'Unravelling the role of cerebellar-motor connections in motor cortical plasticity'

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Abstract

Previous research has suggested that the cerebellum (CB) communicates extensively with interneuronal networks within the motor cortex (M1). These networks are referred to as early and late I-wave networks and are thought to be modified by CB during motor learning. However, it remains unclear which of these I-wave networks are being targeted by CB projections. Hence, the present study used transcranial magnetic stimulation (TMS) to assess the influence of CB on the excitability and neuroplasticity of different I-wave networks. 11 young (21.1 ± 0.46 years), healthy adults participated in the study. We assessed how downregulating CB excitability with transcranial direct current stimulation ($tDCS_{CB}$) influenced the response of early and late I-wave networks to a neuroplasticity-inducing TMS paradigm called I-wave periodicity TMS (iTMS). Changes in early and late I-wave plasticity were assessed by applying single pulse TMS with different coil orientations in addition to the application of the paired pulse TMS measure short intracortical facilitation (SICF). Changes in CB activity were also measured using a TMS measure called cerebellar brain inhibition (CBI). Following the combined application of $tDCS_{CB}$ and iTMS, there was a general increase in the excitability of both early and late I-wave activity ($P < 0.05$). However, these increases featured different temporal dynamics and selectivity between early and late I-wave networks. These findings characterise early and late I-wave networks as separate neuroplastic networks which may both be subject to CB influence.

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Introduction

The ability to modulate patterns of motor behaviour is fundamental for effective motor control. This ability, referred to as error-based motor learning, involves the processing of sensory feedback during motor performance and is thought to be facilitated by the communication between the motor cortex (M1) and the cerebellum (CB), with CB projections targeting and fine-tuning the excitability of neurons within M1[1]. This process allows CB to assess incoming sensory information and update commands in M1 by modifying the strength of communication between its synapses[1] – a critical process referred to as neuroplasticity. To that effect, the communication between M1 and the CB is crucial for motor learning, whereupon CB likely influences M1 plasticity. However, the neuronal elements within M1 that are targeted by CB projections is currently unknown.

To date, transcranial magnetic stimulation (TMS) has been useful in examining specific intracortical networks within M1. TMS is a non-invasive brain stimulation technique that uses electromagnetic pulses to activate neurons within the cortex. When applied over M1, TMS elicits a series of waves within the corticospinal tract which summate at the spinal cord to generate a motor evoked potential (MEP) in the target muscle[2]. Referred to as the descending volley, these waves are believed to reflect the activities of different intracortical networks within M1[3]. The earliest wave of the descending volley is likely due to direct activation of corticospinal axons close to the soma. In contrast, subsequent waves, which typically follow a 1.5 ms latency, are believed to be the result of indirect inputs on to corticospinal neurons from interneuronal networks[3]. These inputs are referred to as indirect (I) waves and are generally divided into early or late based on the order of their appearance in the descending volley[3]. Previous work has demonstrated that early and late I-wave networks can be selectively recruited by changing the direction of the cortical current induced by single pulse TMS[3]. For

example, when the induced current travels in a posterior to anterior (PA) direction across the central sulcus, early I-waves are preferentially recruited[4]. In contrast, a current in the opposite anterior-posterior (AP) direction tends to recruit late I-waves[4]. I-wave excitability has also been assessed using a paired pulse TMS technique called short intracortical facilitation (SICF)[5]. This protocol pairs two TMS pulses at an interstimulus interval (ISI) that corresponds to the I-wave periodicity of 1.5 ms, resulting in an MEP that is facilitated when compared to the response of a single TMS pulse[5].

Previous studies using TMS techniques have suggested that early and late I-waves likely represent activities of separate interneuronal networks that have different roles in neuroplasticity induction and motor learning[3, 6-9]. In particular, learning motor tasks that rely heavily on error-based processes appears to target late I-waves[8]. Furthermore, altering CB excitability has been shown to result in specific changes to late I-wave excitability[9-12], suggesting that the CB may specifically modify the plasticity of these networks. However, direct evidence characterising the relationship between CB and the specific interneuronal networks within M1 is lacking.

The aim of the present study, therefore, was to investigate the influence of CB activity on the excitability and plasticity of the interneurontal networks that produce early and late I-waves. To achieve this, CB activity was downregulated using transcranial direct current stimulation (tDCS) whilst neuroplastic changes within the I-wave generating networks was concurrently induced using a repetitive TMS protocol called I-wave periodicity TMS (iTMS). Effects of this intervention on the early and late I-wave networks were quantified by investigating changes in the response to single pulse TMS applied with different coil orientations, in addition to SICF. Given that previous work suggests that CB projections

specifically influence late I-waves[9-12], it was expected that downregulating CB excitability would result in changes in the excitability and neuroplastic response of late I-wave networks.

Materials & Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the University of Adelaide Human Research Ethics Committee (approval H-2019-252)[13]. Participants were given an information sheet about the experiment and informed consent was attained prior to the experiment.

Subjects

11 young (21.1 ± 0.46 years), right-handed, healthy adults were recruited for this study. All subjects were recruited via advertisements placed online or on notice boards within the University of Adelaide. Exclusion criteria included a history of psychiatric or neurological conditions, current use of medications that affect the central nervous system, or left handedness. Participants were also administered the Transcranial Magnetic Stimulation Adult Safety Screen questionnaire to assess suitability for brain stimulation[14].

Experimental approach

All participants completed three sessions of approximately 2.5 hours. These were separated by at least 1 week and completed at the same time of day to avoid confounding variation in cortisol[13]. The experimental protocol was the same for all three sessions with the exception of the iTMS intervention which was set to target early or late I-wave plasticity, or to have no effect on M1 plasticity (i.e. sham stimulation). The session order was randomised.

