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RESEARCH

Cerebellar Direct Current Stimulation Reveals the Causal Role of the Cerebellum in Temporal Prediction

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Abstract

Temporal prediction (TP) influences our perception and cognition. The cerebellum could mediate this multi-level ability in a context-dependent manner. We tested whether a modulation of the cerebellar neural activity, induced by transcranial Direct Current Stimulation (tDCS), changed the TP ability according to the temporal features of the context and the duration of target interval. Fifteen healthy participants received anodal, cathodal, and sham tDCS (15 min × 2 mA intensity) over the right cerebellar hemisphere during a TP task. We recorded reaction times (RTs) to a target during the task in two contextual conditions of temporal anticipation: rhythmic (i.e., interstimulus intervals (ISIs) were constant) and single-interval condition (i.e., the estimation of the timing of the target was based on the prior exposure of the train of stimuli). Two ISIs durations were explored: 600 ms (short trials) and 900 ms (long trials). Cathodal tDCS improved the performance during the TP task (shorter RTs) specifically in the rhythmic condition only for the short trials and in the single-interval condition only for the long trials. Our results suggest that the inhibition of cerebellar activity induced a different improvement in the TP ability according to the temporal features of the context. In the rhythmic context, the cerebellum could integrate the temporal estimation with the anticipatory motor responses critically for the short target interval. In the single-interval context, for the long trials, the cerebellum could play a main role in integrating representation of time interval in memory with the elapsed time providing an accurate temporal prediction.

Keywords Temporal prediction · Cerebellum · tDCS · Rhythmic · Single-interval · Implicit timing

Introduction

Humans extract temporal regularities from the environment allowing the creation of temporal expectations to interact in an adaptive way with the world [1]. Temporal expectation also known as temporal prediction influences perception and cognition. Indeed, our brain combines recurrent temporal features in a stream of information to optimize the selection of relevant events and to predict their properties [2]. Electroencephalographic studies have shown that temporal expectation is closely related to a modulation of anticipatory neural activity associated with attentional control and motor preparation [3–5]. It has been reported that when a sensory event has a periodic or rhythmic temporal structure (e.g., speech or music), its processing is facilitated [5, 6]. In addition to rhythmic regularities, temporal prediction can be based on the association of temporal relations between single intervals [6–8]. Given an example, it is common experience to automatically shift our attention and estimate the precise time when a traffic light turns from yellow to green [7]. In this sense, the estimation of the temporal occurrence of an event (e.g., a traffic light that turns green) is driven by a previous exposure to the specific time interval that builds a memory template [6, 8]. Such assumption has been supported by studies emphasizing the strong connection between attention, time, and memory [9–12].

In addition to this context-dependent difference (rhythmic vs associations-based temporal context), a further property of temporal prediction is to be based on implicit processes

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[7]. Indeed, temporal prediction requires using temporal information in the absence of explicit instructions to use it. For this reason, this automatic estimation of time information has been described as implicit timing, also known as anticipation of event timing or future-oriented attending [4, 13, 14]. Functional magnetic resonance imaging (fMRI) [15–17] and non-invasive brain stimulation techniques [18, 19] have identified the neuroanatomical networks involved in temporal prediction. fMRI investigations have revealed a critical involvement of action-related and attention-related areas, including premotor cortex, inferior parietal cortex, and cerebellum [20–24]. As an example, when temporal information inherent to the spatial–temporal trajectory of a dynamic visual stimulus is used to predict its final position, fMRI studies revealed activation in different cortical areas and the cerebellum [25–27].

The role of the cerebellum in predictive timing has also been highlighted by clinical observations and neurophysiological investigations. Patients with cerebellar dysfunction, such as those with cerebellar ataxia, are impaired in the temporal control of movement (e.g., saccadic dysmetria and dysarthria) [28]. In healthy individuals, perturbing cerebellar activity using repetitive transcranial magnetic stimulation (1-Hz rTMS) induced altered temporal prediction in a task requiring predicting the outcome of a perceived movement (i.e., handwriting) [29].

