Theta-tACS modulates cerebellar-related motor functions and cerebellar-cortical connectivity

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HIGHLIGHTS

- Cerebellar theta (θ)-tACS reduced movement regularity in a tapping task and prolonged the movement duration of a pointing task.
- Cerebellar θ-tACS enhanced the effectiveness of CBI and the effect of θ-tACS on movement rhythm was correlated with changes in CBI.
- tACS effects potentially involve the modulation of cerebellar neurons resonating with θ rhythm.

ARTICLE INFO

ABSTRACT

Objective: To evaluate the effects of cerebellar transcranial alternating current stimulation (tACS) delivered at cerebellar-resonant frequencies, i.e., theta (θ) and gamma (γ), on upper limb motor performance and cerebellum-primary motor cortex (M1) connectivity, as assessed by cerebellar-brain inhibition (CBI), in healthy subjects.
Methods: Participants underwent cerebellar-tACS while performing three cerebellar-dependent motor tasks: (i) rhythmic finger-tapping, (ii) arm reaching-to-grasp ('grasping') and (iii) arm reaching-to-point ('pointing') an object. Also, we evaluated possible changes in CBI during cerebellar-tACS.
Results: θ-tACS decreased movement regularity during the tapping task and increased the duration of the pointing task compared to sham- and γ-tACS. Additionally, θ-tACS increased the CBI effectiveness (greater inhibition). The effect of θ-tACS on movement rhythm correlated with CBI changes and less tapping regularity corresponded to greater CBI.
Conclusions: Cerebellar-tACS delivered at the θ frequency modulates cerebellar-related motor behavior and this effect is, at least in part, mediated by changes in the cerebellar inhibitory output onto M1. The effects of θ-tACS may be due to the modulation of cerebellar neurons that resonate to the θ rhythm.
Significance: These findings contribute to a better understanding of the physiological mechanisms of motor control and provide new evidence on cerebellar non-invasive brain stimulation.

1. Introduction

Transcranial alternating current stimulation (tACS) is a non-invasive neuromodulation technique that entrains the firing activity of specific neurons and transiently enhances brain oscillations at the frequency of stimulation (Ali et al., 2013; Johnson et al., 2020; Krause et al., 2019). Importantly, tACS provides an opportunity to experimentally investigate the functional role of specific cortical areas and rhythms. For instance, when tACS is delivered over the primary motor cortex (M1) at the beta (β) and gamma (γ) frequencies, it has been shown to modulate the force, velocity and amplitude of hand movements (Guerra et al., 2022, 2018; Joundi et al., 2012; Pogosyan et al., 2009). Similarly, when tACS is applied to the somatosensory cortex at the mu (μ) rhythm, it enhances the somatosensory processing (Fabbrini et al., 2022;

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Gundlach et al., 2016; Saito et al., 2021). Additionally, low-γ tACS over the prefrontal cortex and precuneus improved various cognitive functions (Benussi et al., 2022; Santarnecchi et al., 2016). tACS studies, however, have primarily focused on evaluating the effects of stimulation on the cerebral cortex. There has been limited investigation into whether tACS delivered at the cerebellar-resonant frequencies can modulate cerebellar-related motor behavior. In animal models, intrinsic oscillations within the theta (θ, 4–7 Hz) and γ (30–80 Hz) frequency bands have been observed in the cerebellum. These oscillations are believed to have significant implications for sensorimotor processing and motor control (D'Angelo et al., 2001; De Zeeuw et al., 2008; Manto et al., 2022; Middleton et al., 2008). Also, a recent study on rodents demonstrated that cerebellar tACS modulated the spiking activity of Purkinje cells and entrained neural oscillations across various frequency bands, including θ and γ (Asan et al., 2020). In humans, it has been demonstrated that tACS delivered at 5 Hz (θ-tACS) or 50 Hz (γ-tACS) over the cerebellum has the effect of modulating cerebellar-brain inhibition (CBI) (Naro et al., 2017, 2016; Spampinato et al., 2021), which is a transcranial magnetic stimulation (TMS) measure of cerebellum-M1 connectivity (Ugawa et al., 1995). The present finding, however, was not always confirmed by other authors (Herzog et al., 2022; Spampinato et al., 2021). Moreover, cerebellar γ-tACS has been shown to improve hand-motor task performances in healthy individuals (Miyaguchi et al., 2022; Naro et al., 2017, 2016). In summary, the evidence on the effects of cerebellar θ- and γ-tACS in humans is currently limited and, to some extent, conflicting.

In this double-blind, randomized, sham-controlled study, we objectively assessed upper limb motor performance through kinematic analysis during cerebellar θ- and γ-tACS in healthy subjects. Sham-tACS was a control condition for potential placebo effects or non-specific stimulation-related factors. All subjects underwent the assessment of three distinct cerebellar-dependent motor tasks, encompassing rhythmic finger tapping (Del Olmo et al., 2007) as well as two arm-reaching movements with differing levels of complexity: reaching-to-grasp ('grasping') and reaching-to-point ('pointing') an object (Li Voti et al., 2014). Moreover, we assessed CBI during the three different stimulation conditions to investigate whether any observed behavioral effects of tACS were associated with neurophysiological changes in the cerebellum-M1 connectivity. Investigating these issues can provide valuable insights into cerebellar physiology.

2. Materials and methods

2.1. Participants

We enrolled 15 healthy subjects (five females, age mean ± SD: 27.1 ± 3.5 years) from the Department of Human Neurosciences, Sapienza University of Rome. All participants (except three) were right-handed, as determined by the Edinburgh Handedness Inventory (Oldfield, 1971). No participant included in the study had neuropsychiatric disorders or were taking medications known to alter cortical excitability (Ziemann et al., 2015). The experimental procedures conformed to the Declaration of Helsinki, adhered to international safety guidelines (Antal et al., 2017; Rossi et al., 2021), and were approved by the local institutional review board. Written informed consent was obtained from all participants before they participated in the study.

2.2. tACS

tACS was delivered through a BrainSTIM (E.M.S.) connected to two 5 × 5 cm electrodes enclosed in saline solution-soaked sponges. Like previous cerebellar tACS studies, one electrode was centered over the cerebellar hemisphere ipsilateral to the dominant hand (≈1 cm below and 3 cm lateral to the inion) and the other over the ipsilateral buccinator muscle (Naro et al., 2017, 2016; Spampinato et al., 2021). We used a peak-to-peak amplitude of 1.5 mA, 3-s ramp-up and down periods, and stimulation frequency of 5 Hz and 50 Hz for θ- and γ-tACS, respectively (Spampinato et al., 2021). Sham-tACS consisted of ramp-up and down periods and 1-s stimulation only at 5 Hz. At the end of the experiment, we asked to rate from 0 to 10 the strength and uncomfortableness of stimulation-induced visual and skin sensations. A questionnaire form was used, which was administered by the researcher. No participant reported any sensation during stimulation (0/10), except one subject who referred a mild visual flickering during γ-tACS (strength 3/10, uncomfortableness 2/10). Consequently, the participants were unable to differentiate between the various stimulation conditions.

2.3. TMS

TMS was performed using a Magstim Bistim[2] connected to a figure-of-eight coil (Magstim Company) with the handle pointing posterolaterally. The first dorsal interosseous (FDI) muscle hotspot, resting motor threshold (RMT) and the intensity eliciting motor evoked potentials (MEP) of ∼1 mV amplitude (MT_{1mv}) were identified following international guidelines (Rossini et al., 2015). For the CBI assessment during tACS, we adopted the same methodology and stimulation parameters used in (Spampinato et al., 2021). A Magstim double-cone coil was placed over the cerebellar hemisphere ipsilateral to the dominant hand with an upward current induced to the brain (Ugawa et al., 1995). The first conditioning stimulus (CS) was delivered over the cerebellum 5 ms before the test stimulus (TS) was delivered over the contralateral M1. TS intensity was set at MT_{1mv} and CS intensity at 60% of maximum stimulator output, as this value does not active the brainstem and is easily tolerated by participants (Galea et al., 2009; Spampinato et al., 2021, 2020). We recorded 15 MEP elicited by the TS alone and 15 conditioned MEP (CS + TS) for each CBI assessment. The interstimulus interval was 4.5–5.5 s. The electromyographic activity recorded from the dominant FDI was amplified (Digitimer D360, Digitimer), digitized (CED 1401, Cambridge Electronic Design), and stored on a computer for offline analyses. Peak-to-peak MEP amplitude was measured and averaged for each condition. CBI was expressed as the ratio between the peak-to-peak amplitude of conditioned and unconditioned (TS alone) MEP.