Each session included a set of baseline measures, the intervention, and repeated baseline measures post-intervention (Fig. 1).

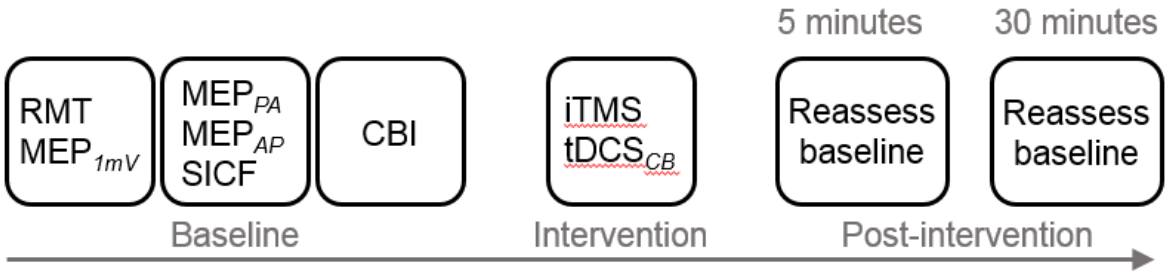

Figure 1. Experimental session procedure. RMT: resting motor threshold. MEP_{1mV}: MEP response that produces 1 mV. MEP_{PA}: MEP response in PA coil orientation. MEP_{AP}: MEP response in AP coil orientation. SICF: short intracortical facilitation. CBI: cerebellar-brain inhibition. iTMS: I-wave periodicity TMS. $tDCS_{CB}$: cerebellar transcranial direct current stimulation.

For each session, participants were seated in a comfortable chair with their right hand in resting position. Surface electromyography (EMG) recordings were made from the first dorsal interosseous (FDI) muscle on the right hand using two Ag-AgCl surface electrodes arranged in a belly-tendon montage. An additional electrode was placed on the styloid process of the right ulnar to ground the electrodes. Subjects were asked to relax the FDI throughout the experiment. EMG signals were amplified (300x) and filtered (bandpass 20-1,000 Hz) using a CED 1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK) then digitised at a rate of 2,000 Hz with a CED 1401 analogue-to-digital converter[13]. Data were stored on a PC for offline analysis[13]. Signal noise within the 50 Hz frequency band was removed via a Humbug mains noise eliminator (Quest Scientific, North Vancouver, Canada)[13].

Experimental protocols

Transcranial magnetic stimulation (TMS)

TMS was applied to the left M1 via a figure-of-8 coil connected to two Magstim 200 stimulators via a BiStim unit. The coil was held tangentially to the scalp at an angle of 45° from the sagittal plane, with the handle rotated 45° from the back to produce a PA current in the cortical representation for the right FDI muscle. The location producing the most consistent MEPs was marked on the scalp and closely monitored throughout the experiment. All baseline and post-intervention TMS was applied at a rate of 0.2 Hz, with a 10% jitter between trials in order to avoid anticipation of the stimulus[13].

Resting motor threshold (RMT) was recorded as the lowest stimulus intensity producing MEPs ≥50 μV in 5 out of 10 consecutive trials. RMT was assessed at the beginning of each session and expressed as a percentage of maximum stimulator output (% MSO). Following RMT, the intensity producing an MEP amplitude of approximately 1 mV when averaged across 20 trials in the PA direction was recorded (MEP_{1mV}). This same intensity was used again at 5 minutes and 30 minutes post-intervention to measure generalised changes in corticospinal excitability.

I-wave excitability

Specific changes in the excitability of early and late I-wave networks were assessed by changing the current direction used for single pulse TMS. When applied using low intensity stimulation, PA orientation preferentially recruits early I-waves (MEP_{PA}) while AP recruits late I-waves (MEP_{AP})[15]. AP stimulation was achieved by rotating the coil 180°. For each orientation, stimulus intensity was adjusted at baseline to produce an MEP of approximately

0.5 mV[16]. Post-intervention responses were then recorded using the same intensity, with changes in response amplitude indicating alterations to interneuronal excitability.

SICF was used as a secondary measure of excitability in early and late I-wave circuits. SICF involved a suprathreshold first stimulus (1 mV response) by a subthreshold second stimulus (90% RMT) at ISIs of 1.5 ms ($SICF_{1.5}$) and 4.5 ms ($SICF_{4.5}$). These intervals were used as they are thought to assess the excitability of early and late I-waves respectively[5]. SICF measures were recorded as a single block of 36 trial, with 12 trials per condition (24 paired, 12 single) at baseline and 5 and 30 minutes post-intervention.

Cerebellar-brain inhibition (CBI)

CBI involved a conditioning stimulus to CB 5 ms preceding a test stimulus (1 mV) to M1[17]. CB stimulation was applied with a double cone coil, with the centre of the coil 3 cm lateral and 1 cm inferior to the inion, ipsilateral to the right FDI. The coil was placed to induce an upwards current. The intensity of CB stimulus was set in the first session by identifying the highest MSO value between 60-70% that was tolerable by the participant[18], but still below the level required to activate the corticospinal tract directly[19]. CBI was assessed as a single block of 30 trials (15 paired, 15 single) at baseline and 5 and 30 minutes post-intervention.