Recently, Breska and Ivry [24] went further, providing neuropsychological evidence for a double dissociation in neural processes underpinning temporal prediction depending on the context. Specifically, they have shown that patients with Parkinson's disease, mainly involving the degeneration of the nigrostriatal system of the basal ganglia, were impaired in the formation of temporal predictions in a rhythmic context but not in a single-interval one. Conversely, patients with cerebellar degeneration were compromised exclusively in building temporal prediction based on single-interval association. This context-dependent (rhythmic versus single-interval) specific role of the cerebellum in a temporal prediction task has not been directly investigated in healthy subjects so far.

To address this issue, in this study, we tested whether modulation of the cerebellar neural activity, by means of cerebellar transcranial Direct Current Stimulation (tDCS), selectively altered the temporal prediction ability differently according to the temporal features of the context (rhythmic vs single-interval) and the target duration interval (short vs long). Fifteen healthy subjects participated in three sessions of tDCS (anodal, cathodal, and sham) during a temporal prediction implicit task (adapted by [24]).

Cerebellar tDCS is a non-invasive technique for inducing prolonged functional changes in the human cerebellum. Cerebellar tDCS could interfere with membrane polarisation in Purkinje cells and in other neurons of the cerebellum, fibres (mossy fibres and climbing fibres), and glial cells. DC stimulation applied to the cerebellar cortex in the decerebrated cat influences Purkinje and granular cell activity in a polarity-specific manner; while anodal DC (0.1–1 mA) flowing in the dendrite–axonal direction increases tonic neuronal activity, cathodal DC decreases it [30].

From a functional point of view, cerebellar tDCS has been used to explore the role of the cerebellum in a variety of tasks ranging from motor functions (e.g., motor adaptation) to non-motor functions (e.g., cognitive functions) [31]. A recent meta-analysis [32] has underlined that cerebellar tDCS is effective in inducing behavioral changes in both motor and non-motor functions. However, the polarity of tDCS still cannot reliably forecast the direction of behavioral changes. Hence, in some experiments, both polarities induced the same effects [31]. In a comprehensive context, it is widely thought that anodal stimulation improves motor and cognitive functions, while cathodal stimulation disrupts functioning (*see* [33]). As an example, an improvement in motor skill learning has been found in a study selectively for anodal tDCS stimulation in contrast to sham or cathodal tDCS [34]. However, in several studies, cathodal stimulation is consistently linked with neurobehavioral effects associated with an enhancement in cerebellar functioning [35–43]. Furthermore, studies using anodal stimulation have also demonstrated compromised cerebellar functioning due to the stimulation [40, 44–47]. Given the heterogeneity in the stimulation polarity effect, it's crucial to recognize that the impact of stimulation is intricately linked to subprocesses that occur in the cortico-pontine-cerebellar-thalamo-cortical circuit associated with the cognitive or motor mechanism under investigation [32, 48]. Pope and co-workers investigated whether modulating the activity of the cerebellum using DC stimulation could influence performance in cognitive tasks that have previously been shown to activate the cerebellum in an MR scanner [30]. Results showed a facilitatory effect of cathodal tDCS (relative to anodal and sham stimulation) on participants' performance in neuropsychological tests assessing arithmetic and language aspects of working memory and attention [37]. The authors speculated that the cerebellum could release cognitive resources in working memory regions of the cortex by disinhibition of the dorsolateral prefrontal cortex: cathodal cerebellar tDCS would hyperpolarize cerebellar cortex, reducing the Purkinje cell outputs which normally exert an inhibitory tone on the cerebral cortex [49]. Indeed, the dorsolateral prefrontal cortex is engaged in working memory and attention, both cognitive functions critical for constructive processes underpinning temporal prediction [2].