2.4. Kinematic recordings

We used an optoelectronic system consisting of infrared cameras, which followed the displacement of 5 reflective markers taped to the participant's dominant hand (SMART motion system, BTS Engineering) (Bologna et al., 2018; Guerra et al., 2022). Subjects were seated on an armchair. The rhythmic tapping task consisted in repetitively opening and closing the index finger and thumb for 30 s at 2 Hz. In the first 15 s, the participants were instructed to synchronize their tapping rhythm with a metronome beating at 2 Hz (synchronization phase), while in the last 15 s the metronome was switched off and they had to continue tapping at the same pace (continuation phase) (Del Olmo et al., 2007). We chose a task duration of 30 s because preliminary observations showed fatigue with longer durations. We measured the finger-tapping frequency and the movement amplitude (degrees) and velocity (degrees/sec) using linear regression techniques (Bologna et al., 2020, 2018; Guerra et al., 2023). Movement rhythm was measured by the coefficient of variation (CV) of the inter-tap intervals (with higher values representing a lower movement

regularity) (Bologna et al., 2020, 2018; Guerra et al., 2023). These parameters were calculated for both the synchronization and continuation phase of the task.

In the grasping task, the subjects were instructed to reach precisely and grasp as fast as possible a cylindrical body using their thumb and index finger. The cylindrical body (∼4 cm in diameter) was placed on the table at sternal height and two-thirds arm's length from the body midline (Li Voti et al., 2014). The pointing task required a more complex and accurate movement. A thin plastic cylinder with a diameter of 1 mm was attached to the index finger. The subjects were instructed to reach and accurately insert the plastic cylinder into a small target (a 2 mm in diameter bull's-eye) positioned over the cylindrical body, aiming for precision and speed (Li Voti et al., 2014). The endpoint marker was placed on the second carpometacarpal joint. Both grasping and pointing tasks consisted of 10 consecutive movements. We measured various parameters reflecting movement performance (i.e., velocity, acceleration and deceleration peaks, duration of the entire movement and the phases of acceleration, deceleration and approach-to-the-target) (Bologna et al., 2015; Li Voti et al., 2014). To estimate the movement quality, we calculated the curvature index (CI - ratio of the curved path length to the straight-line distance between the initial and a determined position) and movement units (MU – number of peaks in the endpoint velocity curve), which reflect the trajectory straightness and smoothness, respectively (Bologna et al., 2015; Deuschl et al., 2000; Li Voti et al., 2014). CI was measured for the entire movement and the acceleration, deceleration and approach-to-the-target movement phases.

2.5. Experimental design

Participants underwent a single experimental session, encompassing kinematic and TMS evaluations. First, we recorded the three behavioral tasks during: i) θ-tACS; ii) γ-tACS; iii) sham-tACS. The assessment of rhythmic tapping consisted of three trials (30-s duration each) per stimulation condition (nine trials in total), while the assessment of the grasping and pointing consisted of one trial (ten movements) per stimulation condition (three trials in total per task). A 3-minute rest period was ensured after each kinematic trial to avoid fatigue. Then, we assessed CBI during θ-, γ- and sham-tACS (15 TS and 15 CS + TS per condition, resulting in 2.5 min of stimulation). Importantly, the order of stimulation conditions for both kinematic and TMS assessments was randomized and counterbalanced across participants. tACS was always switched-on ∼10 s before the beginning of the recording and switched-off immediately after it ended. Participants and the researchers conducting the recordings were blinded to stimulation conditions. Only the additional researcher responsible for setting up tACS knew the stimulation conditions, but (s)he did not actively participate in data collection and analysis (double-blind study design). Finally, although a recent study showed no long-term after-effects following cerebellar θ- and γ-tACS (Spampinato et al., 2021), we decided to wait more than twice the duration of stimulation after each kinematic and TMS assessment (i.e., 3 min after each kinematic trial and 6 min after each CBI assessment) (Fig. 1). The experimental design of our study, involving a single session for testing multiple tACS conditions in a random order, with appropriate wash-out periods, is similar to methodologies employed in other studies (Fabbrini et al., 2022; Feurra et al., 2011; Guerra et al., 2023, 2022, 2018; Manzo et al., 2020).

2.6. Statistical analysis

The sample size was computed using the G*Power software (Faul et al., 2007). We set a desired power of 0.80 and alpha error of 0.05, assuming a 20% change in kinematic and CBI measures between sham (mean and standard deviation (SD) values for tapping and arm reaching kinematic parameters based on (Guerra
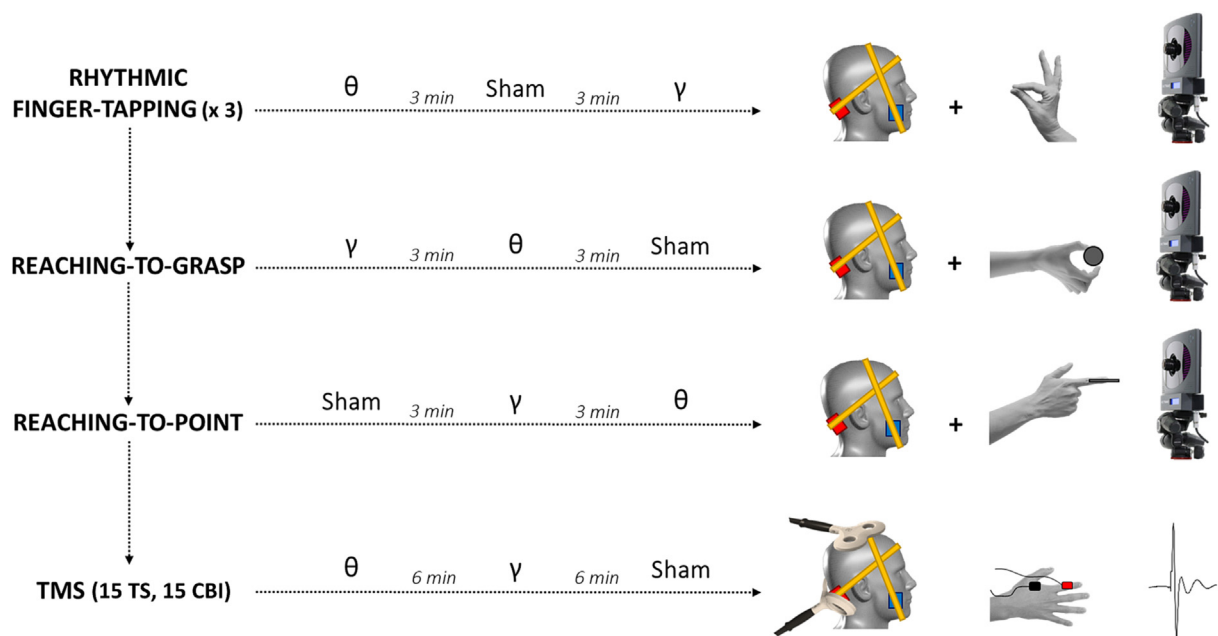

Fig. 1. Experimental design. Participants underwent a single experimental session, encompassing kinematic and transcranial magnetic stimulation (TMS) evaluations (total session duration: ∼2.5 h). Three behavioral tasks, including rhythmic finger tapping, reaching to grasp, and reaching to point tasks were first recorded using kinematic techniques during i) theta (θ)-transcranial alternating current stimulation (tACS); ii) gamma (γ)-tACS; iii) sham-tACS. A 3-minute rest period was ensured after each kinematic trial to avoid fatigue. After that, TMS was used to assess cerebellar brain inhibition (CBI) during θ-, γ- and sham-tACS (15 single TMS pulses - TS - and 15 paired pulses per condition). The sequence of tACS stimulation conditions for both kinematic and TMS assessments was randomized, and both participants and researchers were blinded to stimulation conditions. To exclude any long-term after-effects following cerebellar θ- and γ-tACS, we allowed a waiting period more than twice the duration of stimulation after each assessment, i.e., we waited 3 min after each kinematic trial and 6 min after each CBI assessment.

et al., 2018) and (Li Voti et al., 2014), respectively; mean and SD values for CBI based on (Spampinato et al., 2021)) and real tACS conditions. A sample size of 15 participants was determined to be the minimum required to detect a significant difference between conditions.

Separate repeated-measures analysis of variance (rmANOVA) using the within-group factor 'stimulation condition' (three levels: θ, γ, sham) were adopted to test for possible tACS effects on each kinematic and TMS variable. The within-group factor 'task phase' (two levels: synchronization, continuation) or 'task type' (two levels: grasping, pointing) was added to all the rmANOVAs when analyzing the rhythmic tapping or reaching tasks, respectively. In case of significant interactions, a rmANOVA with 'stimulation condition' as a factor was applied for each task phase or type. Post-hoc analyses were performed using the Tukey's test. To verify whether the CBI protocol produced a significant corticospinal inhibition, we used a paired t-test to compare the peak-to-peak amplitude of MEP evoked by TS alone and the amplitude of MEP evoked by CS + TS in the sham-tACS condition. Pearson's correlation test was adopted to evaluate the possible relationship between kinematic measures and CBI. For this purpose, we quantified tACS effects by computing the ratio between the values obtained during real stimulations (θ- and γ-tACS) and those recorded during sham-tACS (e.g., velocity θ-tACS/sham-tACS). Lastly, to ensure that repeated motor tasks did not alter motor performance per se, irrespective of the stimulation condition, we conducted a one-way rmANOVA with the factor 'trial number.' For the rhythmic finger tapping task, there were nine levels (trials 1–9), while for grasping and pointing tasks, there were three levels (trials 1–3). We assessed various kinematic parameters, including movement velocity and amplitude for the rhythmic tapping task, and velocity peak, acceleration peak, and movement duration for the reaching task. The level of significance was set at $p \leq 0.05$. Statistical analyses were performed using Statistica (TIBCO Software).