I-wave periodicity repetitive TMS (iTMS)

iTMS is a neuroplasticity paradigm involving the repeated application of paired-pulse TMS, with modification to the ISI allowing specific I-waves to be targeted[20, 21]. Here, iTMS consisted of 180 paired stimuli with an ISI of either 1.5 ms (to target early I-waves) or 4.5 ms (to target late I-waves). In addition, a sham iTMS setting ($iTMS_{Sham}$) which was not expected to modulate corticospinal excitability was also used. This involved application of paired stimuli

with ISIs that randomly varied between 1.8, 2.3, 3.3, 3.8 and 4.7 ms. These values were used as they represent the transition point between the peaks and troughs of MEP facilitation within a standard SICF curve[13]. Intensities were set at baseline to evoke a MEP_{1mV} response. As it was impractical to set an intensity that produced a 1 mV MEP for all ISIs during $iTMS_{Sham}$, a single intensity was set using either 1.5 ms or 4.5 ms ISIs, which was randomised between participants.

Cerebellar transcranial direct current stimulation ($tDCS_{CB}$)

tDCS is a neuroplasticity paradigm that uses a low electrical current to modulate neuronal activity[22]. Cathodal tDCS was applied to the right CB ($tDCS_{CB}$) to downregulate CB activity during concurrent iTMS stimulation[13]. A Soterix Medical 1x1 DC stimulator (Soterix Medical, New York, NY) was used to apply $tDCS_{CB}$[13]. The current was applied through two saline-soaked sponge electrodes (EASYpads, 5 x 7 cm), with the cathode positioned 3 cm lateral and 1 cm down from the inion on the right cerebellar hemisphere, and the anode positioned on the right buccinators muscle[23]. Stimulation was applied at 2 mA for 15 minutes alongside iTMS to M1[13].

Data analysis

EMG data was analysed manually with visual inspection of offline recordings. Trials that included muscle activity greater than an amplitude of 25 μV in the 100 ms prior to stimulus were excluded to avoid potential interference with the recorded MEP[13]. All EMG recordings were measured in amplitude (peak-to-peak) and expressed in mV. For baseline SICF, the paired pulse measures were quantified as a ratio of the conditioned MEPs over the mean test MEPs. Baseline CBI measures were similarly expressed as a ratio of the conditioned MEPs over the mean test MEP responses. To quantify changes from baseline, all post-intervention data was

expressed as a percentage of the baseline responses recorded within the same session. MEP responses recorded during iTMS were collapsed into 10 epochs of 12 trials.

Statistical analysis

Kolmogorov-Smirnov tests were applied prior to statistical analysis to assess data normality, with log transformations applied when deviations from normal were indicated ($P < 0.05$)[13]. Linear mixed models (LMM) was used to perform statistical comparisons.

The effects of iTMS session ($iTMS_{1.5}$, $iTMS_{4.5}$ and $iTMS_{Sham}$) on baseline measures of MEP_{1mv}, MEP_{PA}, MEP_{AP} and CBI, and the first 12 trials of the iTMS interventions were each assessed using one-factor repeated-measures LMM (LMM_{RM}). Effects of iTMS session and ISI (1.5 and 4.5 ms) on baseline SICF was assessed using two-factor LMM_{RM}.

Changes in excitability during the intervention were investigated by assessing the effects of iTMS session and time (epochs 1-10)[13]. General changes in corticospinal excitability (MEP_{1mV}) following the intervention were investigated by assessing the effects iTMS session and time (5 and 30 minutes) on baseline-normalised values[13]. Specific changes in the excitability of different I-wave networks measured using different coil orientations (MEP_{PA}, MEP_{AP}) were investigated by assessing the effects of iTMS session, time, and coil orientation (PA, AP) on baseline-normalised values[13]. Changes in SICF were investigated by assessing the effects of iTMS session, time, and ISI on baseline-normalised values[13]. Changes in CB excitability were investigated by assessing the effects of iTMS session and time on baseline-normalised values[13]. Post hoc analyses, with Bonferroni corrections, were performed for significant main effects and interactions. Data are displayed as the estimated marginal means ± standard error of the mean (SEM), and $P < 0.05$ was considered significant.

Results

Baseline subject characteristics are shown in Table 1. There was no difference between sessions for the stimulus intensity required for RMT (P = 1), MEP_{1mV} (P = 0.08), MEP_{PA} (P = 0.9), MEP_{AP} (P = 0.9), and iTMS (P = 0.8).

Characteristic	$iTMS_{1.5}$	$iTMS_{4.5}$	$iTMS_{Sham}$
RMT (% MSO)	44.6 ± 2.0	44.5 ± 2.2	44.6 ± 1.8
MEP_{1mV} (% MSO)	57.4 ± 3.1	57.0 ± 2.9	54.9 ± 3.0
MEP_{PA} (% MSO)	50.7 ± 2.7	50.0 ± 2.2	49.2 ± 2.2
MEP_{AP} (% MSO)	66.7 ± 2.4	68.0 ± 2.9	66.8 ± 2.9
iTMS (% MSO)	51.0 ± 2.8	52.6 ± 2.5	50.0 ± 2.5

Table 1. Baseline characteristics (mean ± STD) between iTMS sessions.

