly correlated from approximately 60 milliseconds. This is consistent with a growing literature<sup>18,19</sup> and may suggest that our masking of auditory input was not completely effective.<sup>20</sup> To avoid the confounding influence of this contamination, we therefore decided to limit TEP analysis to the early components, including N15, P30, and N45. Importantly, developing evidence suggests that these potentials are largely unaffected by sensory contamination.<sup>20,31,32</sup> The results of this approach showed that the amplitude of N45 was decreased by iTMS (Fig. 7). Studies using pharmacologic intervention have suggested that N45 reflects activity of intracortical inhibitory circuits

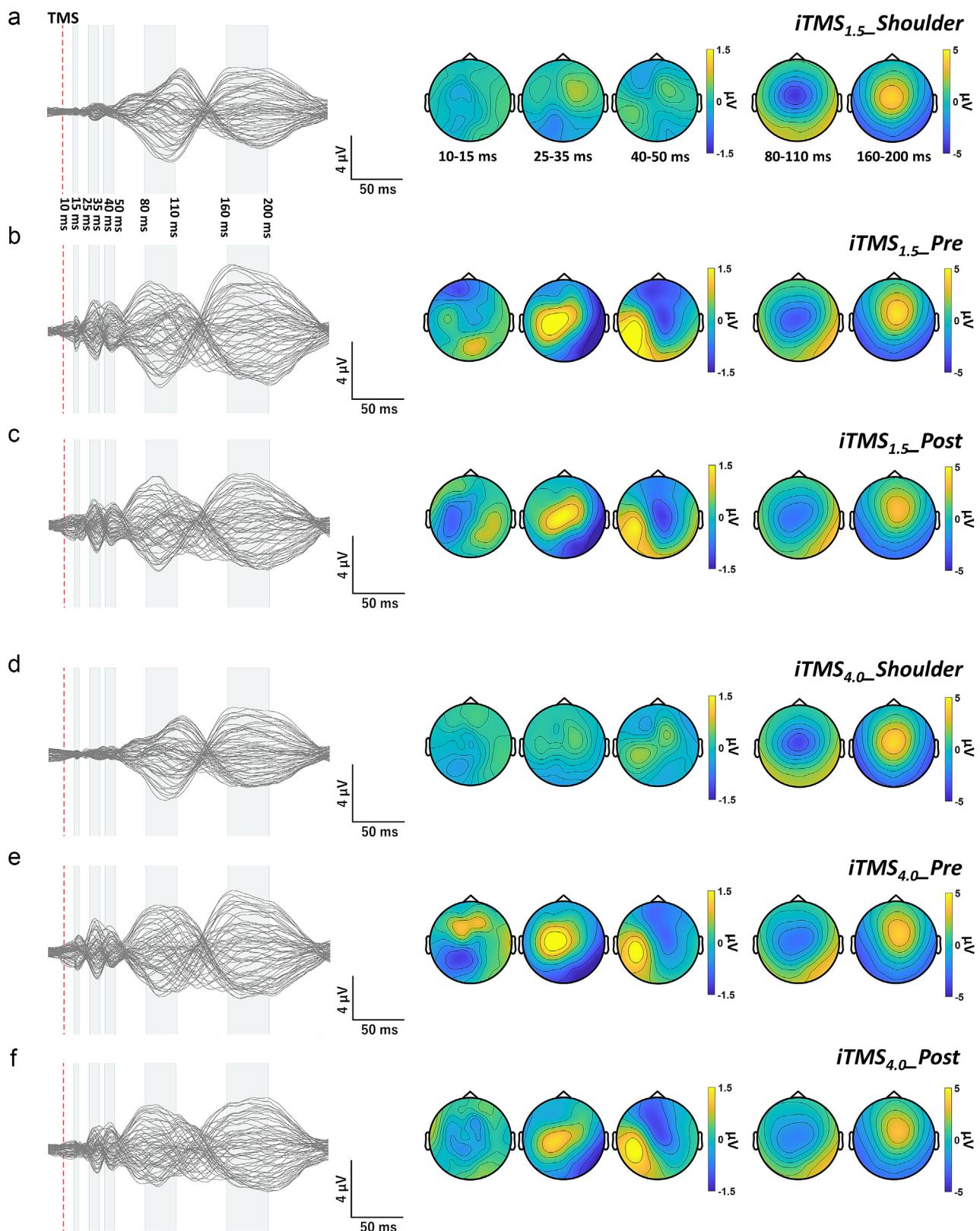
involving GABA<sub>A</sub>.<sup>33–35</sup> In support of discussion within the previous section, our TEP results therefore suggest that application of iTMS produced disinhibition of GABA<sub>A</sub>ergic inhibitory circuits.