3. Results

All participants completed the experimental session without any reported discomfort or adverse effects.

3.1. Effects of tACS on cerebellar-related movements

During the rhythmic tapping, tACS modulated the movement rhythm depending on the task phase and the stimulation condition, as demonstrated by the significant 'stimulation condition' x 'task phase' interaction ($F_{2,28} = 3.63$, $p = 0.04$). More specifically, movement rhythm was similar between conditions in the synchronization phase of the task ('stimulation condition': $F_{2,28} = 0.13$, $p = 0.88$), while it changed during the continuation phase according to the stimulation frequency ('stimulation condition': $F_{2,28} = 7.29$, $p < 0.01$). Post-hoc analysis demonstrated less rhythmic movement (higher CV) during θ-tACS than both sham- ($p = 0.02$) and γ-tACS ($p < 0.01$), while movement rhythm was comparable between sham- and γ-tACS conditions ($p = 0.77$) (Fig. 2). The factor 'task phase' was also significant in the main rmANOVA, indicating, as expected, a greater movement regularity (lower CV) after the subjects effectively synchronized their tapping rhythm to the metronome ($F_{1,14} = 10.22$, $p < 0.01$). The movement frequency, velocity, and amplitude were found to be unaffected by both the task phase and the stimulation condition, as revealed by the lack of significant effects of the factors 'stimulation condition' and 'task phase', and no 'stimulation condition' x 'task phase' interaction in the rmANO-VAs (see Table 1 for details).

During the reaching tasks, tACS exerted a frequency-dependent modulation on both the duration of the entire move-

ment and the duration of the approach-to-the-target phase. Also, the modulation was task-specific, as demonstrated by the significant 'stimulation condition' x 'task type' (duration of the entire movement: $F_{2,28} = 5.77$, $p < 0.01$; duration of the approach-to-the-target phase: $F_{2,28} = 4.89$, $p = 0.01$). These parameters did not change during the grasping task in any stimulation condition ('stimulation condition', duration of the entire movement: $F_{2,28} = 2.14$, $p = 0.14$; duration of the approach-to-the-target phase: $F_{2,28} = 1.16$, $p = 0.33$). Conversely, the duration of the entire movement and the approach-to-the-target phase were modified by tACS during the pointing task ('stimulation condition', duration of the entire movement: $F_{2,28} = 4.46$, $p = 0.02$; duration of the approach-to-the-target phase: $F_{2,28} = 5.46$, $p < 0.01$), with longer durations recorded during θ-tACS than both sham- (duration of the entire movement: $p = 0.02$; duration of the approach-to-the-target phase: $p = 0.01$) and γ-tACS (duration of the entire movement: $p = 0.05$; duration of the approach-to-the-target phase: $p = 0.04$) (Fig. 2). No other kinematic parameter was influenced by the stimulation condition, as revealed by the lack of significant effects of the factors 'stimulation condition' and no 'stimulation condition' x 'task type' interaction in the various rmANOVAs (Table 2). Finally, there was a significant 'task type' factor in the rmANOVAs assessing various kinematic parameters, including peak velocity, acceleration, deceleration, CI of the acceleration phase and MU, which confirmed the higher complexity of the pointing compared to the grasping task (see Table 2 for details).

The rmANOVA conducted to examine the potential impact of repeating the motor tasks on motor performances ('order effect') revealed no significant effect of the factor 'trial number' on any of the tested kinematic parameters ($p > 0.05$ in all cases).

3.2. Effects of tACS on CBI

The participants' RMT and MT_{1mv} were 44.8 ± 10.8% and 55.4 ± 14.8%, respectively. During sham-tACS, the amplitude of MEP elicited by paired-pulse TMS (i.e., CBI) was lower than that elicited by single TMS pulses (t = 5.39, $p < 0.001$). Importantly, tACS did not modify the amplitude of MEP elicited by single TMS pulses, as indicated by the non-significant factor 'stimulation condition' in the rmANOVA ($F_{2,28} = 2.57$, $p = 0.10$). In contrast, the modulation of CBI by tACS was found to be frequency-specific ('stimulation condition': $F_{2,28} = 15.89$, $p < 0.001$). Post-hoc analysis showed that CBI was more effective (i.e., greater inhibition) during θ-tACS than sham- and γ-tACS ($p < 0.001$ for both comparisons). Conversely, CBI did not differ between sham-tACS and γ-tACS conditions ($p = 0.81$) (Fig. 3).

3.3. Correlation analysis

We found a significant correlation between the effect of θ-tACS on movement rhythm in the finger-tapping task (CV continuation phase θ-tACS/sham-tACS) and on CBI (CBI θ-tACS/sham-tACS) ($r = -0.58$, $p = 0.02$). That is, the greater the movement rhythm deterioration (higher CV values), the greater the CBI strengthening (lower values) during θ-tACS. Conversely, no relationship was present between the effect of θ-tACS on movement duration during the reaching-to-point task and on CBI (duration of the entire movement θ-tACS/sham-tACS vs. CBI θ-tACS/sham-tACS: $r = 0.02$, $p = 0.93$; duration of the approach-to-the-target phase θ-tACS/sham-tACS vs. CBI θ-tACS/sham-tACS: $r = -0.14$, $p = 0.61$) (Fig. 3). Finally, there was a strong link between the effect of θ-tACS on the duration of the approach-to-the-target phase and the entire movement during the reaching-to-point task ($r = 0.93$, $p < 0.001$).
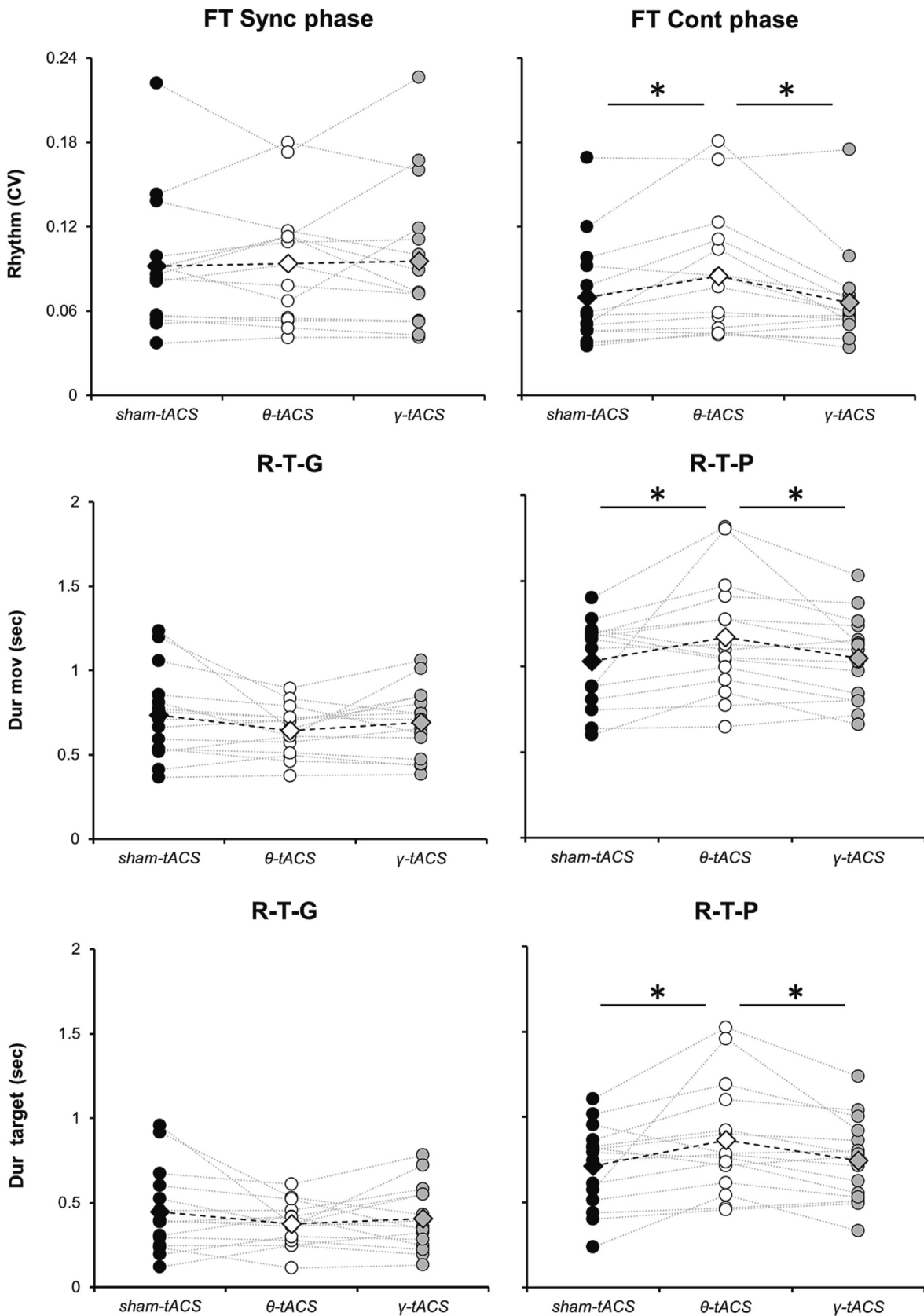
Fig. 2. Kinematic results. The top graphs represent individual kinematic values of finger tapping movement rhythm, expressed by the coefficient of variation (CV), of the participants during sham transcranial alternating current stimulation (sham-tACS) (black circles), theta (θ)-tACS (white circles) and gamma (γ)-tACS (grey circles). Data collected during the synchronization phase of the finger tapping (FT Sync) task are shown on the left, while data collected during the continuation (FT Cont) phase are shown on the right. The middle graphs represent the duration (Dur mov) of the entire reaching to grasp (R-T-G) (on the left) and reaching to point (R-T-P) (on the right) movement during sham-, θ- and γ-tACS. Finally, the bottom graphs represent the duration of the approach-to-the-target phase (Dur target) of the R-T-G (on the left) and R-T-P (on the right) movement during sham-, θ- and γ-tACS. Diamonds indicate mean values. Asterisks indicate significant differences between conditions.