Baseline MEPs are shown in Table 2. There was no significant effect of session on MEP_{PA} ($F_{2,215}$ = 2.6, P = 0.08), MEP_{AP} ($F_{2,482}$ = 0.73, P = 0.9), and CBI ($F_{2,333}$ = 0.30, P = 0.7). However, MEP_{1mV} varied between sessions ($F_{2,199}$ = 3.3, P = 0.04), with *post hoc* comparisons showing that responses for $iTMS_{4.5}$ were significantly larger than $iTMS_{Sham}$ (P = 0.04). The first epoch of iTMS was also different between sessions ($F_{2,296}$ = 14, P < 0.05), with *post hoc* comparisons revealing that $iTMS_{Sham}$ was significantly smaller than both $iTMS_{1.5}$ (P < 0.05) and $iTMS_{4.5}$ (P < 0.05). In addition, SICF varied between sessions ($F_{2,664}$ = 4.0, P = 0.02), with comparisons showing that $iTMS_{Sham}$ was significantly larger than $iTMS_{1.5}$ (P = 0.02). SICF also varied between ISIs ($F_{1,234}$ = 89, P < 0.05), with $SICF_{1.5}$ producing greater facilitation than $SICF_{4.5}$ (P < 0.05). Lastly, there was a significant iTMS session × SICF ISI interaction ($F_{2,694}$ = 8.3, P < 0.05). Comparisons show that $iTMS_{Sham}$ was significantly larger than both $iTMS_{1.5}$ (P < 0.05) and $iTMS_{4.5}$ (P = 0.03) for $SICF_{1.5}$. $SICF_{1.5}$ also produced greater responses than $SICF_{4.5}$ across all conditions (all P < 0.05).

TMS protocol		iTMS$_{1.5}$	iTMS$_{4.5}$	iTMS$_{Sham}$
MEP$_{1mV}$		0.958 ± 0.039	1.01 ± 0.040	0.888 ± 0.037[a]
MEP$_{PA}$		0.527 ± 0.024	0.473 ± 0.027	0.511 ± 0.029
MEP$_{AP}$		0.545 ± 0.026	0.538 ± 0.028	0.584 ± 0.036
SICF	1.5ms	142 ± 7.0	159 ± 6.8	193 ± 10[a, b]
	4.5ms	116 ± 5.1[c]	111 ± 5.4[c]	119 ± 6.2[c]
CBI		80.9 ± 2.9	85.1 ± 2.9	91.1 ± 4.2
iTMS first epoch		1.14 ± 0.047	1.12 ± 0.051	1.01 ± 0.078

Table 2. Baseline MEP (mV) responses (mean ± SEM) between iTMS sessions. [a]$P < 0.05$ compared to iTMS$_{4.5}$, [b] $P < 0.05$ compared to iTMS$_{1.5}$, [c] $P < 0.05$ compared to SICF$_{1.5}$.

Intervention changes in corticospinal excitability

Changes in MEP amplitude during each iTMS session are shown in Figure 2. These values varied between sessions ($F_{2,1080} = 120$, $P < 0.05$), with *post hoc* comparisons showing that iTMS$_{Sham}$ responses were significantly smaller than both iTMS$_{1.5}$ ($P < 0.05$) and iTMS$_{4.5}$ ($P < 0.05$) (Fig. 2A). While MEPs also varied across time ($F_{9,2510} = 2.1$, $P = 0.03$), all *post hoc* comparisons failed to achieve significance (all $P > 0.05$). There was no interaction between factors ($F_{18,2470} = 1.4$, $P = 0.1$).

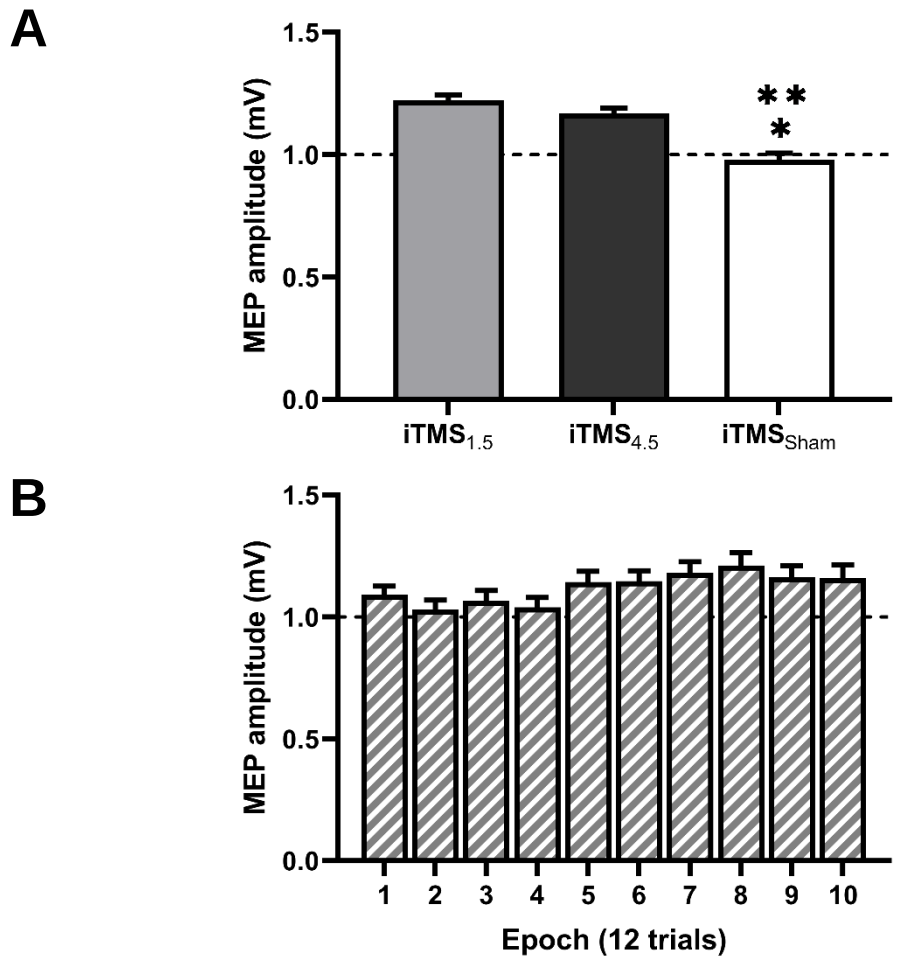

Figure 2. Reducing cerebellar exictability influences corticospinal excitability during plasticity induction. (A) Data shows average MEP responses (mV) during iTMS$_{1.5}$ (light gray bars), iTMS$_{4.5}$ (dark gray bars) and iTMS$_{Sham}$ (white bars). iTMS were set to evoke MEPs of 1 mV (dashed line). *P < 0.05 compared to iTMS$_{1.5}$. **P < 0.05 compared to iTMS$_{4.5}$. **(B)** Data shows average iTMS responses (mV) at each time point.