Consistent with the existing literature and considering the specific demands of our task, it is reasonable to posit that inhibiting the cerebellum through cathodal tDCS may enhance temporal prediction ability. This enhancement is

anticipated through the modulation of circuits associated with attention and working memory, which are crucial to temporal prediction. The opposite effect resulting from anodal tDCS with the same task demands has not been demonstrated [37]. However, also considering the heterogeneity of polarity effect results, we cannot make a strong a priori hypothesis about the directionality of tDCS stimulation. Hence, based on the role of cerebellum in single-interval based temporal prediction [24], we expect that the major effects will be observed when cerebellum is modulated during single-interval temporal prediction task. Our results could support the role of the cerebellum during implicit temporal prediction in a context-dependent manner [50].

Materials and Methods

Main Experiment

Participants

Fifteen participants took part in the study (mean age ± SD: 24.4 ± 5.2 years). Participants reported normal or corrected-to-normal vision, intact color vision, and no professional musical training or engagement in amateur-level musical activities in the 3 years before testing [5]. All participants were naïve to the purposes of the experiment and provided written informed consent. Prior to the experimental session, all participants were screened for any general contraindications to non-invasive brain stimulation [51, 52] using a safety-screening questionnaire [53] and to a general questionnaire to investigate for neurological, psychiatric, or other medical problems. None of the participants had neurological, psychiatric, other medical problems or any contraindication to tDCS [51]. All participants were right-handed according to the standard handedness inventory [54]. The experimental protocol was approved by the ethics committee at the University of Genoa and was carried out in agreement with legal requirements and international norms stated in the adjourned declaration of Helsinki (2001) [55]. The sample size for our within-subjects experiment was determined a priori considering the mean sample size of previous studies reported in a recent meta-analysis on the tDCS effects on non-motor functions [32] and a recent work by Clausi et al. [56] on the effect of cerebellar tDCS on reaction times at a psychophysiological task.

Procedure

The experiment took place in a quiet room, participants sat comfortably at 60 cm from the screen. Stimuli presentation and response acquisition were controlled using E-Prime software (E-Prime 3.0, www.pstnet.com/eprime). Upon arrival, participants provided written informed consent and completed the State-Trait Anxiety Inventory Questionnaire (STAI: [57]), used as a manipulation check for the presence of evaluation apprehension, in each experimental condition [58]. All subjects were submitted to three tDCS conditions (anodal, cathodal, and sham) over the right cerebellum, on three different days, 1 week apart (7 ± 2 days). In the experimental sessions, tDCS neuromodulation started 5 min before the temporal prediction task and continued for the entire duration of the task (15 min) (Fig. 1).

Temporal Prediction (TP) Task

Participants performed a temporal prediction task adapted to that proposed by Breska and Ivry [24]. The stimuli consisted of filled squares (green or red) that appeared on a 17-inch computer screen (~3.5° visual angle per side) for 100 ms. Each trial started with two or three red squares, followed by a fixation cross (served as a Warning Signal (WS)), and then a green square (served as a target).

Two different trials were included in the task based on the interval between the WS and the target appearance: (i) short trial (600 ms interval) and (ii) long trial (900 ms interval). Participants were asked to press with the right hand the spacebar on the keyboard as quickly as possible once the target appeared (Fig. 1**).** The trials were divided into blocks that differed in their temporal structure: rhythmic and single-interval. In the rhythmic block, the interstimulus intervals (ISIs) between all stimuli were the same as the WS-target interval (i.e., interval between the fixation cross and the target); in this condition, the target timing was fully predictable. The single-interval block consisted of only two red squares, instead of three, and the interval between the last stimulus before the warning signal and the warning signal was randomly jittered with a mean that was 2.5 times the WS-target interval (− 13.3%, − 6.6%, 0%, + 6.6%, + 13.3 of 1.500 or 2.250 ms for short and long trials, respectively, uniform distribution). In this block, the rhythmicity of stimuli train was reduced and the estimation of the timing of the target was based on the prior exposure of the stream of red squares.