Table 1
Kinematic data and statistics for the rhythmic finger tapping task.

		Raw data		rmANOVA
		Sync Phase	Cont Phase	
Frequency (Hz)	Sham	2.00 ± 0.01	2.03 ± 0.05	STIM COND: $F_{2,28}$ = 1.54, P = 0.23
	θ	1.99 ± 0.01	2.02 ± 0.07	PHASE: $F_{1,14}$ = 3.35, P = 0.09
	γ	2.00 ± 0.01	2.03 ± 0.06	STIM COND x PHASE: $F_{2,28}$ = 0.04, P = 0.96
Amplitude (degrees)	Sham	51.5 ± 12.8	52.3 ± 13.3	STIM COND: $F_{2,28}$ = 0.56, P = 0.58
	θ	52.3 ± 14.0	52.3 ± 14.0	PHASE: $F_{1,14}$ = 0.87, P = 0.37
	γ	51.1 ± 13.1	52.1 ± 13.5	STIM COND x PHASE: $F_{2,28}$ = 2.67, P = 0.09
Velocity (degrees/sec)	Sham	928 ± 298	894 ± 328	STIM COND: $F_{2,28}$ = 1.51, P = 0.24
	θ	961 ± 309	908 ± 327	PHASE: $F_{1,14}$ = 3.36, P = 0.09
	γ	942 ± 312	920 ± 345	STIM COND x PHASE: $F_{2,28}$ = 1.61, P = 0.22
Rhythm (CV)	Sham	0.092 ± 0.047	0.070 ± 0.037	STIM COND: $F_{2,28}$ = 2.36, P = 0.11
	θ	0.094 ± 0.043	0.085 ± 0.045	**PHASE: $F_{1,14}$ = 10.22, P < 0.01**
	γ	0.095 ± 0.053	0.066 ± 0.034	**STIM COND x PHASE: $F_{2,28}$ = 3.63, P = 0.04**

Sync Phase: synchronization phase; Cont Phase: continuation phase; Sham: sham-tACS; θ: theta-tACS; γ: gamma-tACS; CV: coefficient of variation; STIM COND: factor 'Stimulation condition'; PHASE: factor 'Task phase'; rmANOVA: repeated-measures analysis of variance; sec: seconds; STIM COND x PHASE: 'Stimulation condition' x 'Task phase' interaction. Data reflect mean values ± 1 standard deviation. Significant factors and interactions are in bold.

Table 2
Kinematic data and statistics for the reaching tasks.

		Raw data		rmANOVA
		R-T-G	R-T-P	
Peak vel	Sham	1.10 ± 0.22	0.57 ± 0.17	STIM COND: $F_{2,28}$ = 1.58, P = 0.22
	θ	1.13 ± 0.24	0.59 ± 0.19	**TASK: $F_{1,14}$ = 84.35, P < 0.001**
	γ	1.10 ± 0.23	0.58 ± 0.19	STIM COND x TASK: $F_{2,28}$ = 0.41, P = 0.67
Peak accel	Sham	9.84 ± 2.52	3.49 ± 1.23	STIM COND: $F_{2,28}$ = 1.24, P = 0.30
	θ	10.92 ± 3.28	3.63 ± 1.42	**TASK: $F_{1,14}$ = 140.67, P < 0.001**
	γ	10.27 ± 3.06	3.56 ± 1.25	STIM COND x TASK: $F_{2,28}$ = 0.68, P = 0.51
Peak decel	Sham	−8.49 ± 3.85	−2.55 ± 0.75	STIM COND: $F_{2,28}$ = 1.02, P = 0.37
	θ	−7.67 ± 3.33	−2.74 ± 0.94	**TASK: $F_{1,14}$ = 68.92, P < 0.001**
	γ	−6.95 ± 2.21	−2.72 ± 0.92	STIM COND x TASK: $F_{2,28}$ = 1.89, P = 0.17
Dur mov	Sham	0.73 ± 0.26	1.03 ± 0.25	STIM COND: $F_{2,28}$ = 0.69, P = 0.51
	θ	0.64 ± 0.14	1.17 ± 0.34	**TASK: $F_{1,14}$ = 35.42, P < 0.001**
	γ	0.69 ± 0.21	1.05 ± 0.25	**STIM COND x TASK: $F_{2,28}$ = 5.77, P < 0.01**
Dur accel	Sham	0.04 ± 0.02	0.05 ± 0.04	STIM COND: $F_{2,28}$ = 0.50, P = 0.61
	θ	0.04 ± 0.02	0.05 ± 0.04	TASK: $F_{1,14}$ = 1.06, P = 0.32
	γ	0.04 ± 0.03	0.05 ± 0.04	STIM COND x TASK: $F_{2,28}$ = 0.19, P = 0.82
Dur decel	Sham	0.27 ± 0.06	0.27 ± 0.06	STIM COND: $F_{2,28}$ = 1.92, P = 0.17
	θ	0.24 ± 0.07	0.27 ± 0.05	TASK: $F_{1,14}$ = 0.65, P = 0.43
	γ	0.26 ± 0.04	0.26 ± 0.06	STIM COND x TASK: $F_{2,28}$ = 2.18, P = 0.13
Dur target	Sham	0.45 ± 0.25	0.71 ± 0.24	STIM COND: $F_{2,28}$ = 1.26, P = 0.30
	θ	0.37 ± 0.13	0.86 ± 0.33	**TASK: $F_{1,14}$ = 30.12, P < 0.001**
	γ	0.41 ± 0.19	0.74 ± 0.24	**STIM COND x TASK: $F_{2,28}$ = 4.89, P = 0.01**
CI mov	Sham	1.06 ± 0.04	1.04 ± 0.02	STIM COND: $F_{2,28}$ = 1.38, P = 0.27
	θ	1.06 ± 0.03	1.05 ± 0.02	TASK: $F_{1,14}$ = 1.38, P = 0.26
	γ	1.06 ± 0.03	1.05 ± 0.03	STIM COND x TASK: $F_{2,28}$ = 1.57, P = 0.22
CI accel	Sham	0.76 ± 0.23	0.90 ± 0.10	STIM COND: $F_{2,28}$ = 0.85, P = 0.44
	θ	0.79 ± 0.15	0.94 ± 0.10	**TASK: $F_{1,14}$ = 8.96, P < 0.01**
	γ	0.80 ± 0.16	0.93 ± 0.07	STIM COND x TASK: $F_{2,28}$ = 0.11, P = 0.90
CI decel	Sham	1.00 ± 0.04	0.98 ± 0.05	STIM COND: $F_{2,28}$ = 0.79, P = 0.46
	θ	0.99 ± 0.04	0.97 ± 0.07	TASK: $F_{1,14}$ = 0.99, P = 0.37
	γ	0.99 ± 0.04	0.96 ± 0.11	STIM COND x TASK: $F_{2,28}$ = 0.37, P = 0.69
CI target	Sham	1.10 ± 0.10	1.07 ± 0.07	STIM COND: $F_{2,28}$ = 1.49, P = 0.24
	θ	1.16 ± 0.27	1.10 ± 0.07	TASK: $F_{1,14}$ = 1.20, P = 0.29
	γ	1.09 ± 0.08	1.08 ± 0.06	STIM COND x TASK: $F_{2,28}$ = 0.44, P = 0.65
MU	Sham	2.13 ± 0.83	5.89 ± 3.33	STIM COND: $F_{2,28}$ = 1.13, P = 0.34
	θ	1.99 ± 0.98	5.36 ± 2.49	**TASK: $F_{1,14}$ = 48.87, P < 0.001**
	γ	2.07 ± 1.18	5.43 ± 2.23	STIM COND x TASK: $F_{2,28}$ = 0.30, P = 0.74

