Cerebellar noninvasive neuromodulation influences the reactivity of the contralateral primary motor cortex and surrounding areas: a TMS-EMG-EEG study

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Abstract

Understanding cerebellar–cortical physiological interactions is of fundamental importance to advance the efficacy of neurorehabilitation strategies for patients with cerebellar damage. Previous works have aimed to modulate this pathway by applying transcranial electrical or magnetic stimulation (TMS) over the cerebellum and probing the resulting changes in the primary motor cortex (M1) excitability with motor-evoked potentials (MEPs). While these protocols produce changes in cerebellar excitability, their ability to modulate MEPs has produced inconsistent results, mainly due to the MEP being a highly variable outcome measure that is susceptible to fluctuations in the excitability of M1 neurons and spinal interneurons. To overcome this limitation, we combined TMS with electroencephalography (EEG) to directly record TMS-evoked potentials (TEPs) and oscillations from the scalp. In three sessions, we applied intermittent theta-burst stimulation (iTBS), cathodal direct current stimulation (c-DC) or sham stimulation to modulate cerebellar activity. To assess the effects on M1 and nearby cortex, we recorded TMS-EEG and MEPs before, immediately after (T1) and 15 min (T2) following cerebellar neuromodulation. We found that cerebellar iTBS immediately increased TMS-induced alpha oscillations and produced lasting facilitatory effects on TEPs, whereas c-DC immediately decreased TMS-induced alpha oscillations and reduced TEPs. We also found increased MEP following iTBS but not after c-DC. All of the TMS-EEG measures showed high test–retest repeatability. Overall, this work importantly shows that cerebellar neuromodulation influences both cortical and corticospinal physiological measures; however, they are more pronounced and detailed when utilizing TMS-EEG outcome measures. These findings highlight the advantage of using TMS-EEG over MEPs when assessing the effects of neuromodulation.

Keywords Cerebellum · Motor cortex · Transcranial magnetic stimulation · Electroencephalography · TMS-EEG · Transcranial evoked potentials · Brain oscillations

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Introduction

The communication between the cerebellum and the motor cortex is essential for the execution and control of everyday movements [1]. Therefore, understanding the physiological properties of the cerebellar–motor cortex (M1) pathways and how they respond to neuromodulatory interventions, is of fundamental importance to advance the efficacy of neurorehabilitation strategies for patients with cerebellar damage.

One way in which the cerebellum exerts its modulatory control over the primary motor cortex (M1) is through the di-synaptic, excitatory dentato-thalamo-cortical (DTC) projections [2]. Activity in Purkinje cells, the output neurons of the cerebellar cortex, results in inhibition of the dentate nucleus and, ultimately, of the contralateral M1. This phenomenon can be studied in humans by means of transcranial

magnetic stimulation (TMS), using a paired-pulse protocol known as cerebellar-brain inhibition (CBI), which requires the cerebellum to be stimulated 5–7 ms before the contralateral M1 [3–7].

Previous works have aimed to modulate this pathway by applying either repetitive TMS [8–10], paired associative stimulation [11, 12] or electrical stimulation [4, 7] over the cerebellum, determining the neurophysiological effects indirectly by probing M1 excitability with motor-evoked potentials (MEPs). While these protocols are capable of inducing long-lasting excitability changes within the cerebellum, their ability to modulate MEPs has produced inconsistent results [13]. Beyond the differences amongst protocols administered, a key source of variability is due to using the MEP as a readout measure. While MEPs result from the depolarization of the corticospinal neurons following stimulation, they also reflect the excitability of both the cortex and spinal cord [14, 15], which presents a confound when interpreting changes in the MEP response. On these premises, there is a need for new TMS indexes that can reflect more directly cortical excitability and have a higher intra- and inter-subject reliability.

In the present study, we combined TMS with electroencephalography (EEG) to directly record cortical activity evoked by TMS from the scalp. In detail, we used different measures to test the effects of cerebellar neuromodulation over the contralateral hemisphere using specific cortical dynamics in the temporal and frequency domain. To assess cortical excitability, we recorded TMS-evoked potentials (TEPs), which reflect the neurophysiological state of the stimulated area and of its interconnected areas [16, 17]. TEPs were analyzed in terms of local mean field power (LMFP), a reference-free measure that is commonly used to assess the local excitability from a cluster of electrodes [18–20]. We were also interested in assessing the thalamo-cortical circuits, given their critical importance in the connections between the cerebellum and the cortex. To this aim, we computed the TMS-related spectral perturbation (TRSP) and inter-trial coherence (ITC), two measures that reflect the power and the phase synchronization of cortical oscillations originating from thalamo-cortical circuits [21]. We were also interested in comparing the novel TMS-EEG measures with traditional MEPs that were recorded during simultaneous EMG recording from the first dorsal interosseous of the hand contralateral to the stimulation. Additionally, since recent work has shown two distinct cerebellar–cerebral interactions using cerebellar stimulation in combination with directional TMS over M1 [22] we also recorded MEPs and TMS-EEG measures with both posterior–anterior (PA) and anterior–posterior (AP) currents to assess the effects on different interneuronal populations [23, 24]. We selected two different protocols which previously produced the most consistent effects on cerebellar and M1 excitability: (1) cerebellar intermittent theta burst stimulation (iTBS), which induces long-term potentiation (LTP) like changes in cerebellar excitability and other cortical areas [9, 20, 25–29]; (2) cerebellar cathodal direction current stimulation (c-DC), which induces a long-term depression (LTD) like effects on the cerebellum without changing M1 excitability [4, 30]. Finally, to assess the reliability of our results, we explored the test–retest reproducibility of our physiological measures.