Before the experimental session, participants performed a practice session of the TP task. The practice session encompassed two blocks (rhythmic and single-interval block) including short and long trials (8 trials for each duration). After the practice session, the experimental session started. It included two blocks (rhythmic and single-interval) with short and long trials. Each block consisted of 32 trials (i.e., 32 for rhythmic and 32 for single interval conditions), 16 with the short interval and 16 with the long interval. The order of the block and the order of presentation of short and long trials were randomized across participants. Within the two intervals, four trials for each duration (25%) were

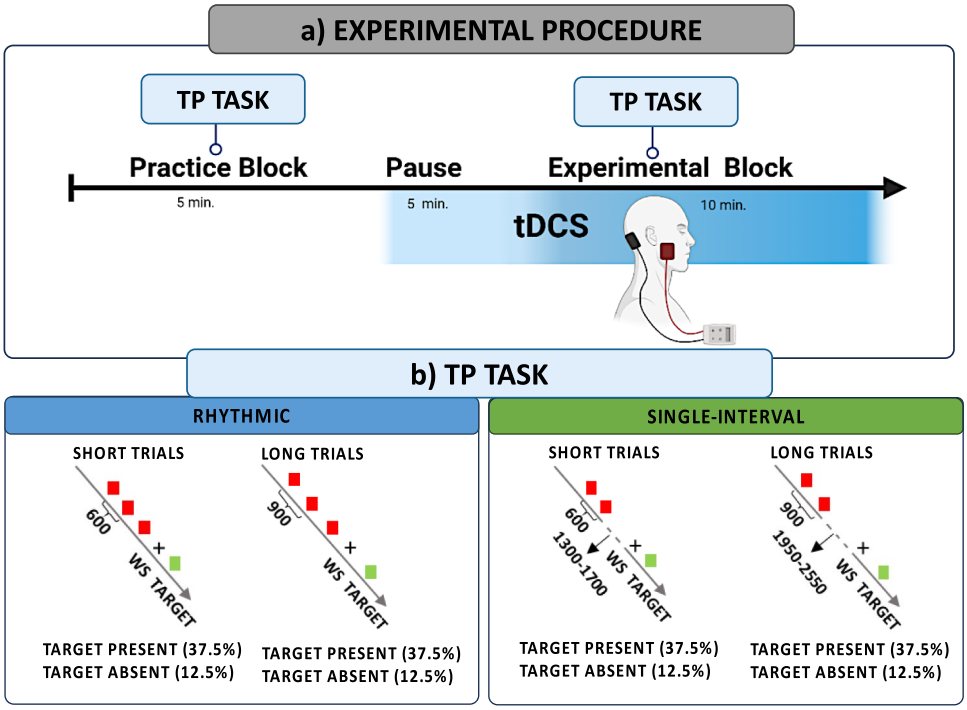

Fig. 1 Schematic representation of the experimental procedure (**a**) and the experimental task (i.e., Temporal Prediction (TP) task) (**b**). tDCS, transcranial direct current stimulation; WS, warning signal

catch trials (4 short and 4 long) to minimize the anticipatory responses. Each time participants responded too late (3 s from target onset), too early (before the onset of the target) or they responded on a catch trial, a feedback message was sent to the screen.

Transcranial Direct Current Stimulation (tDCS)

tDCS was applied using a battery-driven direct current stimulator (BrainSTIM, E.M.S. s.r.l. Italy) through a pair of sponge surface saline-soaked electrodes (25 cm^2). The electrodes were held in position on the scalp with elastic rubber bands and an electroconductive paste was applied to reduce contact impedance [31]. All subjects received 15 min of anodal, cathodal, and sham tDCS in three different sessions, 1 week apart (7 ± 2 days). The order of the stimulation sessions was randomized and counterbalanced across subjects. In the active conditions (anodal and cathodal), the intensity of stimulation was set at 2 mA (current density of 0.08 mA/cm^2), with a 30 s of ramp up and ramp down, complying with safety recommendations [59]. For anodal stimulation, the anode was placed 3 cm laterally to the inion (I2 according to the 10/20 EEG system) over the right cerebellar hemisphere and the cathode was placed over the ipsilateral buccinator muscle [49]. For cathodal stimulation, the opposite montage was used (i.e., anode over the buccinator muscle and cathode over the cerebellar hemisphere). This tDCS setup has proved suitable to effectively stimulate the cerebellum [31, 49, 59, 60].