CI mov: curvature index of the entire movement; CI accel: curvature index of the acceleration phase; CI decel: curvature index of the deceleration phase; CI target: curvature index of the approach-to-target phase; Dur accel: duration of the acceleration phase; Dur decel: duration of the deceleration phase; Dur mov: duration of the entire movement; Dur target: duration of the approach-to-target phase; MU: movement units; Peak accel: acceleration peak; Peak decel: deceleration peak; Peak vel: velocity peak; R-T-G: reaching-to-grasp task; R-T-P: reaching-to-point task; rmANOVA: repeated-measures analysis of variance; Sham: sham-tACS; θ: theta-tACS; γ: gamma-tACS; STIM COND: factor 'Stimulation condition'; TASK: factor 'Task type'; STIM COND x TASK: 'Stimulation condition' x 'Task type' interaction. Data reflect mean values ± 1 standard deviation. Significant factors and interactions are in bold.
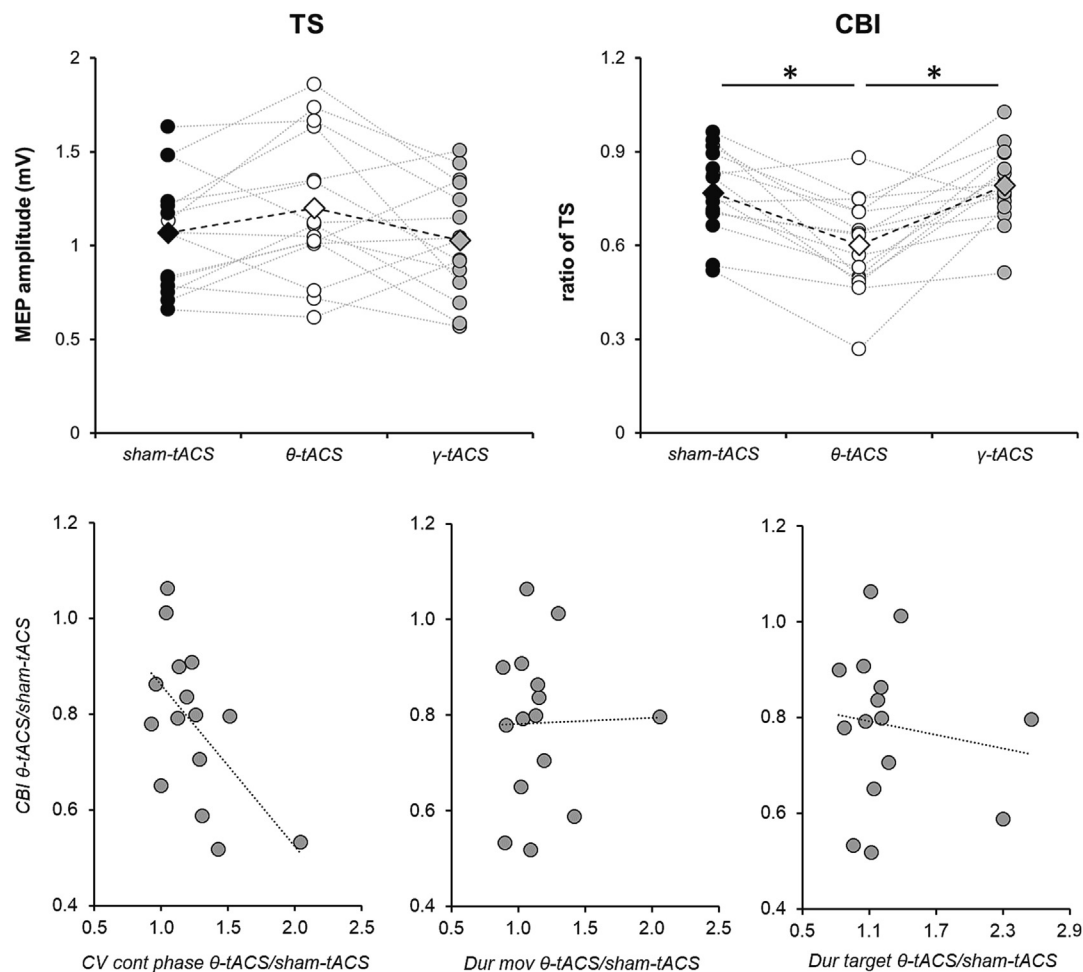
Fig. 3. TMS results and correlation. The top graph on the left side represents individual values of the peak-to-peak amplitude of motor evoked potentials (MEP) elicited by single TMS pulses (TS), expressed in mV, during sham transcranial alternating current stimulation (sham-tACS) (black circles), theta (θ)-tACS (white circles) and gamma (γ)-tACS (grey circles). The top graph on the right side represents individual values of cerebellar-brain inhibition (CBI), expressed as the ratio between the amplitude of conditioned and unconditioned MEP (TS alone) during sham-, θ- and γ-tACS. Diamonds indicate mean values. Asterisks indicate significant differences between conditions. The bottom graphs show correlation analysis results. The y-axes represent the ratio between the CBI during θ-tACS and during sham-tACS. The x-axes indicate the ratio between the coefficient of variation (CV) values (continuation phase of the finger tapping task), obtained during θ-tACS and during sham-tACS (left-side graph); the ratio between the duration of the entire movement (Dur mov - middle graph) and of the approach-to-the-target phase (Dur target - right-side graph) of the reaching to point task obtained during θ-tACS and during sham-tACS. Note that the greater the movement rhythm deterioration (higher CV values), the greater the CBI strengthening (lower values) during θ-tACS.

4. Discussion

In this study, we aimed to assess the possible effects of cerebellar tACS delivered at θ and γ frequencies on different motor tasks. Also, we investigated whether the behavioral effects of tACS were associated with neurophysiological changes in cerebellum-M1 connectivity, as assessed by CBI. We found that tACS delivered at the θ frequency had a detrimental effect on movement regularity, i.e., it decreased movement regularity of rhythmic tapping and increased the movement duration of a pointing task, particularly the approach-to-the-target phase duration. Furthermore, we found that θ-tACS increased the effectiveness of CBI (more M1 inhibition) and the effect correlated with changes in tapping regularity induced by the stimulation. In contrast, we did not observe any change in movement kinematic parameters or CBI during the application of γ-tACS.

Our study was performed using a randomized experimental design, which ensured no influence of the stimulation condition order on the obtained results. Importantly, no participant reported any relevant visual or skin sensation during tACS, so it was impossible to distinguish between real and sham stimulation conditions. In addition, the researchers involved in data collection and analysis were unaware of the tACS conditions. These factors guarantee a proper double-blind study design. The three motor tasks we tested were not particularly long or tiring, and a 3-min rest period was ensured after each kinematic trial to avoid fatigue. The overall corticospinal excitability, as reflected by single pulse MEP amplitude, was similar between θ-, γ- and sham-tACS, which allowed us to exclude that possible changes in corticospinal excitability during stimulation influenced our findings. Again, a recent study using the same TMS-tACS setup and stimulation parameters as in our study demonstrated that changes in cerebellar-M1 connectivity induced by cerebellar-tACS occur during stimulation only (Spampinato et al., 2021). Therefore, it is unlikely that any potential aftereffects could have influenced our data. Finally, the detrimental effect on finger tapping movements rhythm observed during θ-tACS cannot be considered secondary to nonspecific changes in other kinematic parameters, including modifications of the duration of the single tapping movement or changes in movement velocity, being the tapping frequency and speed the same during all the different stimulation conditions.

The most innovative finding of our study concerns the effect of cerebellar-tACS on motor behavior. We objectively analyzed three specific upper limb tasks related to the cerebellar activity, i.e., the rhythmic tapping and two arm-reaching movements that differed in complexity (grasping and pointing) (Bologna et al., 2016, 2015; Del Olmo et al., 2007; Deuschl et al., 2000; Li Voti et al., 2014). We provided the first evidence that cerebellar-tACS can interfere with cerebellar-related behavior during stimulation. First, tACS decreases the movement regularity during repetitive finger movements. This effect was particularly evident when participants were asked to perform tapping at the predefined rate of 2 Hz without the auditory cue (Rao et al., 1997). Performing a rhythmic movement without auditory cues, which is a more challenging task, requires significant involvement of the cerebellum and potentially involves short-term learning processes (Cheron et al., 2016). Consequently, executing rhythmic movements without auditory cues may be more susceptible to the detrimental effects of non-invasive brain stimulation. Also, previous evidence showed that tACS can modulate learning mechanisms both at the cortical (Bologna et al., 2019; Giustiniani et al., 2019; Pollok et al., 2015; Sugata et al., 2018) and cerebellar level (Giustiniani et al., 2021; Miyaguchi et al., 2020; Wessel et al., 2020).