Methods

Participants and procedure

Seventeen healthy volunteers (four females, mean age 29 ± 5 years) were enrolled in the study after giving written informed consent and were screened for TMS exclusion criteria [31]. All the participants were completely naïve about brain stimulation techniques. The experimental procedure was approved by the University College of London ethics committee and was in accordance with the Declaration of Helsinki (Sixth revision, 2008). The appropriateness of our sample size was established by a power calculation performed with G*Power software. The effect size, obtained as the post–pre means over pooled standard deviations, was taken from a previous study with a similar experimental design, in which we compared the amplitude of LMFP-M1 before and after cerebellar iTBS in 10 healthy volunteers (pre-iTBS: mean = 0.133, sd = 0.312; post-iTBS: mean = -0.116, sd = 0.172; d = 0.988; [20]). Considering this effect size, adopting a two-tailed paired t test, with type I error alpha = 0.05, the minimum sample for reaching a power of 0.9 was n = 13, and n = 15 to ensure a power of 0.95. Following a crossover design experiment, all participants underwent three experimental sessions on three different days, at least 1 week apart. In a particular session, participants either received either iTBS, c-DC or sham stimulation over the right cerebellar lobe. The order of the three sessions was counterbalanced across participants. To assess the effects of the three protocols, we applied TMS over the left M1, during EEG and EMG recordings, before (T0), right after (T1) and 15 min after cerebellar neuromodulation (T2) (see details below). Each TMS-EMG-EEG session consisted of two blocks of stimulation (100 pulses each) delivered in two current directions: PA and AP. The order of the two TMS-EMG-EEG blocks (PA and AP) was counterbalanced across the subjects. During all recordings, participants were seated on a comfortable armchair and were asked to fixate a white cross (6 × 6 cm) presented on a computer monitor to minimize eye movements. To avoid auditory responses caused by TMS, participants were given earplugs that continuously played a masking noise composed of white noise mixed with specific time-varying frequencies of the TMS

click [32, 33]. The masking noise volume was adjusted to ensure that participants could not decipher the TMS click, or as much as tolerated (always below 90 dB) [34, 35].

Cerebellar neuromodulation

Cerebellar neuromodulation was performed by using iTBS [9] and c-DC [4]. The iTBS protocol was carried out with a 70 mm figure-of-eight coil connected to a Magstim Rapid^2 stimulator (Magstim Company Limited, Whitland, UK), which produces a biphasic waveform with a pulse width of ~ 0.1 ms. iTBS consists of bursts containing 3 pulses at 50 Hz repeated at 200 ms intervals for 2 s. A 2-s train was repeated every 10 s, for a total of 190 s and 600 pulses [36]. To apply iTBS over the cerebellum, the coil was placed 1 cm inferior and 3 cm right to the inion, thus targeting the right cerebellar hemisphere, with the handle pointing superiorly [9, 20, 29, 37]. These coordinates were adopted in several studies in which MRI reconstruction and neuronavigation showed that cerebellar TMS in this site predominantly targets the posterior and superior lobules of the lateral cerebellum [9, 20, 28, 29, 37, 38]. The intensity of iTBS was set to 80% of the active motor threshold (AMT), determined with single-pulse TMS over the left FDI M1 hotspot while the participant contracted the right FDI muscle at 20% of the maximum force. AMT was defined as the intensity at which MEPs with a peak-to-peak amplitude of 200 μV were elicited in 5 out of 10 consecutive trials [39].

The c-DC protocol was delivered using a battery-driven programmable direct current stimulator (neuroConn GmbH, Ilmenau, Germany) connected to two sponge electrodes of 25 cm^2 embedded in a saline-soaked solution. The cathode was centered on the same right cerebellar spot that we used for the iTBS protocol, while the anode was positioned on the right buccinator muscle. The intensity of stimulation was set at 2 mA for a duration of 20 min. Current was increased in a ramp-like manner for both the cathodal and sham conditions; however, during the sham condition, DC was ramped up and down for 30 s at the beginning and end of stimulation [4].

Single-pulse transcranial magnetic stimulation (TMS)

Single-pulse TMS was carried out with a Magstim 200^2 device connected to a 70 mm figure-of-eight coil (Magstim Company Limited, Whitland, UK), which produces monophasic pulses with a pulse width of ~80 μs. For M1 stimulation, the coil was positioned tangentially to the scalp at an angle of 45° from the midline over the hand motor area of the left M1, defined as the point where stimulation evoked the largest MEPs of the right FDI muscle. The coil orientation upon this spot was different depending on the condition: in the PA current condition, the coil handle pointed posteriorly with respect to the focus of stimulation; in the AP current condition, the coil handle was rotated 180° from the PA direction. Stimulus intensity was set to 120% of resting motor threshold (RMT), which was determined by stimulating M1 with single TMS pulses until an MEP of 50 μV peak to peak amplitude was visible in the FDI in 5 out of 10 consecutive trials. 100 TMS single-pulses were delivered at a random inter-stimulus interval (ISI) of 5 ± 20% s.

Electromyography (EMG) recordings and analysis

Surface EMG was acquired from the right first dorsal interosseous (FDI) muscle via Ag/AgCl electrodes in a belly-tendon montage using a Digitimer D360 Amplifier (Digitimer Ltd, Welwyn Garden City, UK); raw signals were sampled at 5000 Hz and band-pass filtered at 5–2000 Hz. EMG signal was monitored online with Signal software (Cambridge Electronic Devices, Cambridge UK). MEP amplitude was computed by taking the peak-to-peak amplitude after each TMS pulse and then averaged for each TMS-EMG-EEG block. MEP amplitudes were log-transformed to have a normal distribution of the data before applying parametrical statistic tests.

Electroencephalography (EEG) recordings and analysis

EEG was acquired using a TMS-compatible, EEG DC amplifier (Advanced Neuro Technology, Enschede, Netherlands). The amplifier was optically connected to a PC for online EEG monitoring, and to a 32-channel EEG cap ('Wave-Guard', ANT). EEG was continuously recorded from 30 TMS-compatible passive Ag/AgCl pellet electrodes mounted on the cap. The electrodes were placed according to the international 10–20 system including: Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, POz, O1, Oz, O2. The impedance from each electrode was kept below 5 kΩ. All recordings were referenced to the AFz electrode, while the ground electrode was placed at POz. The EEG signal was bandpass filtered at 0.1–1000 Hz and the sampling frequency was 2048 Hz.