Post-intervention changes in corticospinal and intracortical excitability

Post-intervention changes for corticospinal excitability (MEP$_{1mV}$) are shown in Figure 3. Analysis revealed a significant main effect of session ($F_{2,287}$ = 4.8, P = 0.009) but no main effects for time ($F_{1,348}$ = 0.5, P = 0.5) or interaction between factors ($F_{2,320}$ = 0.5, P = 0.6). *Post hoc* comparisons showed that changes in MEP$_{1mV}$ were larger for iTMS$_{1.5}$ compared to iTMS$_{Sham}$ (P = 0.007).

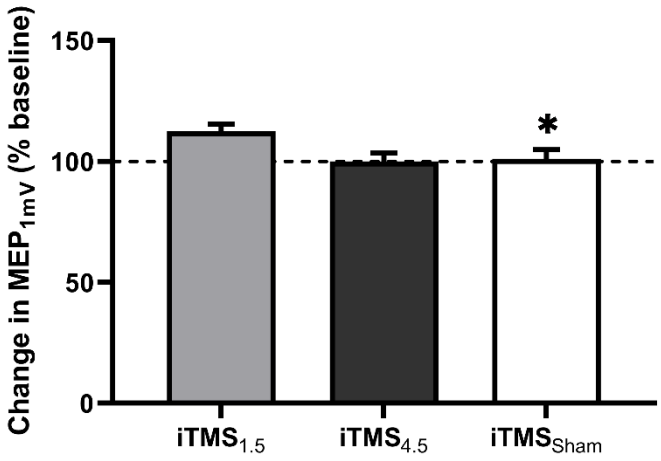

Figure 3. Reducing cerebellar excitability has limited effects on corticospinal excitability. Data shows average changes in MEP_{1mV} after application of $iTMS_{1.5}$ (light gray bars), $iTMS_{4.5}$ (dark gray bars) and $iTMS_{Sham}$ (white bars). Values are expressed as a percentage of the baseline response, indicated by dashed horizontal line. *$P < 0.05$ when compared to $iTMS_{1.5}$.

Post-intervention changes in MEP_{PA} and MEP_{AP} are shown in Figure 4 A and B respectively. There was a significant three-way interaction between session, time, and coil orientation ($F_{2,2030} = 4.9$, $P = 0.0008$). For MEP_{PA}, *post hoc* comparisons within the post 5-minute time point showed that responses following $iTMS_{1.5}$ were significantly increased compared to $iTMS_{4.5}$ ($P = 0.03$) and $iTMS_{Sham}$ ($P < 0.05$), whereas the response to $iTMS_{4.5}$ was also increased compared to $iTMS_{Sham}$ ($P < 0.05$). At the 30-minute time point, responses to $iTMS_{4.5}$ were greater than $iTMS_{Sham}$ ($P = 0.005$). For MEP_{AP}, responses at the 5-minute time point were increased following $iTMS_{4.5}$ compared to $iTMS_{Sham}$ ($P = 0.01$). At 30 minutes, both $iTMS_{1.5}$ and $iTMS_{4.5}$ were significantly greater than $iTMS_{Sham}$ (both $P < 0.05$). Comparisons between time points revealed that the response to MEP_{PA} was greater at 5 minutes than 30 minutes ($P = 0.001$). Finally, the response to $iTMS_{1.5}$ was greater for MEP_{PA} than MEP_{AP} at the 5-minute time point ($P = 0.001$). There were no other significant main effects or interactions.