Further demonstrating the detrimental effect of cerebellar tACS on motor execution, we found that stimulation increased the duration of arm-reaching movements. Importantly, this effect depended on the complexity of the reaching task and was related to the specific phase of movement considered. We found that during tACS movement duration increased in the pointing but not in the grasping task, which is a less complex reaching movement (Li Voti et al., 2014). The lower complexity of the grasping task is supported by our data, which indicates higher peak velocity and amplitude, as well as a shorter movement duration in this task compared to the reaching-to-point task. Also, we found that the increased movement duration in the reaching-to-point task mainly affected the approach-to-the-target phase. The correlation we found between tACS-induced modulation of the entire movement duration and the approach-to-the-target phase supports the idea that there is a link between these two effects, i.e., the lengthening of the approach-to-the-target phase likely results in the increase of the entire movement duration. The specificity of tACS effects observed in the reaching-to-point task, particularly during the approach-to-target phase, prompts the hypothesis that cerebellar-tACS may predominantly modulate high-demand movements, characterized by increased complexity and precision requirements. Importantly, the level of cerebellar activation is known to correlate with the complexity of motor performance (Manto et al., 2012). Additionally, the approach-to-target phase, requiring substantial movement adjustments, is closely associated with heightened cerebellar activation (Becker and Person, 2019). Thus, we hypothesize that cerebellar-tACS may exhibit enhanced effectiveness when the level of cerebellar activation is higher. A relevant aspect of our results is that tACS effects were frequency-specific, being present only when the stimulation was delivered at the θ frequency. Indeed, repetitive finger movement had a decreased movement regularity during θ-tACS than during sham- and γ-tACS, while comparable values were observed between sham- and γ-tACS. Similarly, in the pointing task, the movement duration increased during θ-tACS compared to both sham- and γ-tACS, while it was similar between the other conditions. The mechanism of action of tACS involves the modulation of the firing rate and timing discharge of susceptible neurons, as well as the entrainment of their oscillatory activity to the stimulation frequency (Johnson et al., 2020; Krause et al., 2019; Reato et al., 2013). Importantly, tACS effectiveness depends on the degree to which the stimulation frequency matches the natural frequency of the targeted neuronal populations (Ali et al., 2013; Fabbrini et al., 2022; Ozen et al., 2010; Witkowski et al., 2016). Both animal and computational studies have provided evidence that cerebellar granule and Golgi cells express a preferential response frequency in the θ band (Courtemanche et al., 2013; De Zeeuw et al., 2008; Dieudonné and Dumoulin, 2000; Dugué et al., 2009; Hoffmann and Berry, 2009; Medina and Mauk, 2000). It has been hypothesized that θ frequency bursting and resonance in granule cells play a significant role in synchronization, rhythmicity, and learning processes in the cerebellum (D'Angelo et al., 2013, 2001; Koziol et al., 2014). Hence, it is reasonable to speculate that these neurons also possess inherent oscillatory properties at the θ frequency in humans. tACS could specifically target θ resonant neurons within the cerebellum, and their modulation could account for the observed effects during stimulation. The significance of θ oscillations in the human cerebellum is underscored by a recent study revealing notable changes in θ activity during visuomotor adaptation (Tzvi et al., 2022). Regarding cerebellar physiology, there is also evidence that cerebellar Purkinje cells express oscillatory properties at the γ frequency (De Zeeuw et al., 2008; Middleton et al., 2008; Ruigrok, 2011; Shin et al., 2007). However, we found no effect of γ-tACS on cerebellar-related motor tasks, possibly due to a higher threshold of Purkinje cells to be activated by γ-tACS or, alternatively, to unresponsiveness of these neurons to be entrained for neuroanatomical reasons, i.e., the layer location and specific orientation of dendrites and axons. Another plausible explanation is that γ oscillations in the cerebellum may not be involved in the specific motor tasks we tested.

Another significant finding of our study pertains to the effect of tACS on CBI and its correlation with the modulation of motor behavior. Confirming previous results (Spampinato et al., 2021), we found a more effective CBI during θ-tACS than sham- and γ-tACS, indicating a greater inhibitory drive from the cerebellum to M1 during stimulation. As discussed above and previously hypothesized by others (Spampinato et al., 2021), cerebellar θ-tACS may induce a stronger CBI by increasing the recruitment of granule cell activity, which in turn would boost Purkinje cells' inhibitory drive. Interestingly, we demonstrated a significant correlation between the effect of θ-tACS on CBI and movement rhythm in the finger-tapping task. That is, the greater the CBI (increased inhibition), the less rhythmic the finger-tapping during stimulation. In healthy humans, CBI decreases (reduced inhibition) in active muscles during voluntary movements. This mechanism is believed to contribute to the physiological activation of movements (Kassavetis et al., 2011; Panyakaew et al., 2016). The neuroanatomical substrate of rhythmic finger tapping encompasses a network that includes the cerebellum, sensorimotor and supplementary motor areas, along with subcortical regions (Del Olmo et al., 2007; Rao et al., 1997). Notably, recent data propose that the cerebellum regulates neuronal activity in the cerebral cortex, influencing movement timing and predicting the timing of rhythmic events (Tanaka et al., 2021). Our findings indicate that the θ-tACS-induced impairment in movement regularity is associated with alterations in cerebellum-M1 connectivity. In particular, we hypothesize that enhancing the inhibitory output from the cerebellum to M1 during stimulation could alter the balance between inhibition and excitation within the cortex. This disruption may lead to dysregulated M1 activity, thereby affecting the ability to maintain a constant and regular rhythm during repetitive movements. The lack of correlation between the effect of θ-tACS on CBI and movement duration in the reaching-to-point task may reflect a secondary role of cerebellum-M1 connectivity in this motor function, which could be instead codified by intrinsic cerebellar mechanisms or alternative cerebellar circuits not projecting to M1.

This study has some limitations to mention. First, we have interpreted our results as due to the activation of a cerebellar

subpopulation of neurons resonant to the θ rhythm; however, we did not provide a direct biological demonstration supporting this hypothesis. Also, since θ and γ oscillatory activities are those more consistently reported in the cerebellum (D'Angelo et al., 2001; De Zeeuw et al., 2008; Manto et al., 2022; Middleton et al., 2008), we focused on testing the possible effects of θ- and γ-tACS. Importantly, other rhythms have been recorded in the cerebellum in animals, like β and high-frequency oscillations (Cheron and Cheron, 2018; Dalal et al., 2013; Herrojo Ruiz et al., 2017; Middleton et al., 2008). However, there is currently no consistent data connecting these activities to cerebellar-related measures or functions in humans. Investigating the effects of cerebellar-tACS delivered at frequencies other than θ and gamma γ would be an intriguing objective for future studies. For example, it remains uncertain whether tACS delivered at β and very high frequencies may also induce behavioral effects. Also, we tested rhythmic tapping and arm-reaching movements as reflecting cerebellar-related behavioral measures. The assessment of different tasks or other body districts (e.g., lower limb, gait, posture, etc.) was beyond the aim of our study and could be tested in future research. Lastly, the sample size in our study was determined to be the minimum required for detecting any potential effects of tACS on cerebellar-related behavioral and neurophysiological measures. We acknowledge that testing small sample sizes may lead to an overestimation of effects, and enhancing reproducibility in neuroscience is a pertinent concern (Button et al., 2013). Future studies with larger sample sizes are needed to validate the reliability of cerebellar θ-tACS effects on motor behavior.

5. Conclusions

We here provide evidence that tACS delivered at the θ frequency over the cerebellum modulates motor performance in various cerebellar-dependent upper limb tasks. This effect relies, at least in part, on the strengthening of inhibitory cerebellar drive to M1 and could be due to the activation of cerebellar granule cells which project on Purkinje cells. Notably, cerebellar-tACS modulated specific kinematic parameters, i.e., movement rhythm for the finger-tapping task and movement duration for the reaching-to-point task, while all the other measures did not change. These results support the idea that different movement parameters (e.g., velocity, rhythm, timing, trajectory smoothness, etc.) do not necessarily reflect the activity of the same areas/similar neurophysiological mechanisms in humans. Rather, they could be codified by distinct networks and different neural processes. Overall, the relevance of our findings improve the understanding of specific neurophysiological mechanisms underlying human motor control, i.e., the role of cerebellar oscillations in cerebellar-related functions. Importantly, our results serve as a proof-of-principle for the development of innovative methods to modulate cerebellar functions through non-invasive brain stimulation, especially in movement disorders (Manto et al., 2022). For instance, θ-tACS could be explored in conditions such as Parkinson's disease and dystonia, which are characterized by impaired cerebellum-M1 inhibition (Ni et al., 2010; Sondergaard et al., 2023).

Declarations of interest

None of the authors have potential conflicts of interest to be disclosed.

References

Ali MM, Sellers KK, Frohlich F. Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance. J Neurosci 2013;33:11262–75. https://doi.org/10.1523/JNEUROSCI.5867-12.2013.

Antal A, Alekseichuk I, Bikson M, Brockmöller J, Brunoni AR, Chen R, et al. Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol 2017;128:1774–809. https://doi.org/10.1016/j.clinph.2017.06.001.