Off-line EEG pre-processing was performed with MATLAB (version 2017b, MathWorks Inc., Natick, USA) compatible EEGLAB 13.3.2 [40] and Fieldtrip toolbox [41] that run in MATLAB environment (version 2017b, MathWorks Inc., Natick, USA). Data were segmented into epochs starting 1 s before the TMS pulse and ending 1 s after it. The TMS artifact was removed from 1 ms before to 10 ms after the pulse, and missing data were interpolated with a cubic function. Afterwards, data were downsampled to 1000 Hz and band-pass filtered between 1 and 80 Hz (fourth order Butterworth zero phase filters). A 50 Hz notch filter was

applied to reduce noise from electrical sources. Then, all the epochs were visually inspected and those with excessively noisy EEG were excluded from the analysis. Independent component analysis (INFOMAX-ICA) was applied to the EEG signal to identify and remove residual TMS-locked (voltage decay, EMG activity from scalp muscles) and non-TMS-locked (e.g., continuous muscle activity, eyeblinks) artifacts, based on criteria such as amplitude, scalp distribution and frequency content [42]. Finally, the signal was re-referenced to the average signal of all the electrodes.

Subsequent analyses of the EEG signal were conducted in the time-domain and time–frequency domain. Time-domain analysis of the cortical activation induced by single-pulse TMS of M1 was conducted by computing the LMFP as the square root of the squared TEPs averaged across three electrodes surrounding the site of stimulation, i.e., C3, FC1 and CP1 as done in previous works [18, 19, 43, 44]. For LMFP, we considered a time window from 10 to 200 ms after TMS, which corresponded to the TMS-evoked response.

To evaluate changes in the oscillatory domain, we performed a time/frequency decomposition based on Morlet wavelet (parameters cycles = 3; 23 linear 2 Hz steps from 4 to 50 Hz), and then, we computed the TRSP and the ITC. TRSP was computed as:

$$TRSP(f,t) = \frac{1}{n}\sum_{k=1}^{n} F_k(f,t)^2$$

where, for n trials, the spectral estimate F was computed at trial k, at frequency f and time t. ITC was computed as:

$$ITC(f,t) = \sum_{k=1}^{n} \frac{F_k(f,t)}{F_k(f,t)}$$

ITC takes values between 0 and 1, where 0 represents the absence of EEG synchronization across trials and 1 represents perfect synchronization [45]. Analysis of TRSP and ITC values was conducted over the same electrodes used for LMFP analysis. TRSP and ITC values were averaged over a time window that corresponded to the timing of the oscillatory activity, i.e., 20–70 ms for beta (14–30 Hz) and gamma (31–45 Hz) activity, and 20–200 ms for alpha (8–13 Hz) [23, 29, 44].

Statistical analysis

All data were analyzed using SPSS version 22 (SPSS Inc., Chicago, USA). Prior to undergoing ANOVA procedures, we tested if all neurophysiological data were normally distributed by using the Shapiro–Wilks' test. The level of significance was set at $\alpha = 0.05$. Sphericity of the data was tested with Mauchly's test; when sphericity was violated (i.e., Mauchly's test < 0.05) the Huynh–Feldt ε correction was used. Post hoc pairwise comparisons were corrected with the Bonferroni method.

MEP amplitude, LMFP, TRSP and ITC were analyzed by means of three-way repeated-measures ANOVAs with "stimulation" (c-DC, iTBS and sham), "direction" (AP, PA) and "time" (T0, T1 and T2). To further explore the timing of the LMFP differences found in the ANOVA (see results section), we conducted an analysis with multiple paired t test comparing the LMFP waveform at the single time point level, between the baseline (T0) and the two following time points (T1 and T2) in each condition (iTBS, sham and c-DC). For this analysis, to reduce the occurrence of false I type error, time points were considered as significant when at least 10 successive t tests reached the significant threshold [23], given that we observed that 10 ms is the mean duration of the first TMS-evoked components [17, 19, 23]. TRSP and ITC analyses were conducted separately for each frequency band (alpha, beta and gamma). RMT was analyzed by means of a two-way repeated-measures ANOVA with factors "stimulation" and "direction." In order to investigate possible relationships between corticospinal and cortical excitability (i.e., MEPs, LMFP, TRSP and ITC), we used the Pearson's coefficient. Test–retest reliability of MEPs, LMFP, TRSP and ITC was assessed by means of intra-class correlation coefficient (ICC) using the first two sessions, separately for each current direction.

Results

Corticospinal excitability

Figure 1A depicts the mean RMT values for each condition. ANOVA conducted on the RMT values showed a significant main effect of direction [$F(1,16) = 75.721$; $p < 0.001$; $\varepsilon = 0.826$], revealing that RMT was significantly higher when pulses were delivered in AP direction. ANOVA conducted on MEP amplitude showed a significant stimulation × time interaction [$F(4,64) = 3.942$; $p = 0.006$; $\varepsilon = 0.198$]. Post hoc analysis revealed that MEPs after iTBS were higher both at T1 (post hoc $p < 0.001$) and at T2 (post hoc $p = 0.006$) compared to T0. Since there were no main effects or interactions with TMS direction (all ps > 0.05), Fig. 1B depicts the %mean MEP change for each condition and time, collapsed across AP and PA directions.

Cortical excitability

ANOVA conducted on LMFP activity showed a significant stimulation × time interaction [$F(4,64) = 5.151$; $p = 0.001$; $\varepsilon = 0.244$]. Post hoc analysis revealed that LMFP after iTBS was significantly higher at T2 compared to T0 (post hoc $p = 0.006$), whereas it was significantly lower after