For the sham condition, no current was delivered in the stimulation sites except for 10 s of ramp up and ramp down. Thus, the participants experienced the sensation initially associated with the onset of stimulation (mild local tingling), without producing effects on brain excitability [61]. Figure 2 represents tDCS electrodes montage used and the computational model of voltage distribution of electric field induced by tDCS. The simulation of electric field distribution was performed with SimNIBS software [62] using a realistic head model (called "Ernie")

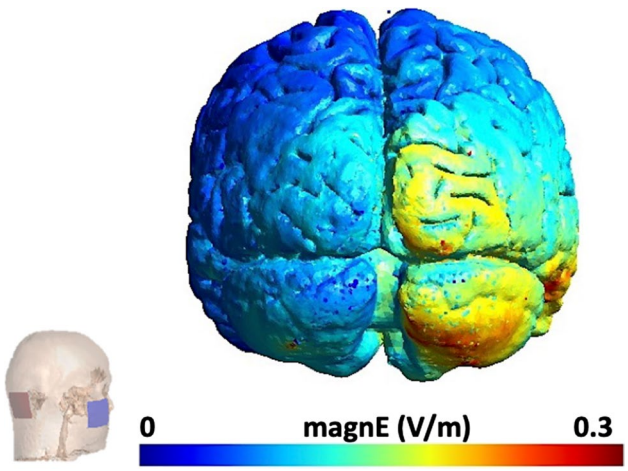

Fig. 2 Transcranial direct current stimulation (tDCS) montage and the computational model of voltage distribution of electric field induced by tDCS. Simulations were performed with SimNIBS software [62]

provided by SimNIBS (SimNIBS4: www.simnibs.org) as an example dataset. In the model, the conductivities for different tissue segments were set using standard values: ($\sigma_{WM} = 0.126$ S/m, $\sigma_{GM} = 0.275$ S/m, $\sigma_{CSF} = 1.654$ S/m, $\sigma_{scalp} = 0.465$ S/m, $\sigma_{skull} = 0.01$ S/m, σ_{eye} balls = 0.500 S/m, $\sigma_{electrode}$ rubber = 29.4 S/m, $\sigma_{sponge/gel} = 1.0$ S/m) [63]. In keeping with the tDCS montage used, the simulation of the spatial distribution of the electric field showed an accurate propagation of the stimulation over the right cerebellar hemisphere but a weak focality in the stimulation.

Data Analysis

We collected the reaction time (RTs) in response to the target. RTs shorter than 100 ms or longer than 3000 ms were discarded (4% of trials, no difference between conditions). Then, RTs greater or less than two SDs above or below the mean RTs has been removed (2% of trials, no between difference conditions).

Statistical Analysis

Firstly, we used the Shapiro–Wilk test to evaluate if all variables were normally distributed and the Mauchly's test to verify if the sphericity was respected. Then, to verify that baseline anxiety was not different among sessions, we carried out a repeated measure ANOVA (RM ANOVA) with Session (3 levels: Sham, Anodal and Cathodal) as within-factors on STAI Y-1 scores.

Mean RTs data were analysed via a $3 \times 2 \times 2$ RM ANOVA with Stimulation (3 levels: Sham, Anodal and Cathodal), Task (2 levels: Rhythmic and Single-interval) and Duration (2 levels: Short and Long) as within factors. tDCS induced formation of temporal predictions in each of the predictive conditions should be expressed in faster RTs relative to the sham condition. Statistical analysis was performed using SPSS Statistics 23.0 (IBM, Somers, USA). The significant level was set at 0.05 and significant interactions were analysed using post hoc comparisons with Bonferroni correction for multiple comparisons.