Asan AS, Lang EJ, Sahin M. Entrainment of cerebellar purkinje cells with directional AC electric fields in anesthetized rats. Brain Stimul 2020;13:1548–58. https://doi.org/10.1016/j.brs.2020.08.017.

Becker MI, Person AL. Cerebellar control of reach kinematics for endpoint precision. Neuron 2019;103:335–348.e5. https://doi.org/10.1016/j.neuron.2019.05.007.

Benussi A, Cantoni V, Grassi M, Brechet L, Michel CM, Datta A, et al. Increasing brain gamma activity improves episodic memory and restores cholinergic dysfunction in Alzheimer's disease. Ann Neurol 2022;92:322–34. https://doi.org/10.1002/ana.26411.

Bologna M, Di Biasio F, Conte A, Iezzi E, Modugno N, Berardelli A. Effects of cerebellar continuous theta burst stimulation on resting tremor in Parkinson's disease. Parkinsonism Relat Disord 2015;21:1061–6. https://doi.org/10.1016/j.parkreldis.2015.06.015.

Bologna M, Guerra A, Colella D, Cioffi E, Paparella G, Di Vita A, et al. Bradykinesia in Alzheimer's disease and its neurophysiological substrates. Clin Neurophysiol 2020;131:850–8. https://doi.org/10.1016/j.clinph.2019.12.413.

Bologna M, Guerra A, Paparella G, Colella D, Borrelli A, Suppa A, et al. Transcranial alternating current stimulation has frequency-dependent effects on motor learning in healthy humans. Neuroscience 2019;411:130–9. https://doi.org/10.1016/j.neuroscience.2019.05.041.

Bologna M, Guerra A, Paparella G, Giordo L, Alunni Fegatelli D, Vestri AR, et al. Neurophysiological correlates of bradykinesia in Parkinson's disease. Brain 2018;141:2432–44. https://doi.org/10.1093/brain/awy155.

Bologna M, Paparella G, Fabbrini A, Leodori G, Rocchi L, Hallett M, et al. Effects of cerebellar theta-burst stimulation on arm and neck movement kinematics in patients with focal dystonia. Clin Neurophysiol 2016;127:3472–9. https://doi.org/10.1016/j.clinph.2016.09.008.

Button KS, Ioannidis JPA, Mokrysz C, Nosek BA, Flint J, Robinson ESJ, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 2013;14:365–76. https://doi.org/10.1038/nrn3475.

Cheron G, Márquez-Ruiz J, Dan B. Oscillations, timing, plasticity, and learning in the cerebellum. Cerebellum 2016;15:122–38. https://doi.org/10.1007/s12311-015-0665-9.

Cheron J, Cheron G. Beta-gamma burst stimulations of the inferior olive induce high-frequency oscillations in the deep cerebellar nuclei. Eur J Neurosci 2018;48:2879–89. https://doi.org/10.1111/ejn.13873.

Courtemanche R, Robinson JC, Aponte DI. Linking oscillations in cerebellar circuits. Front Neural Circuits 2013;7:125. https://doi.org/10.3389/fncir.2013.00125.

Dalal SS, Osipova D, Bertrand O, Jerbi K. Oscillatory activity of the human cerebellum: the intracranial electrocerebellogram revisited. Neurosci Biobehav Rev 2013;37:585–93. https://doi.org/10.1016/j.neubiorev.2013.02.006.

D'Angelo E, Nieus T, Maffei A, Armano S, Rossi P, Taglietti V, et al. Theta-frequency bursting and resonance in cerebellar granule cells: experimental evidence and modeling of a slow k+-dependent mechanism. J Neurosci 2001;21:759–70. https://doi.org/10.1523/JNEUROSCI.21-03-00759.2001.

D'Angelo E, Solinas S, Mapelli J, Gandolfi D, Mapelli L, Prestori F. The cerebellar Golgi cell and spatiotemporal organization of granular layer activity. Front Neural Circuits 2013;7:93. https://doi.org/10.3389/fncir.2013.00093.

De Zeeuw CI, Hoebeek FE, Schonewille M. Causes and consequences of oscillations in the cerebellar cortex. Neuron 2008;58:655–8. https://doi.org/10.1016/j.neuron.2008.05.019.

Del Olmo MF, Cheeran B, Koch G, Rothwell JC. Role of the cerebellum in externally paced rhythmic finger movements. J Neurophysiol 2007;98:145–52. https://doi.org/10.1152/jn.01088.2006.

Deuschl G, Wenzelburger R, Löffler K, Raethjen J, Stolze H. Essential tremor and cerebellar dysfunction clinical and kinematic analysis of intention tremor. Brain 2000;123(Pt 8):1568–80. https://doi.org/10.1093/brain/123.8.1568.

Dieudonné S, Dumoulin A. Serotonin-driven long-range inhibitory connections in the cerebellar cortex. J Neurosci 2000;20:1837–48. https://doi.org/10.1523/JNEUROSCI.20-05-01837.2000.

Dugué GP, Brunel N, Hakim V, Schwartz E, Chat M, Lévesque M, et al. Electrical coupling mediates tunable low-frequency oscillations and resonance in the cerebellar Golgi cell network. Neuron 2009;61:126–39. https://doi.org/10.1016/j.neuron.2008.11.028.

Fabbrini A, Guerra A, Giangrosso M, Manzo N, Leodori G, Pasqualetti P, et al. Transcranial alternating current stimulation modulates cortical processing of somatosensory information in a frequency- and time-specific manner. Neuroimage 2022;254:119119. https://doi.org/10.1016/j.neuroimage.2022.119119.

Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175–91. https://doi.org/10.3758/BF03193146.

Feurra M, Bianco G, Santarnecchi E, Del Testa M, Rossi A, Rossi S. Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J Neurosci 2011;31:12165–70. https://doi.org/10.1523/JNEUROSCI.0978-11.2011.

Galea JM, Jayaram G, Ajagbe L, Celnik P. Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. J Neurosci 2009;29:9115–22. https://doi.org/10.1523/JNEUROSCI.2184-09.2009.

Giustiniani A, Tarantino V, Bonaventura RE, Smirni D, Turriziani P, Oliveri M. Effects of low-gamma tACS on primary motor cortex in implicit motor learning. Behav Brain Res 2019;376:112170. https://doi.org/10.1016/j.bbr.2019.112170.

Giustiniani A, Tarantino V, Bracco M, Bonaventura RE, Oliveri M. Functional role of cerebellar gamma frequency in motor sequences learning: a tACS study. Cerebellum 2021;20:913–21. https://doi.org/10.1007/s12311-021-01255-6.

Guerra A, Bologna M, Paparella G, Suppa A, Colella D, Di Lazzaro V, et al. Effects of transcranial alternating current stimulation on repetitive finger movements in healthy humans. Neural Plast 2018;2018:1–10. https://doi.org/10.1155/2018/4593095.

Guerra A, Colella D, Cannavacciuolo A, Giangrosso M, Paparella G, Fabbrini G, et al. Short-term plasticity of the motor cortex compensates for bradykinesia in Parkinson's disease. Neurobiol Dis 2023;182:106137. https://doi.org/10.1016/j.nbd.2023.106137.

Guerra A, Colella D, Giangrosso M, Cannavacciuolo A, Paparella G, Fabbrini G, et al. Driving motor cortex oscillations modulates bradykinesia in Parkinson's disease. Brain 2022;145:224–36. https://doi.org/10.1093/brain/awab257.

Gundlach C, Müller MM, Nierhaus T, Villringer A, Sehm B. Phasic modulation of human somatosensory perception by transcranially applied oscillating currents. Brain Stimul 2016;9:712–9. https://doi.org/10.1016/j.brs.2016.04.014.

Herrojo Ruiz M, Maess B, Altenmüller E, Curio G, Nikulin VV. Cingulate and cerebellar beta oscillations are engaged in the acquisition of auditory-motor sequences. Hum Brain Mapp 2017;38:5161–79. https://doi.org/10.1002/hbm.23722.

Herzog R, Berger TM, Pauly MG, Xue H, Rueckert E, Münchau A, et al. Cerebellar transcranial current stimulation – an intraindividual comparison of different techniques. Front Neurosci 2022;16:987472. https://doi.org/10.3389/fnins.2022.987472.

Hoffmann LC, Berry SD. Cerebellar theta oscillations are synchronized during hippocampal theta-contingent trace conditioning. Proc Natl Acad Sci U S A 2009;106:21371–6. https://doi.org/10.1073/pnas.0908403106.

Johnson L, Alekseichuk I, Krieg J, Doyle A, Yu Y, Vitek J, et al. Dose-dependent effects of transcranial alternating current stimulation on spike timing in awake nonhuman primates. Sci Adv 2020;6. https://doi.org/10.1126/sciadv.aaz2747.

Joundi RA, Jenkinson N, Brittain J-S, Aziz TZ, Brown P. Driving oscillatory activity in the human cortex enhances motor performance. Curr Biol 2012;22:403–7. https://doi.org/10.1016/j.cub.2012.01.024.