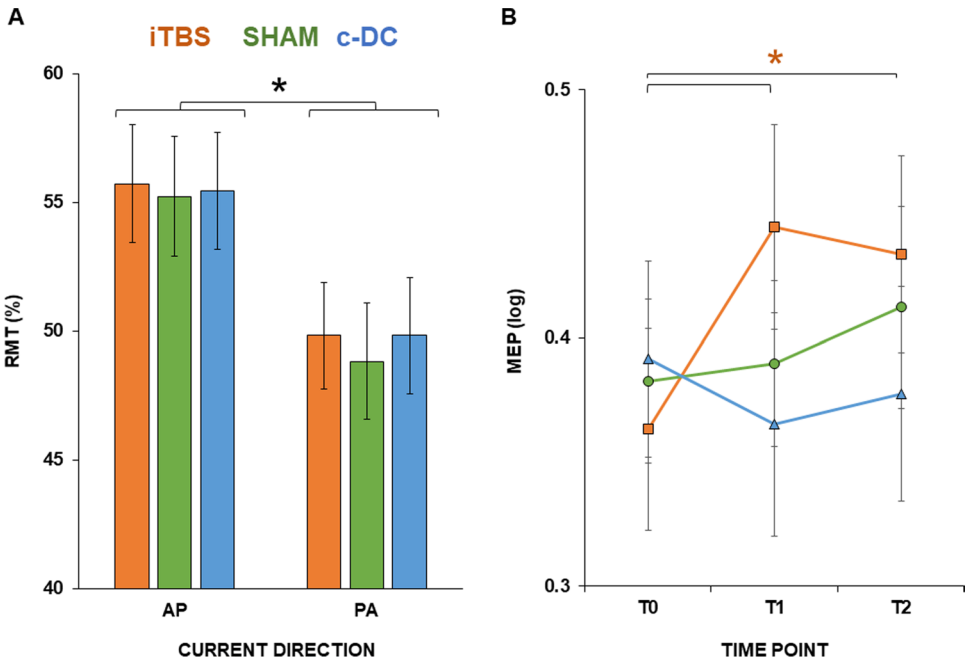

Fig. 1 Corticospinal spinal analysis. *Panel A:* mean RMT for iTBS (orange bars), sham (green bars) and c-DC condition (blue bars) in the two current directions (anterior–posterior AP, posterior–anterior PA). *Panel B:* mean % change of MEP amplitude right after the stimulation (T1) and 15 min after (T2), with respect to the baseline (T0), for the iTBS, sham and c-DC condition. *p < 0.05, error bars depict the standard error of the mean

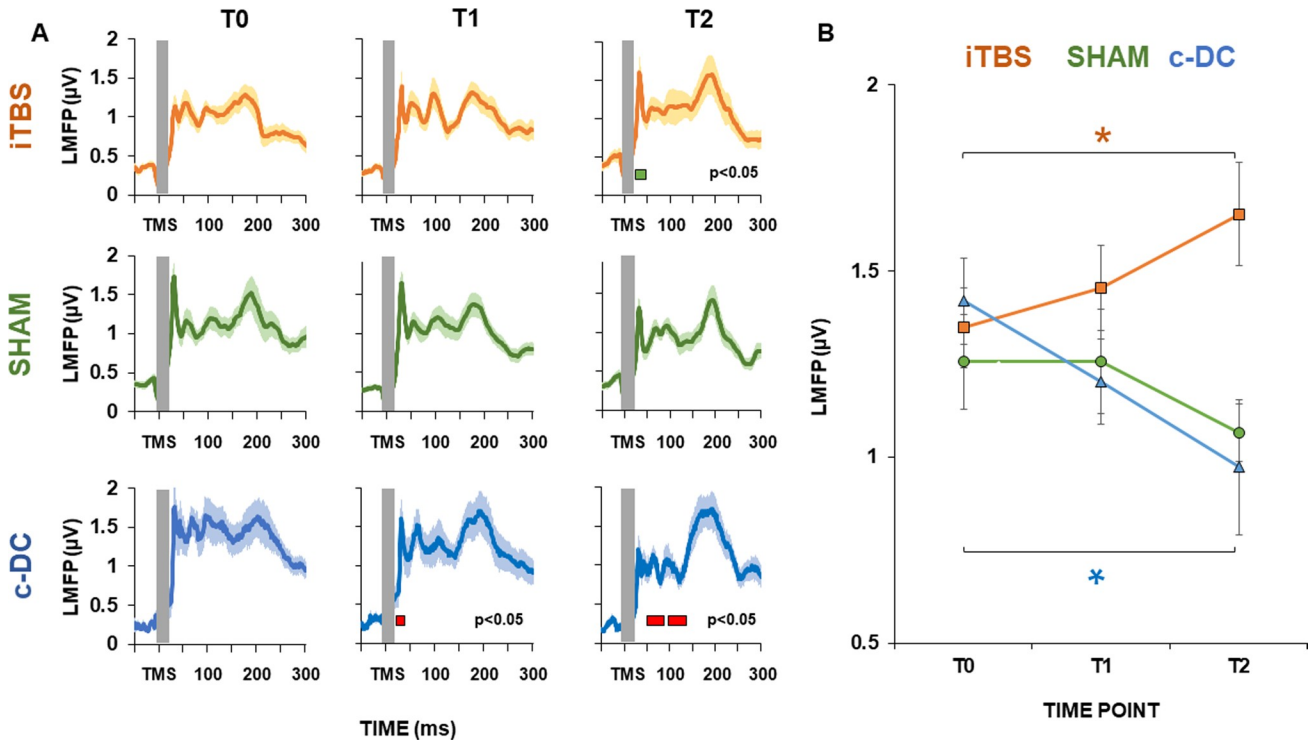

Fig. 2 Cortical excitability analysis. *Panel A:* local mean field power (LMFP) waveform for the iTBS (orange lines), sham (green lines) and c-DC condition (blue lines) in the three time points (T0, T1, T2). Green and red squares represent the time windows at which we found a significant LMFP increase (green squares) o decrease (red squares) between T0 and T1 or T2. *Panel B:* mean % change of total LMFP amplitude right after the stimulation (T1) and 15 min after (T2), with respect to the baseline (T0), for the iTBS, sham and c-DC condition. *p < 0.05, error bars depict the standard error of the mean