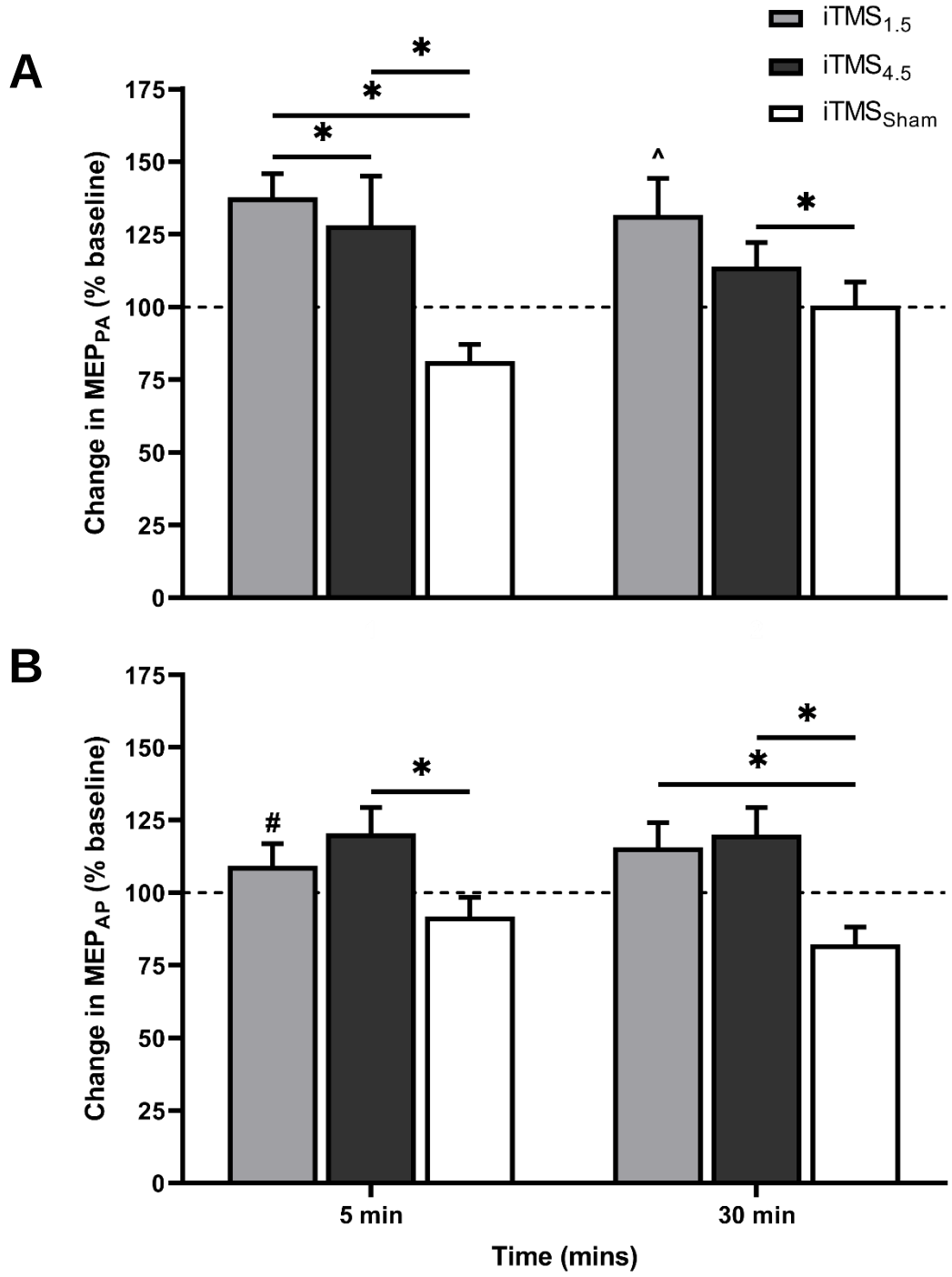

Figure 4. Reducing cerebellar excitability differentially influences plasticity of early and late intracortical motor networks. Data shows changes in MEP_{PA} (A) and MEP_{AP} (B) 5 and 30 mins after application of $iTMS_{1.5}$ (light gray bars), $iTMS_{4.5}$ (dark gray bars) and $iTMS_{Sham}$ (white bars). Values are expressed as a percentage of the baseline response, indicated by dashed horizontal line. $*P < 0.05$. $\#P < 0.05$ compared to MEP_{PA} at same time point. $\wedge P < 0.05$ compared to 5 min in same iTMS condition.

Post-intervention changes in SICF are shown in Figure 5. Analysis of these data identified an interaction between session and SICF ISI ($F_{2,985}$ = 6.2, P = 0.02). *Post hoc* comparisons showed that $SICF_{4.5}$ was increased for $iTMS_{Sham}$ (P = 0.002) and $iTMS_{4.5}$ (P = 0.04) compared to $iTMS_{1.5}$. In addition comparisons between SICF ISI were all significantly different ($P < 0.05$).

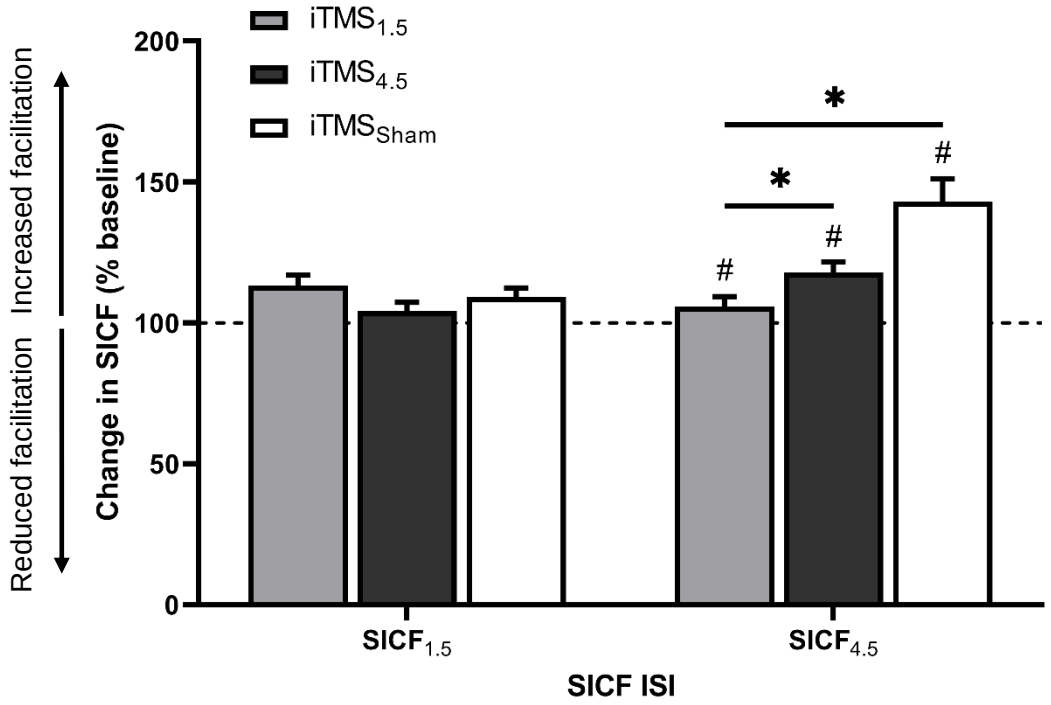

Figure 5. Reducing cerebellar excitability influences plasticity of late I-wave networks. Data shows average changes in SICF after of $iTMS_{1.5}$ (light gray bars), $iTMS_{4.5}$ (dark gray bars) and $iTMS_{Sham}$ (white bars) for $SICF_{1.5}$ and $SICF_{4.5}$. Values are expressed as a percentage of the mean baseline SICF responses, indicated by dashed horizontal line. *$P < 0.05$. #$P < 0.05$ for comparisons to $SICF_{1.5}$ within the same iTMS session.