As suggested above, the lower stimulus intensities we applied during iTMS may have resulted in effects on disinhibitory circuits that may not be as apparent after interventions applied with higher stimulus intensities. Consequently, it remains possible that using higher stimulus intensities during iTMS may reveal a different TEP response, possibly more focused on indices of motor cortical excitation such as the P30.<sup>13</sup> Despite this, it is interesting that changes in the N45 were only apparent in responses generated with AP stimulation after iTMS<sub>1.5</sub>. Although the reason for this remains to be determined, one explanation may be sensitivity to GABAergic circuits. For example, previous work using MEPs to assess short-interval intracortical inhibition has shown that AP responses are more sensitive to activity of GABAergic inhibitory circuits, possibly owing to preferential activation of late I-waves.<sup>18,36,37</sup> Furthermore, the AP session of the iTMS<sub>1.5</sub> intervention applied the lowest intensity stimulation (see “iTMS intensity” in Table 1), suggesting that its activation of low threshold inhibitory circuits would have been relatively higher than the other sessions. Although speculative, this could suggest that manipulating stimulus intensity may be one way in which the effects of iTMS could be targeted to different intracortical circuits.

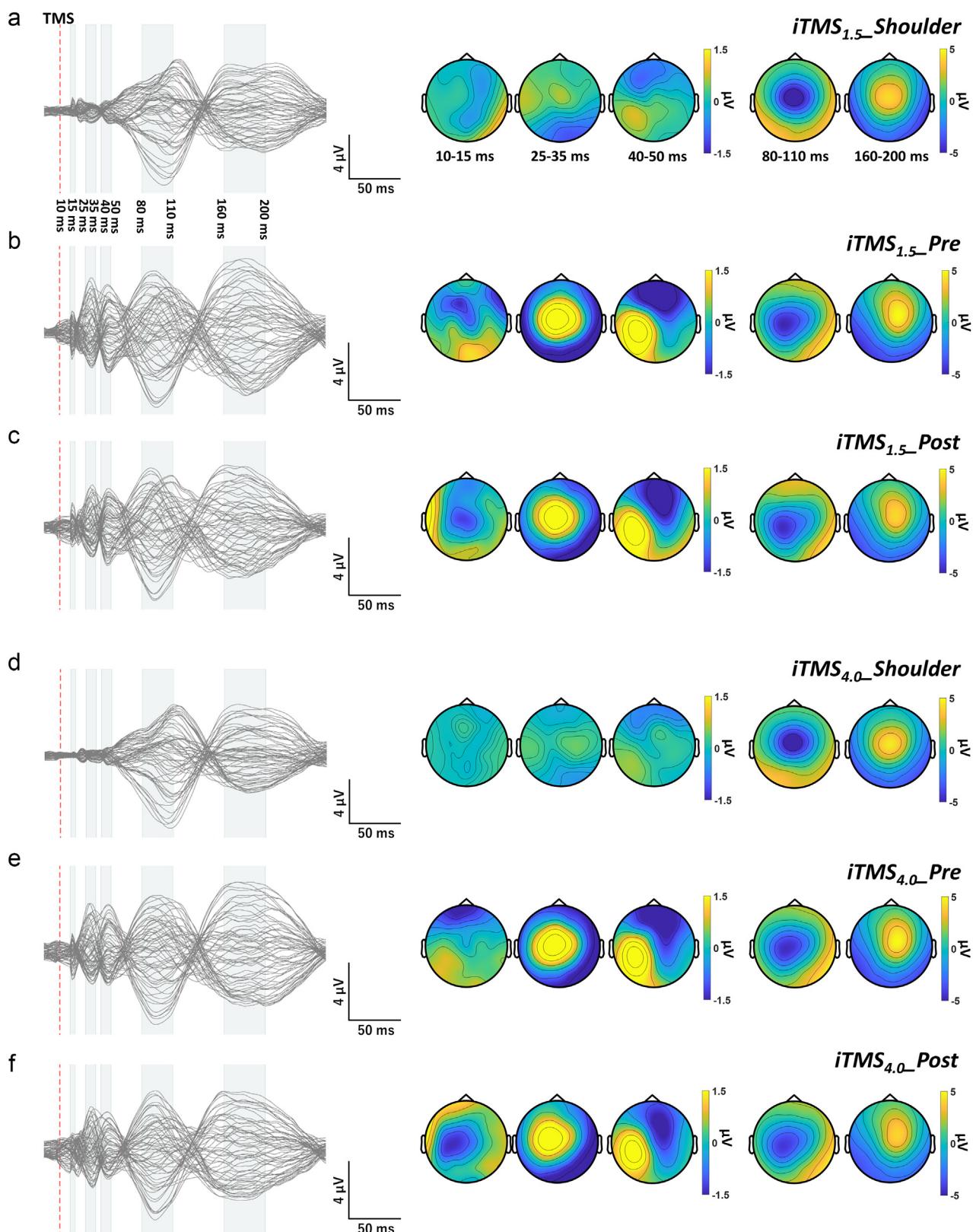
### Response Specificity After iTMS Differs Between MEPs and TEPs