c-DC at T2 compared to T0 (post hoc $p = 0.048$; Fig. 2B). Figure 2A depicts the LMFP waveform for each condition and time, collapsed for AP and PA direction, since there was no effect of current direction. Single time-point analysis revealed that, after iTBS, the LMFP increased at T2 compared to T0 in a time window ranging from 10 to 45 ms after the TMS pulse (average p = 0.031). The same analysis showed that, in the c-DC condition, the LMFP decreased at T1 (10–27 ms, average p = 0.04) and T2 (50–80 ms, average p = 0.014, and 95–130 ms, average p = 0.033) compared to baseline (Fig. 2A). Figure 2B depicts the %mean LMFP change with respect to the baseline level (T0). Figure 3A depicts TRSP for each condition and time. ANOVA conducted on mean α-TRSP activity showed a significant stimulation × time interaction [$F(4,64) = 4.666$; $p = 0.002$; $\varepsilon = 0.226$]. Post hoc analysis revealed that α-TRSP was enhanced right after iTBS (post hoc $p = 0.05$) but this effect disappeared at T2 ($p > 0.05$). Figure 3B depicts %mean α-TRSP change with respect to the baseline level (T0). Following these results, we conducted an exploratory ANOVA to analyze the effects right after the cerebellar modulation (T1) without considering after effects (T2). In this case, the stimulation × time interaction was still significant [$F(2,32) = 6.793$; $p = 0.003$; $\varepsilon = 0.298$] and post hoc analysis showed a significant modulation of α-TRSP, which was enhanced after iTBS (post hoc $p = 0.018$) and reduced after c-DC (post hoc $p = 0.028$). No effects were observed for the other frequency bands (all ps > 0.05). Analysis of ITC did not reveal any significant effect (all ps > 0.05).

Correlations and reliability of corticospinal and cortical excitability measures

Correlation analysis between MEPs, LMFP, TRSP and ITC did not reveal any significant linear relationship at baseline, nor after cerebellar neuromodulation (all ps > 0.05). Figure 4 depicts test–retest plots for MEPs, LMFP; TRSP and ITC. Analysis of reliability revealed a high ICC for LMFP both in PA (0.870; $p < 0.001$) and AP direction (0.932; $p < 0.001$); for TRSP both in PA (0.913; $p < 0.001$) and AP direction (0.903; $p < 0.001$) and for ITC both in PA (0.969; $p < 0.001$) and AP direction (0.965; $p < 0.001$). ICC was weak for MEPs in PA direction (0.517; $p < 0.078$) and medium in AP direction (0.643; $p < 0.024$).

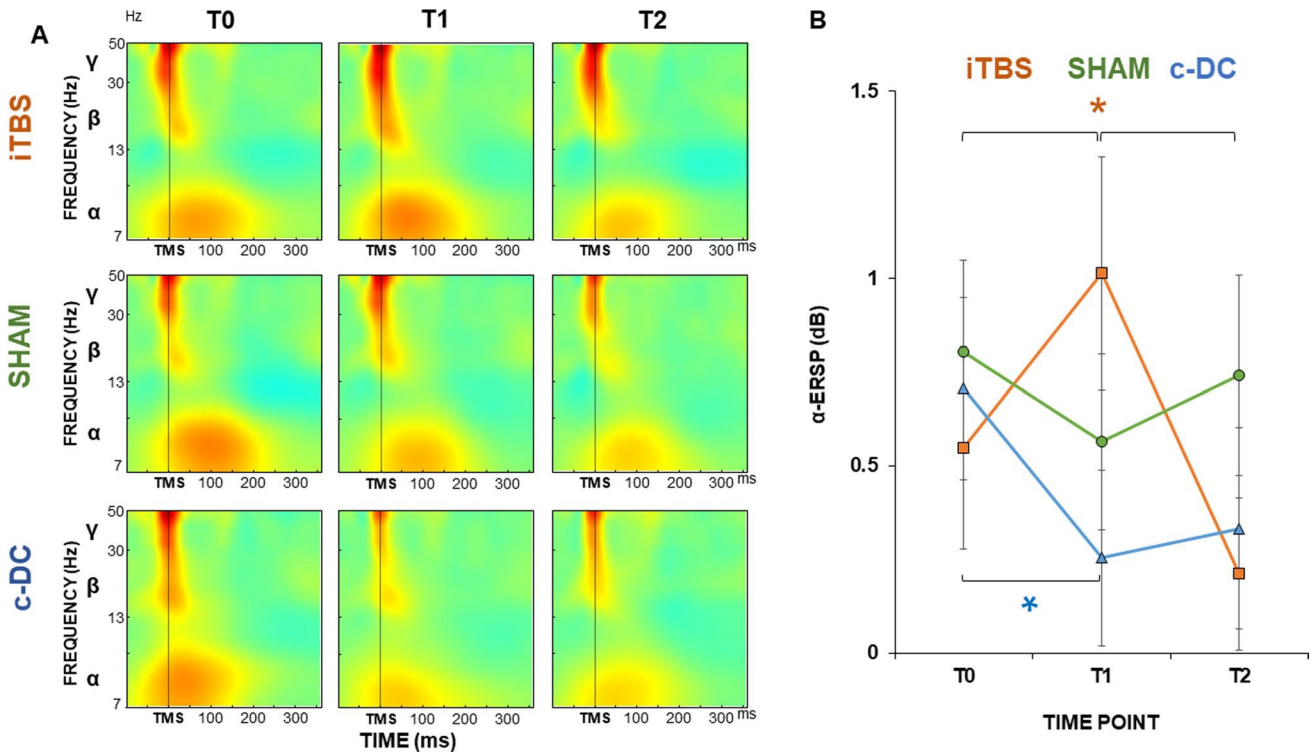

Fig. 3 Cortical oscillations analysis. *Panel A:* TMS-related spectral perturbation (TRSP) plots for the iTBS (upper plots), sham (central plots) and c-DC condition (below plots) in the three time points (T0, T1, T2). *Panel B:* mean % change of total α-TRSP amplitude right after the stimulation (T1) and 15 min after (T2), with respect to the baseline (T0), for the iTBS (orange lines), sham (green lines) and c-DC condition (blue lines). *p < 0.05, error bars depict the standard error of the mean