Changes in CBI following the intervention are displayed in Figure 6. An interaction between iTMS session and time ($F_{2,846}$ = 6.4, P = 0.002) was found for these data, with p*ost hoc* analysis showing that $iTMS_{1.5}$ had reduced inhibition compared to $iTMS_{4.5}$ (P = 0.003)

after 30 minutes. Further, the response to iTMS$_{1.5}$ was greater at 30 minutes compared to 5 minutes (P = 0.001).

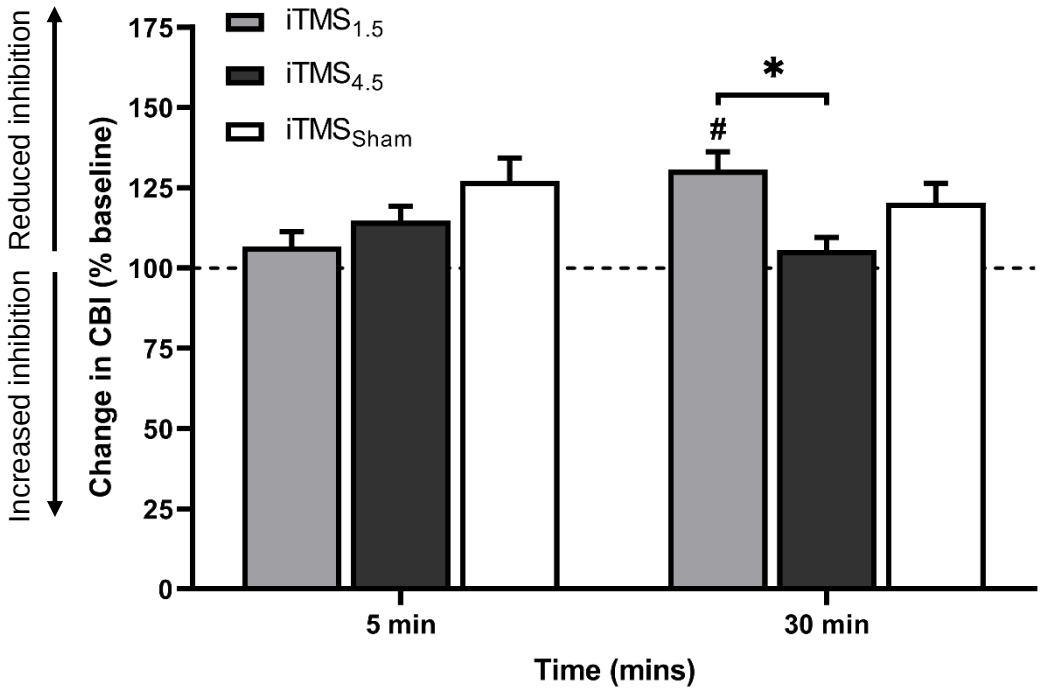

Figure 6. Reducing cerebellar excitability reduces cerebellar inhibition. Data shows changes in CBI 5 and 30 mins after application of iTMS$_{1.5}$ (light gray bars), iTMS$_{4.5}$ (dark gray bars) and iTMS$_{Sham}$ (white bars). Values are expressed as a percentage of the baseline response, indicated by dashed horizontal line. *P < 0.05. #P < 0.05 for comparison to 5 min of the same iTMS condition.

Discussion

The present study investigated the influence of CB on the excitability and plasticity of early and late I-wave networks within M1. We assessed the effects of downregulating CB excitability (tDCS$_{CB}$) during a concurrent I-wave plasticity inducing protocol (iTMS). Changes in I-wave excitability were assessed using single pulse TMS in different coil orientations (PA, AP) and SICF. Changes in CB activity were measured with CBI. Results show that cathodal tDCS$_{CB}$, when paired with iTMS, resulted in a generalised increase in I-wave activity. Further

inspection shows that early and late I-wave networks feature different temporal dynamics and selectivity.

Cerebellar stimulation has a limited effect on corticospinal excitability

MEP amplitudes during $iTMS_{1.5}$ and $iTMS_{4.5}$ were significantly increased compared to $iTMS_{Sham}$ (Fig. 2A). This suggests that corticospinal excitability increased during the intervention, which is consistent with previous studies applying iTMS[24]. Conversely, increases in MEP_{1mV} were only observed following $iTMS_{1.5}$ compared to $iTMS_{Sham}$ (Fig. 3). These changes likely reflect a sustained increase in corticospinal excitability following the intervention, which is also consistent with previous iTMS findings[21]. A lack of facilitation following $iTMS_{4.5}$ may be due to the selectivity of $iTMS_{4.5}$ to AP stimulation[25]. Taken together, these findings suggest that the changes in corticospinal excitability may be predominantly a result of iTMS, as the effects of $tDCS_{CB}$ have not been clearly demonstrated. Therefore, further studies featuring alternative methods that more precisely disentangle the effects of M1 and CB plasticity paradigms, such as including a sham condition for $tDCS_{CB}$, are needed to confirm the effects of $tDCS_{CB}$.