Although the changes discussed above show that MEPs and TEPs were both modified by iTMS, the nature of these changes varied between outcome measurements: while MEPs/SICF showed generalized facilitation across both current directions, TEPs instead revealed a specific profile of response that was limited to AP stimulation. Although MEPs and TEPs are initiated by activation of the same intracortical networks after TMS, they provide unique characterization of these (and subsequent) events. For MEPs, the complex intracortical activity generated by TMS is integrated at the corticospinal neuron and then summated at the spinal cord, with the additional potential for modulation by spinal circuits. Consequently, MEPs reflect a net measure of excitability that summarizes the activity of individual intracortical circuits and is subject to potential subcortical input. In contrast, TEPs index activity within discrete intracortical networks (both excitatory and inhibitory)<sup>13</sup> over a broader period. They therefore provide a higher resolution measure of the initial response to TMS, in addition to consequent activity within the broader network that occurs outside the MEP time window. The differential response of MEPs and TEPs may therefore not be surprising, possibly reflecting the unique sensitivity of each measure.

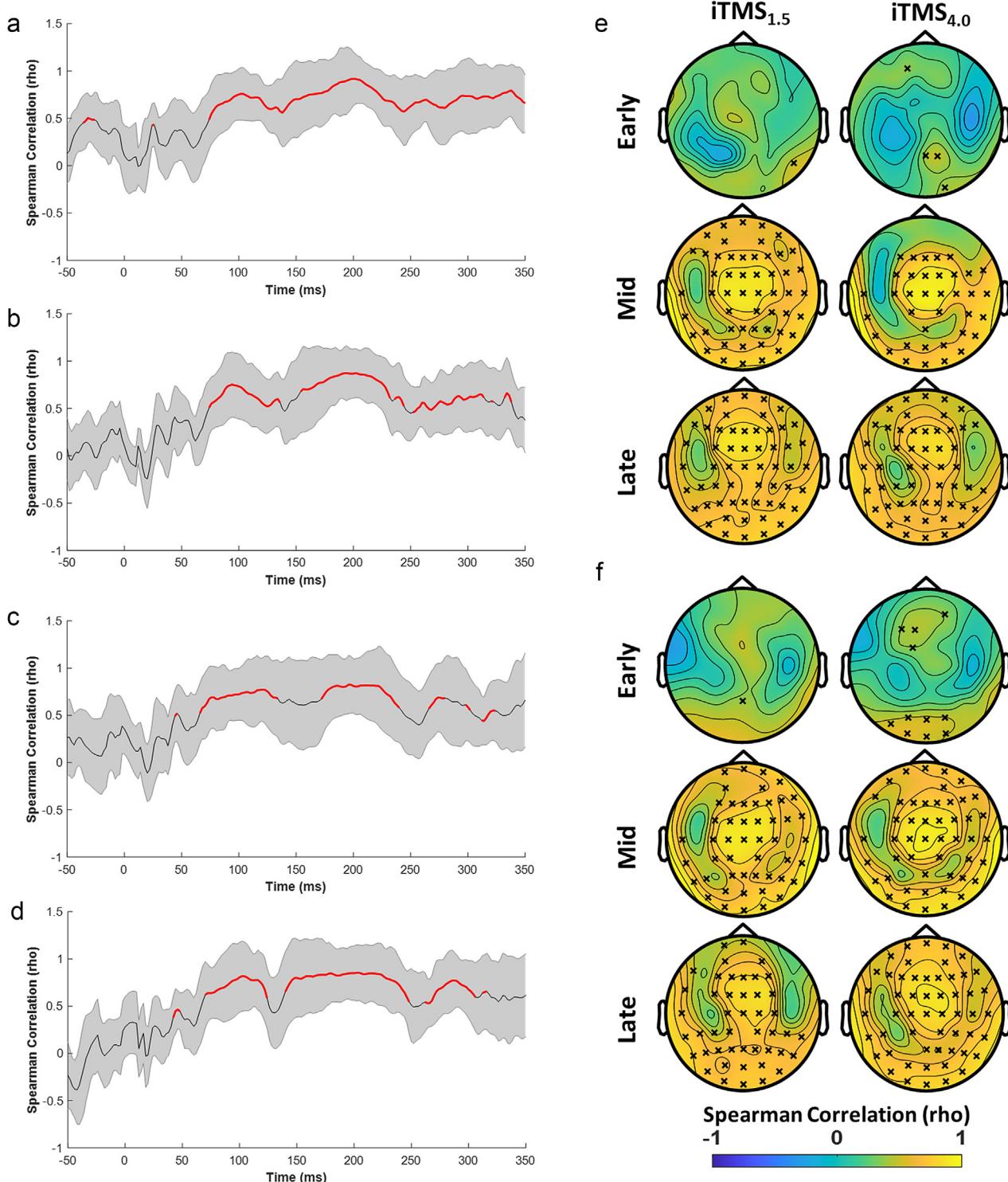
Given that stimulus intensity influences TEP amplitude<sup>38–40</sup> and intensities varied between current directions (Table 1), an alternative explanation for the specific effects on AP TEPs may be that AP stimulation produced larger amplitude components that provided an increased signal-to-noise ratio for discerning effects of the intervention. This possibility highlights some of the technical difficulties implicit within our experimental design (ie, the need for stimulus intensity to vary between current directions), in addition to issues that need to be addressed within the field more generally (ie, an ability to standardize TEP amplitude, similar to MEP recordings). The development of new methods that take these factors into account (eg,<sup>41</sup>) will be critical for confirming the role of different intracortical circuits in the response to iTMS.