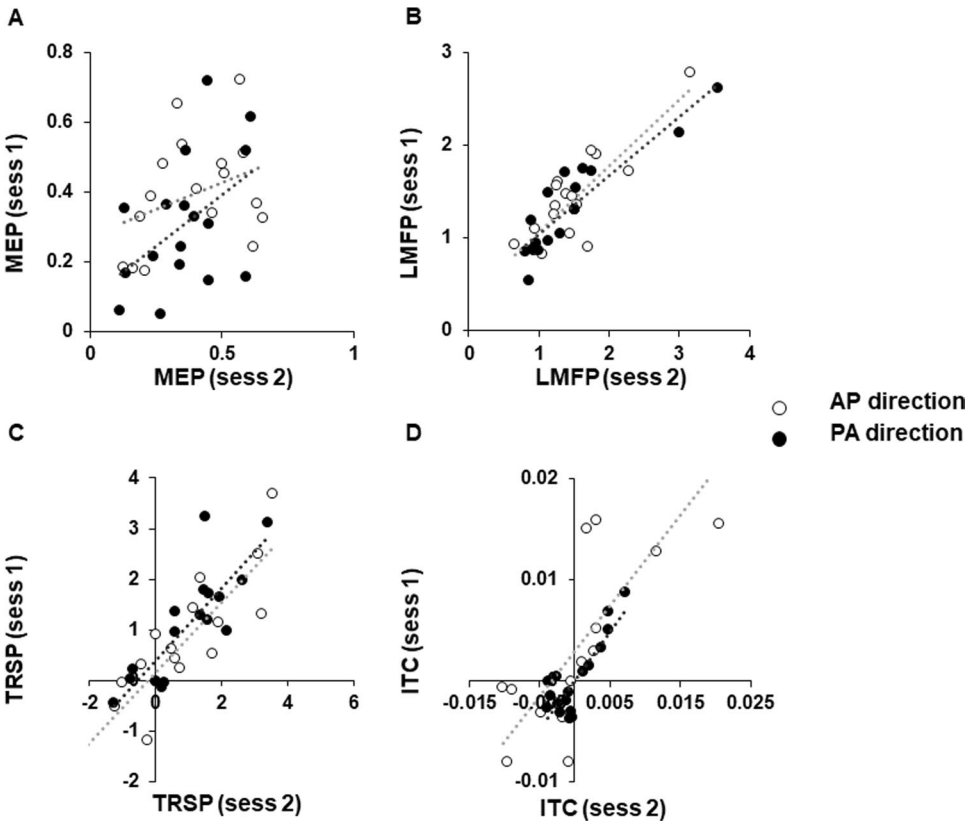

Fig. 4 Reliability of corticospinal and cortical measures. Test–retest analysis of MEP (*panel A*), LMFP (*panel B*), α-TRSP (*panel C*) and α-ITC (*panel D*) for the anterior–posterior (AP, white dots) and the posterior–anterior (PA, black dots) current direction

Discussion

Here, we present novel findings on how two different neuromodulatory protocols over the cerebellum (i.e., iTBS and c-DC) affect both cortical (LMFP, TRSP and ITC) and corticospinal (MEP) physiological measures. Additionally, we examined whether applying different current directions over M1 (AP and PA currents) reveal differences across these measures. As shown in previous work, we found that iTBS produced excitatory changes in corticospinal excitability [9], whereas no MEP changes were detected following c-DC [4]. Notably, this result was independent of the current direction applied over the brain with TMS, suggesting that cerebellar plasticity protocols produce similar effects over distinct interneuronal cortical networks. This result is likely due to cerebellar excitability changes producing a non-specific effect upon the distinct pathways reaching M1 [22], thereby stimulation modulates these circuits in a similar manner. Interestingly, a major novel finding of this study revealed that both cerebellar iTBS and c-DC modulate TMS-EEG measures of cortical physiology in a bidirectional manner. Namely, cerebellar iTBS immediately increased alpha oscillations and produced lasting facilitatory effects on cortical activation (e.g., LMFP), whereas c-DC immediately decreased alpha oscillations and reduced cortical activity following stimulation. These results were also found independent of the current direction applied to the brain. Overall, this work importantly shows that cerebellar neuromodulatory techniques can produce results on cortical and corticospinal physiological measures, indicating that the specific effects of these techniques may be clearer when proved with TMS-EEG outcome measures.

Corticospinal Excitability Responses to Cerebellar Neuromodulation

In our measures of corticospinal excitability, we found that cerebellar iTBS modulated the MEP response well after protocol termination, whereas no changes in MEP excitability were detected following c-DC. These results are in line with a pair of findings from seminal work demonstrating that: (1) cerebellar iTBS facilitates corticospinal excitability via the DTC pathway for up to 30 min following stimulation [9]; and (2) c-DC modulates cerebellar excitability without producing changes in corticospinal excitability [4]. While the contrasting effects on corticospinal excitability may appear conflicting, it is likely that iTBS and DC produce varying effects on the cerebellar cortex since the former protocol depolarizes the stimulated neurons, whereas the latter is thought to change cell polarity [46]. Thus, the repetitive depolarization of cerebellar Purkinje cells via iTBS may engage the DTC pathway, leading to changes in corticospinal

excitability [9, 20]. On the other hand, it is possible that c-DC may produce tonic changes at the Purkinje cell level without activating DTC pathways [47]. The limiting factor of this hypothesis using the MEP as an outcome measure, as it reflects both the excitability of cortical and spinal inputs of the corticospinal tract. Thus, potential effects of cerebellar modulation on the cortex may not be detected when probing this specific pathway. Rather, we argue that the integration of TMS-EEG outcome measures may more precisely reveal the response of cortical activity to cerebellar neuromodulation.

We additionally considered how each cerebellar neuromodulatory technique may have affected different inputs to the corticospinal tract by applying different currents to the brain. Similar to previous findings, we show that the RMT was significantly lower when elicited with PA currents compared to AP currents [23, 48]. Since the RMT mainly reflects membrane excitability of corticospinal neurons [49] [49], the effect of current direction is likely related to the different axonal orientations of these neurons, which influences their recruitment [23, 48, 50, 51]. Notably, we did not observe any effect of cerebellar neuromodulation in RMT [9]; however, the effects of cerebellar iTBS (or lack thereof c-DC) on the MEP were independent of the current applied to the brain. This result is interesting since it is well known that the cerebellum interacts with M1 through a variety of anatomically and functionally distinct pathways [22, 52]. In other words, cerebellar modulation does not selectively target a specific pathway, but rather it likely affects corticospinal excitability through the entire complex network of cerebellar inputs to M1.