I-wave excitability is altered following the intervention

Compared to MEP_{1mV} during and after the intervention, MEPs recorded with different coil orientations revealed a more complex response. MEP_{PA} showed strong facilitation 5 minutes after both $iTMS_{1.5}$ and $iTMS_{4.5}$, with these responses reducing at 30 minutes (Fig. 4). In contrast, early facilitation of MEP_{AP} was only demonstrated for $iTMS_{4.5}$, before a general facilitation was shown for both $iTMS_{1.5}$ and $iTMS_{4.5}$ at 30 minutes. Furthermore, there appeared to be inhibitory effects following $iTMS_{Sham}$ across both coil orientations. These findings are consistent with previous work and suggest that early and late I-wave networks are

likely separate networks[26]. Moreover, the complex responses from MEP_{PA} and MEP_{AP} compared to MEP_{1mV} may likely be due to the differences in TMS intensity. These measures are believed recruit a mix of early and late I-waves[27]. However, lower intensities are suggested to be more selective than higher intensities[28, 29]. Therefore, the present findings support the idea that MEP_{PA} and MEP_{AP} are likely recruiting from different neuronal populations[27].

The findings of MEP_{PA} and MEP_{AP} suggest that CB may influence both early and late I-wave networks. Notably, inhibition of MEP_{PA} and MEP_{AP} following $iTMS_{Sham}$ suggests that cathodal $tDCS_{CB}$ may result in M1 inhibition. In addition, it is also possible that this effect may have reduced the extent of facilitation following $iTMS_{1.5}$ and $iTMS_{4.5}$. However, evidence of the polarity effects of cathodal $tDCS_{CB}$ is unclear and is seemingly dependent on numerous factors such as current size[23, 30]. Therefore, further investigation involving a sham $tDCS_{CB}$ is required. Despite this, inhibition during $iTMS_{Sham}$ is suggestive of CB influence to both early and late I-wave networks, as suggested by previous work[26]. As such, the differences in temporal characteristics between MEP_{PA} and MEP_{AP} may reflect different processing of CB inputs. However, further characterisation of how tDCS influences CB is required to better understand the nature of these projections.

While both MEP_{PA} and MEP_{AP} facilitated following the intervention, previous work has also shown that modulating cerebellar excitability specifically modulates late I-waves. One possibility for this inconsistency is that MEP_{PA} and MEP_{AP} still recruited a mix of both early and late I-wave networks at 0.5 mV. In particular, these measures were made from a resting muscle, thus raising the threshold of corticospinal activation[2]. In contrast, a general alternative approach to recording MEP_{PA} and MEP_{AP} uses active muscle recordings which requires lower intensities[31]. However, resting muscle was necessary to avoid confounding influence of muscle

activation on the response to plasticity induction[32]. Therefore, the responses of MEP_{PA} and MEP_{AP}, recruited at higher stimulus intensities, may reflect an overlap of early and late I-wave facilitation.

Late I-waves in SICF are modulated by cerebellar stimulation

In contrast to the measures of I-wave excitability with different coil orientations, $tDCS_{CB}$ alone ($iTMS_{Sham}$) resulted in a specific facilitation of SICF at 4.5 ms (Fig. 5). As they likely target the same I-wave networks, it is unclear why the responses for SICF were different. One possibility may be due to the different intensities used for these measures. Despite this, the effect is consistent with previous work[11], suggesting that CB projections target late I-waves, at least those recruited by SICF. However, it is unclear why $SICF_{4.5}$ following $iTMS_{4.5}$ had reduced facilitation while $iTMS_{1.5}$ showed no facilitation. It is possible that this may be due to an interaction with $tDCS_{CB}$. Further, it is also unclear why $SICF_{1.5}$ did not facilitate as previous work suggests that $SICF_{1.5}$ can be modulated by iTMS[21]. As $iTMS_{Sham}$ did not result in facilitation, this suggests that $tDCS_{CB}$ did not influence $SICF_{1.5}$, consistent with previous findings[11]. Consequently, further investigation into the effects of iTMS and $tDCS_{CB}$ on SICF are required.

Cerebellar stimulation has a limited effect on cerebellar excitability

CBI was reduced at the 30-minute time point following $iTMS_{1.5}$ compared to $iTMS_{4.5}$ (Fig. 6). This is inconsistent with previous work, which demonstrated that CBI was reduced immediately following different motor learning tasks[26]. One possibility for this difference may be due to the way in which CBI data was calculated in this study. Here, CBI was quantified as a ratio of the mean single-pulse test MEP_{1mV} to paired-pulse MEPs within the same block at each time point. As the mean test MEP_{1mV} increased following the intervention, the CBI ratio

was increased. Effectively, the change in CBI may have been driven by the change in test MEP_{1mV} as a result of $iTMS_{1.5}$. This is consistent with our other finding for MEP_{1mV} which also increased following $iTMS_{1.5}$. Consequently, this infers a limited efficacy of $tDCS_{CB}$ as no other significant differences were observed. Though $tDCS_{CB}$ has been shown to influence M1 plasticity[9], its effects are highly variable and further research is needed to understand its mechanisms of action[30].

Conclusion

In conclusion, the present study investigated the role of CB-M1 communication in the plasticity of I-wave networks. Our results suggest that early and late I-wave networks are distinct, separate networks which are both possibly targeted by the cerebellum. Further, these results indirectly suggest that early and late I-wave networks may process CB inputs differently. These findings reiterate the complexity of CB-M1 connections and the need for further examination, but suggest that it may be possible to modulate I-wave plasticity by modulating CB excitability.

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