**Figure 4.** Grand average TEP waveforms and topographies with PA stimulation. a–c. Shoulder (a) and M1 stimulation before and after iTMS<sub>1.5</sub> (b, c). d–f. Shoulder (d) and M1 stimulation before and after iTMS<sub>4.0</sub> (e, f). Baseline TEP waveforms show several typical TEP components, named as N15, P30, P45, N100, and P180. [Color figure can be viewed at [www.neuromodulationjournal.org](http://www.neuromodulationjournal.org)]



**Figure 5.** Grand average TEP waveforms and topographies with AP stimulation. a–c. Shoulder (a) and M1 stimulation before and after iTMS<sub>1.5</sub> (b, c). d–f. Shoulder (d) and M1 stimulation before and after iTMS<sub>4.0</sub> (e, f). Baseline TEP waveforms show several typical TEP components, named as N15, P30, P45, N100, and P180. [Color figure can be viewed at [www.neuromodulationjournal.org](http://www.neuromodulationjournal.org)]

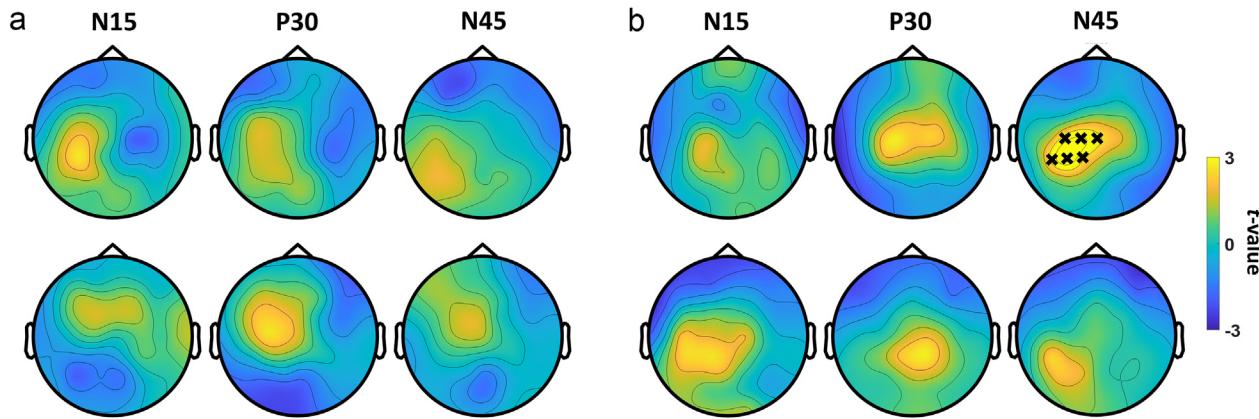


**Figure 6.** TEPs and sensory correlations. a-d. Spatial correlations between EEG response to M1 and shoulder stimulation with PA current in iTMS<sub>1.5</sub> (a) and iTMS<sub>4.0</sub> (b) sessions and that with AP current in iTMS<sub>1.5</sub> (c) and iTMS<sub>4.0</sub> (d) sessions across all EEG electrodes. Red line segments indicate time periods that are significantly related between stimulation conditions. e, f. Temporal correlations between EEG response to M1 and shoulder stimulation with PA (e) and AP (f) during Early (15–60 milliseconds), Mid (60–180 milliseconds), and Late (180–280 milliseconds) time periods. Black crosses show that electrodes were significantly related between conditions. [Color figure can be viewed at [www.neuromodulationjournal.org](http://www.neuromodulationjournal.org)]

### Limitations

This study included several limitations that need to be acknowledged. First, although the amplitude of our early TEP

components is consistent with the literature (eg, <sup>42</sup>), they are nonetheless relatively small, possibly indicating decreased reliability across subjects. This issue is apparent within much of the



**Figure 7.** Comparison of TEPs between before and after using cluster analysis. a, b. Cluster-based permutation *t*-test comparing the TEP amplitudes with PA (a) and AP stimulation (b) before and after iTMS<sub>1.5</sub> (top row) and iTMS<sub>4.0</sub> (bottom row). Black crosses show a significant cluster between pre- and post-iTMS TEP amplitude. [Color figure can be viewed at [www.neuromodulationjournal.org](http://www.neuromodulationjournal.org)]

TMS-EEG literature, likely owing to poor signal optimization with respect to the presence of artifacts and response amplitude.