Cortical Excitability Responses to Cerebellar Neuromodulation

Cortical excitability was assessed in terms of evoked potentials (TEPs) and oscillations (TRSP and ITC). TEPs analysis was conducted locally to the stimulated area using LMFP, as previously done in TMS-EEG studies investigating cortical excitability after cerebellar [20] and cortical neuromodulation [19]. We found that LMFP evoked by stimulating M1 was bidirectionally modulated by the two cerebellar protocols but not from the current direction. In particular, we observed an enhancement of LMFP after iTBS and a reduction after c-DC, interestingly both the effects were still significant after 15 min. Notably, this effect was prominent for the early components (< 80 ms), as revealed by the single time-point analysis. From a physiological point of view, LMFP reflects the synchronous activation of a neural population [19, 53, 54]. When TMS is applied, the number of neurons recruited by a single pulse is dependent on the excitability threshold of the neuronal population [56]. Thus, the changes in the cortical population response after M1 TMS likely depend on the bidirectional excitability shift led by the two cerebellar neuromodulation protocols. Changes in cerebellar excitability may subsequently influence activity of various cortical areas via the DTC pathway. Indeed, iTBS may have induced plastic changes in parallel-fiber Purkinje cell synapses or in cerebellar inhibitory interneurons at the molecular layer [9]. For instance, if stimulation were to increase the activity of basket and stellate cells that regulate Purkinje cell output, the overall net effect would "release" the inhibitory tone that Purkinje cells exert on the deep cerebellar nuclei. This in turn should increase the firing of deep cerebellar nuclei, which sends projections to the ventro-lateral nucleus of the thalamus [2]. Here, the thalamic relay project to several cortical areas, such as motor and parietal areas [57, 58]. Thus, our hypothesis is that iTBS could have influenced M1 and surrounding areas through the CTC pathway, as several studies suggested before [10, 20, 28, 59–61]. On the other hand, c-DC may have produced the opposite effect on Purkinje cell activity or inhibitory cerebellar interneurons, which in turn would result in decreased cortical excitability.

It is important to stress why this effect was only observed with TEPs but not in MEPs. First, it is very likely that MEPs and TEPs reflect two different physiological processes; indeed, the present study confirms the lack of linear relationship between the two measures observed in previous reports [19, 44, 62–65]. Indeed, MEP amplitudes partially depend on spinal motoneuron excitability [66] and their inter-trial responses show large variability [67]. Therefore, it is possible that high variability in MEPs amplitudes was not sensible enough to changes in cortical excitability being influenced by the whole corticospinal tract. On the other hand, TEPs do not rely on responses on the corticospinal tract response (i.e., no influence of spinal excitability), but rather measures EEG voltage difference induced by TMS from a cluster of electrodes over the brain. Signals in these electrodes likely reflect output from a cortical area wider than M1, which might have been influenced as well by cerebellar conditioning, due to the widespread distribution of CTC pathways [2, 20, 52, 57]. Thus, the TEPs represent a compound signal of cortico-cortical activity that may be more receptive to changes in cerebellar–thalamic inputs to the cortex than MEPs, either due to the different cellular elements involved in the generation of the two outputs, or because LMFP reflects activation of a larger cortical area.

As in the case of corticospinal excitability, changing the TMS current direction did not show any change in the LMFP amplitude. This result is in line with a previous study of our group in which we tested the effect of current direction, waveform and pulse width in the stimulation of M1 [23]. In this study, we found that cortical excitability was not different with AP and PA pulses. However, this result should be unexpected, given that LMFP is sensible to stimulus intensity [68]. Thus, given that absolute stimulus intensities were

higher for AP pulses, we should have expected a greater amplitude in the LMFP. When TMS is applied over M1, it activates neurons both in the pre-central and post-central gyrus [69]. Here, we compute the LMFP from a cluster of electrodes surrounding the stimulation site that reflects the strength of this neuronal recruitment, which should be larger at higher TMS intensities. A possible explanation of the lack of this effect could lie in the fact that the current direction itself, with respect to axonal orientation, influences the neuronal recruitment threshold and thus their response. Along these lines, it has been demonstrated that some neurons in the rolandic area have different excitability thresholds [70]. An additional explanation might lie in the low spatial specificity of LMFP, which is likely generated not only in M1, but also in surrounding areas, where pyramidal neurons might have less directional sensitivity to stimulation.

Cortical oscillations were assessed with two different indexes, TRSP and ITC. These two indexes reflect two different aspects of oscillatory activity. While TRSP is a measure of TMS-evoked changes in terms of spectral power of a specific frequency, ITC reflects frequency coherence among the trials, i.e., the TMS pulses. We found that TRSP in alpha frequency was specifically enhanced by iTBS and reduced by c-DC. Although the exact TMS mechanism of action on cortical oscillations is still debated, several studies support the idea that, when applied in single-pulses, TMS evokes a predominant response in a specific frequency, depending on the stimulated area [21]. This frequency should represent the "natural frequency" of the stimulated area, that is the predominant frequency at which activity of the area oscillates [71]. In our study, single-pulse TMS of M1 evoked a sustained response in the alpha/beta range, as we observed in several previous studies [20, 29, 54, 65, 72].

Interestingly, we found that the power of alpha activity was also modulated by iTBS and c-DC in a bidirectional manner. The results of cerebellar iTBS enhancing TMS-evoked alpha activity confirm the results of previous work [20]; on the other hand, we are the first to show that c-DC reduces alpha activity. Notably, both these effects occurred only immediately after cerebellar neuromodulation and not after 15 min. This indicates that the naturally occurring frequencies of the stimulated region are immediately perturbed by changes in cerebellar–thalamic inputs. Indeed, cortical oscillations originate from the thalamo-cortical circuits, through which the cortex communicates with several subcortical structures [73]. These thalamo-mediated interactions between several cortical and subcortical regions are known to produce oscillatory rhythms [74]. In this process, the interplay of excitatory and inhibitory neurons is of fundamental importance, in particular of GABAergic thalamic neurons, being the main gateway of cortical projections to the thalamus. GABAergic neurons are also implicated in the origination of TMS-evoked response, in particular between 30 and 150 ms after TMS, as shown by TMS-EEG investigations using GABA-agonists [75, 76] or by measuring GABA-ergic dependent MEP measures [77, 78]. In this view, it could be hypothesized that modulation of cerebellar output could have affected the excitability of GABA-ergic interneurons at thalamic and/or cortical level, and in turn the natural frequency of oscillation of M1 and surrounding cortex [20, 79]. Finally, it is also important to note that cerebellar neuromodulation affected only the spectral power and not the phase synchronization, as previously observed [20].