Kassavetis P, Hoffland BS, Saifee TA, Bhatia KP, van de Warrenburg BP, Rothwell JC, et al. Cerebellar brain inhibition is decreased in active and surround muscles at the onset of voluntary movement. Exp Brain Res 2011;209:437–42. https://doi.org/10.1007/s00221-011-2575-5.

Koziol LF, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu H, et al. Consensus paper: the cerebellum's role in movement and cognition. Cerebellum 2014;13:151–77. https://doi.org/10.1007/s12311-013-0511-x.

Krause MR, Vieira PG, Csorba BA, Pilly PK, Pack CC. Transcranial alternating current stimulation entrains single-neuron activity in the primate brain. Proc Natl Acad Sci USA 2019;116:5747–55. https://doi.org/10.1073/pnas.1815958116.

Li Voti P, Conte A, Rocchi L, Bologna M, Khan N, Leodori G, et al. Cerebellar continuous theta-burst stimulation affects motor learning of voluntary arm movements in humans. Eur J Neurosci 2014;39:124–31. https://doi.org/10.1111/ejn.12391.

Manto M, Argyropoulos GPD, Bocci T, Celnik PA, Corben LA, Guidetti M, et al. Consensus paper: novel directions and next steps of non-invasive brain stimulation of the cerebellum in health and disease. Cerebellum 2022;21:1092–122. https://doi.org/10.1007/s12311-021-01344-6.

Manto M, Bower JM, Conforto AB, Delgado-García JM, da Guarda SNF, Gerwig M, et al. Consensus paper: roles of the cerebellum in motor control–the diversity of ideas on cerebellar involvement in movement. Cerebellum 2012;11:457–87. https://doi.org/10.1007/s12311-011-0331-9.

Manzo N, Guerra A, Giangrosso M, Belvisi D, Leodori G, Berardelli A, et al. Investigating the effects of transcranial alternating current stimulation on primary somatosensory cortex. Sci Rep 2020;10:17129. https://doi.org/10.1038/s41598-020-74072-2.

Medina JF, Mauk MD. Computer simulation of cerebellar information processing. Nat Neurosci 2000;3(Suppl):1205–11. https://doi.org/10.1038/81486.

Middleton SJ, Racca C, Cunningham MO, Traub RD, Monyer H, Knöpfel T, et al. High-frequency network oscillations in cerebellar cortex. Neuron 2008;58:763–74. https://doi.org/10.1016/j.neuron.2008.03.030.

Miyaguchi S, Inukai Y, Matsumoto Y, Miyashita M, Takahashi R, Otsuru N, et al. Effects on motor learning of transcranial alternating current stimulation applied over the primary motor cortex and cerebellar hemisphere. J Clin Neurosci 2020;78:296–300. https://doi.org/10.1016/j.jocn.2020.05.024.

Miyaguchi S, Inukai Y, Mitsumoto S, Otsuru N, Onishi H. Gamma-transcranial alternating current stimulation on the cerebellum and supplementary motor area improves bimanual motor skill. Behav Brain Res 2022;424:113805. https://doi.org/10.1016/j.bbr.2022.113805.

Naro A, Bramanti A, Leo A, Manuli A, Sciarrone F, Russo M, et al. Effects of cerebellar transcranial alternating current stimulation on motor cortex excitability and motor function. Brain Struct Funct 2017;222:2891–906. https://doi.org/10.1007/s00429-016-1355-1.

Naro A, Leo A, Russo M, Cannavò A, Milardi D, Bramanti P, et al. Does transcranial alternating current stimulation induce cerebellum plasticity? Feasibility, safety and efficacy of a novel electrophysiological approach. Brain Stimul 2016;9:388–95. https://doi.org/10.1016/j.brs.2016.02.005.

Ni Z, Pinto AD, Lang AE, Chen R. Involvement of the cerebellothalamocortical pathway in Parkinson disease. Ann Neurol 2010;68:816–24. https://doi.org/10.1002/ana.22221.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.

Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, et al. Transcranial electric stimulation entrains cortical neuronal populations in rats. J Neurosci 2010;30:11476–85. https://doi.org/10.1523/JNEUROSCI.5252-09.2010.

Panyakaew P, Cho HJ, Srivanitchapoom P, Popa T, Wu T, Hallett M. Cerebellar brain inhibition in the target and surround muscles during voluntary tonic activation. Eur J Neurosci 2016;43:1075–81. https://doi.org/10.1111/ejn.13211.

Pogosyan A, Gaynor LD, Eusebio A, Brown P. Boosting cortical activity at Beta-band frequencies slows movement in humans. Curr Biol 2009;19:1637–41. https://doi.org/10.1016/j.cub.2009.07.074.

Pollok B, Boysen A-C, Krause V. The effect of transcranial alternating current stimulation (tACS) at alpha and beta frequency on motor learning. Behav Brain Res 2015;293:234–40. https://doi.org/10.1016/j.bbr.2015.07.049.

Rao SM, Harrington DL, Haaland KY, Bobholz JA, Cox RW, Binder JR. Distributed neural systems underlying the timing of movements. J Neurosci 1997;17:5528–35. https://doi.org/10.1523/JNEUROSCI.17-14-05528.1997.

Reato D, Rahman A, Bikson M, Parra LC. Effects of weak transcranial alternating current stimulation on brain activity-a review of known mechanisms from animal studies. Front Hum Neurosci 2013;7:687. https://doi.org/10.3389/fnhum.2013.00687.

Rossi S, Antal A, Bestmann S, Bikson M, Brewer C, Brockmöller J, et al. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: expert guidelines. Clin Neurophysiol 2021;132:269–306. https://doi.org/10.1016/j.clinph.2020.10.003.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Clin Neurophysiol 2015;126:1071–107. https://doi.org/10.1016/j.clinph.2015.02.001.

Ruigrok TJH. Ins and outs of cerebellar modules. Cerebellum 2011;10:464–74. https://doi.org/10.1007/s12311-010-0164-y.

Saito K, Otsuru N, Yokota H, Inukai Y, Miyaguchi S, Kojima S, et al. α-tACS over the somatosensory cortex enhances tactile spatial discrimination in healthy subjects with low alpha activity. Brain Behav 2021;11:e02019.

Santarnecchi E, Muller T, Rossi S, Sarkar A, Polizzotto NR, Rossi A, et al. Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. Cortex 2016;75:33–43. https://doi.org/10.1016/j.cortex.2015.11.003.

Shin S-L, Rotter S, Aertsen A, De Schutter E. Stochastic description of complex and simple spike firing in cerebellar Purkinje cells. Eur J Neurosci 2007;25:785–94. https://doi.org/10.1111/j.1460-9568.2007.05308.x.

Sondergaard RE, Strzalkowski NDJ, Gan LS, Jasaui Y, Furtado S, Pringsheim TM, et al. Cerebellar brain inhibition is associated with the severity of cervical dystonia. J Clin Neurophysiol 2023;40:293–300. https://doi.org/10.1097/WNP.0000000000000884.

Spampinato D, Avci E, Rothwell J, Rocchi L. Frequency-dependent modulation of cerebellar excitability during the application of non-invasive alternating current stimulation. Brain Stimul 2021;14:277–83. https://doi.org/10.1016/j.brs.2021.01.007.

Spampinato DA, Celnik PA, Rothwell JC. Cerebellar-motor cortex connectivity: one or two different networks? J Neurosci 2020;40:4230–9. https://doi.org/10.1523/JNEUROSCI.2397-19.2020.

Sugata H, Yagi K, Yazawa S, Nagase Y, Tsuruta K, Ikeda T, et al. Modulation of motor learning capacity by transcranial alternating current stimulation. Neuroscience 2018. https://doi.org/10.1016/j.neuroscience.2018.09.013.

Tanaka M, Kunimatsu J, Suzuki TW, Kameda M, Ohmae S, Uematsu A, et al. Roles of the cerebellum in motor preparation and prediction of timing. Neuroscience 2021;462:220–34. https://doi.org/10.1016/j.neuroscience.2020.04.039.

Tzvi E, Gajiyeva L, Bindel L, Hartwigsen G, Classen J. Coherent theta oscillations in the cerebellum and supplementary motor area mediate visuomotor adaptation. Neuroimage 2022;251:118985. https://doi.org/10.1016/j.neuroimage.2022.118985.

Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. Ann Neurol 1995;37:703–13. https://doi.org/10.1002/ana.410370603.

Wessel MJ, Draaisma LR, de Boer AFW, Park C-H, Maceira-Elvira P, Durand-Ruel M, et al. Cerebellar transcranial alternating current stimulation in the gamma range applied during the acquisition of a novel motor skill. Sci Rep 2020;10:11217. https://doi.org/10.1038/s41598-020-68028-9.

Witkowski M, Garcia-Cossio E, Chander BS, Braun C, Birbaumer N, Robinson SE, et al. Mapping entrained brain oscillations during transcranial alternating current stimulation (tACS). Neuroimage 2016;140:89–98. https://doi.org/10.1016/j.neuroimage.2015.10.024.

Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol 2015;126:1847–68. https://doi.org/10.1016/j.clinph.2014.08.028.