<sup>41</sup> Second, sensory contamination within TEPs of this study was assessed using the peripherally evoked potential generated by shoulder stimulation. Although this approach has been used in previous literature to identify generalized contamination from sensory sources,<sup>18,19</sup> it is blind to the source of this contamination and cannot replicate the specific somatosensory input associated with TMS applied to the head. Despite this, studies that have applied realistic sham stimulation to the head have shown that somatosensory and auditory contamination influences later segments of the TEP than were investigated in the current study (ie, > 60 milliseconds).<sup>20,32,38</sup> Consequently, it is unlikely that variations in sensory input contributed to our results. Nonetheless, we are unable to completely exclude this possibility, and it will therefore be important for our outcomes to be replicated in future studies that include realistic sham stimulation, in addition to improved shielding from auditory input (eg, ear defenders<sup>20</sup>). Third, this study did not use foam spacing on the face of the TMS coil, which may have resulted in bone conduction of the TMS click and exacerbation of multimodal vertex responses. Although the influence of this limitation is likely to be minor,<sup>31</sup> its impact should nonetheless be controlled for in future work. Fourth, the intensity of stimulation applied during iTMS varied between PA and AP sessions, despite iTMS always involving PA stimulation. However, because the magnitude of difference was small (ie, 1%–2% MSO) and there was no difference in MEP amplitude between conditions at the start of iTMS, it is unlikely that this limitation influenced our outcomes.

In conclusion, the application of iTMS with short and longer ISIs increased corticospinal and intracortical excitability, irrespective of iTMS interval. Although these findings suggest that modifying the timing of iTMS has limited effects on which circuits are targeted by the intervention, clarification of how stimulus intensity influences contributions from intracortical inhibitory circuits is required. In support of this, iTMS also produced specific decreases in the N45 produced by AP stimulation, suggesting that disinhibition of GABA<sub>A</sub>ergic circuits contributes to the neuroplastic effects of this paradigm.

## Authorship Statements

Ryoki Sasaki, John G. Semmler, and George M. Opie conceived and designed the experiment. Ryoki Sasaki and Brodie J. Hand

collected the data. Ryoki Sasaki analyzed the data, created the figures, and drafted the manuscript. Ryoki Sasaki and George M. Opie critically revised the manuscript. All authors reviewed and approved the final manuscript.

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