Another important result of the present study lies in the demonstration of the high reproducibility of our TMS-EEG measures, as assessed with ICC [80]. Our analysis showed high repeatability both for LMFP, TRSP and ITC regardless of the current direction but not for MEPs, whose repeatability was weak for PA direction and medium for AP. Previous studies suggested a high reproducibility of TMS-EEG measures in terms of TEPs [81], or dynamic measures such as interhemispheric signal propagation (ISP) and balance (IHB) [65, 72]. Here, we showed a high reproducibility of TMS-evoked EEG activity also when considering more complex measures such as LMFP, TRSP and ITC. On the other hand, we could hypothesize that the relatively low reproducibility of MEPs could be due to the constant fluctuations in the excitability of corticospinal neurons, which is reflected in the variability of the MEP amplitude [82, 83].

Limitations

Our study presents some limitations. First of all, it is important to underline that our conclusions were relative only to the cerebellar influence on M1 and contiguous cortical areas. Due to the relatively low number of recording electrodes, we did not have a sufficient spatial resolution to draw conclusions about the response of specific cortical areas. In addition, we did not test any direct measure of cerebellar-brain connectivity, such as the CBI, since our primary aim was to compare TMS-EMG responses with those recorded with TMS-EEG. In principle, this would require us to assess cerebellar-M1 connectivity with TMS-EEG, whose feasibility remains unclear. Indeed, a recent study attempting to record connectivity via TMS-EEG with the double cone-coil (widely accepted as the appropriate coil to yield consistent CBI) observed large coil-driven artifacts and sensory contamination [84]. Thus, a substantial amount of work still needs to be carefully done before cerebellar-M1 connectivity can be measured with TMS-EEG.

Some limitations are intrinsic to the TMS-EEG methodology used. As we did not use any forms of spatial filters, the topographical specificity of the LMFP may be limited by a degree of volume conduction. Additionally, the TMS pulse activates somatosensory afferent fibers in the scalp, therefore potentially giving rise to somatosensory evoked responses,

which may contaminate the TEP. However, these responses have recently been suggested to be very small and located at the vertex [33], thus outside our M1 region of interest, or absent [85]. It is also to note that the same TMS intensity was used in all stimulation conditions; this implies a similar degree of somatosensory stimulation of the scalp, which, therefore, is unlikely to have driven the differential effects following our stimulation protocols. Another possible bias might have been represented by contamination of the TEP by afferent somatosensory activity from hand muscles following the MEP. This has been shown to potentially affect the spectral properties of the TMS-EEG signal, but in a time window not compatible with our results (around 300 ms) [43]. Another study suggested the P60 component of TEPs may be modulated by MEP amplitude [86]; however, this result was not confirmed elsewhere [87]. At least two pieces of evidence in the present paper suggest that our results were not significantly biased by contamination caused by somatosensory feedback. The first is the observation that some changes in LMFP due to cerebellar conditioning occurred in time windows too early to be accounted for by reafferent activity. Secondly, we did not find any correlation between MEP and LMFP, which would have likely occurred in case a substantial amount of the latter was the result of cortical stimulation by the afferent somatosensory volley caused by muscle twitch. Therefore, while we acknowledge that the latter may represent a confounding factor, we do not think that it significantly influenced our results.

A final limitation pertains to the sham stimulation, which was designed to control for the c-DC, but not for the iTBS, since the latter entails a degree of cortical input due to stimulation of the skin and muscles in the posterior neck region which is not induced by our sham protocol. Thus, we cannot fully exclude that the mentioned somatosensory stimulation might be partly responsible for our results. However, we consider this possibility unlikely, since previous evidence has suggested that the somatosensory input induced by theta burst stimulation does not induce changes in cortical excitability [9, 88, 89].

Conclusions

Overall, the present data show that cortical excitability and oscillatory activity are influenced by two diverse forms of cerebellar neuromodulatory protocols. While the effects of cerebellar iTBS have been well documented for the neurophysiological outcomes of cortical (TMS-EEG) and corticospinal activity (TMS-EMG), previous work has suggested that the effects of c-DC are limited to the cerebellar cortex. While our results confirm that c-DC does not elicit changes in traditional measures of corticospinal excitability (MEP), we observed that cortical activity is indeed influenced by this neuromodulatory protocol. This important result highlights the advantage of using TMS-EEG strategies over traditional measures to reveal the influence of cerebellar activity on the cerebral cortex. Moreover, our results reveal that cerebellar neuromodulation produces only immediate changes in cortical oscillations, whereas changes in cortical excitability were more prominent with the passage of time. Future works need to investigate whether changes in oscillatory activity are involved in plasticity-related mechanisms or their changes are a mere epiphenomenon.

Acknowledgements We thank all the participants to the experiment.

Authors' contribution Elias P Casula, Lorenzo Rocchi, Danny A Spampinato, John C Rothwell, Michael Orth and Patrizia S Bisiacchi were involved in *conceptualization*; Elias P Casula and Lorenzo Rocchi helped in *methodology*; Elias P Casula and Valentina Pezzopane contributed to *formal analysis and investigation*; Elias P Casula, Lorenzo Rocchi and Danny A Spampinato were involved in *writing*; John C Rothwell helped in *resources*; Elias P Casula, John C Rothwell, Michael Orth and Patrizia S Bisiacchi contributed to *supervision*.

Funding EPC is supported by the European Commission with Marie Sklodowska-Curie Individual Fellowship (H2020-MSCA-IF Grant No. 798957).

Declarations

Conflict of interest The authors have not relevant financial or non-financial interests to disclose.

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