Supplemental Experiment

Eight participants took part in the supplemental experiment (mean age ± SD: 26 ± 4.1 years). Sample size was calculated based on data from the main experiment. To obtain a 95% chance of detecting a significant decrease of RT after cathodal with respect to sham stimulation at $\alpha = 0.01$, 8 participants are required. They were submitted to two sessions of tDCS conditions (cathodal and sham), 1 week apart (7 ± 2 days), during a modified version of the TP task. The tDCS montage and the experimental procedure were the same as the main experiment. To explore issues related to the specificity of the target intervals used in the TP task within the main experiment (600 ms for short and 900 ms for long trials) we adopted a modified version of TP. Hence, we used two different durations of the interval between the WS and the target: 500 ms (short trials) and 1000 ms (long trials). As the main experiment, the trials were divided into two blocks: rhythmic and single-interval. In the rhythmic block, the interstimulus intervals (ISIs) between all stimuli were the same as the WS-target interval (500 or 1000 ms). In the single-interval block, the interval between the last stimulus before the warning signal and the warning signal was randomly jittered with a mean that was 2.5 times the WS-target interval (− 13.3%, − 6.6%, 0%, + 6.6%, + 13.3% of 1.250 or 2.500 ms for short and long trials, respectively, uniform distribution). Like the main experiment, participants were required to press the space bar, with the right hand, as quickly and accurately as possible when they saw the target (i.e., green square). Before the experimental session, participants performed a practice session of the TP task. The number of trials of the practice and experimental sessions was the same as the main experiment.

Data Analysis

The data analysis was carried out in the same way as the main experiment.

Statistical Analysis

We used the Shapiro–Wilk test to evaluate if all variables were normally distributed and the Mauchly's test to verify if the sphericity was respected. In the supplemental experiment, mean RTs data were analyzed via a $2 \times 2 \times 2$ RM ANOVA with Stimulation (2 levels: Sham and Cathodal), Task (2 levels: Rhythmic and Single-interval), and Duration (2 levels: Short and Long) as within factors.

Comparison Between Main and Supplemental Experiments

To compare data obtained from main and supplemental experiments, we calculated the percentage of improvement in the performance respect to the baseline (i.e., sham) in the cathodal tDCS sessions, by extracting an index of the percentage of RTs decrease as follows: {[(Mean RTs CATHODAL tDCS – Mean RTs SHAM)/ Mean RTs SHAM] *100}. Mean RTs index data were analysed via mixed RM ANOVA with Task (2 levels:

Rhythmic and Single-interval) and Duration (2 levels: Short and Long) as within factors and Experiment (2 levels: main and supplemental) as between subjects factor.

Results

Main Experiment

State-Trait Anxiety Inventory Questionnaire (STAI)

Participants reported average STAI Y-1 scores of 31.5 ± 8.9 (CATHODAL session), 30.3 ± 9.3 (ANODAL session) and 31.9 ± 8.6 (SHAM session). The RM ANOVA revealed no significant effect of the Session [$F_{(2,28)} = 2.105$; $p = 0.141$; $p\eta^2 = 0.39$] indicating no significant difference in the STAY Y-1 scores across the experimental conditions.

Reaction Times

No discomfort or adverse effects during tDCS were reported or noticed in any of the participants. Reaction times data are shown in Figs. 3 and 4. The RM ANOVA on mean RTs revealed a significant effect of Stimulation [$F_{(2,28)} = 6.71$; $p = 0.004$; $p\eta^2 = 0.32$], of Task [$F_{(1,14)} = 4.71$; $p = 0.045$; $p\eta^2 = 0.25$] and a significant interaction Stimulation × Task × Duration [$F_{(2,28)} = 3.34$; $p = 0.045$; $p\eta^2 = 0.19$]. Post hoc analysis of the main effects revealed that RTs during the cathodal tDCS condition (322.6 ± 10.9) were decreased compared to the sham stimulation (347.9 ± 11.7; $p = 0.01$) (Fig. 3a). Furthermore, RTs were shorter in the rhythmic condition (327.2 ± 10.1) compared to the single-interval one (343.4 ± 12.5; $p = 0.048$) (Fig. 3b).

Post hoc analysis of the Stimulation × Task × Duration interaction showed that cathodal tDC induced a significant improvement of performance during the TP task, accounted for by faster RTs, specifically in the rhythmic block only for the short ISIs trials (306.4 ± 10.3, $p = 0.01$) compared

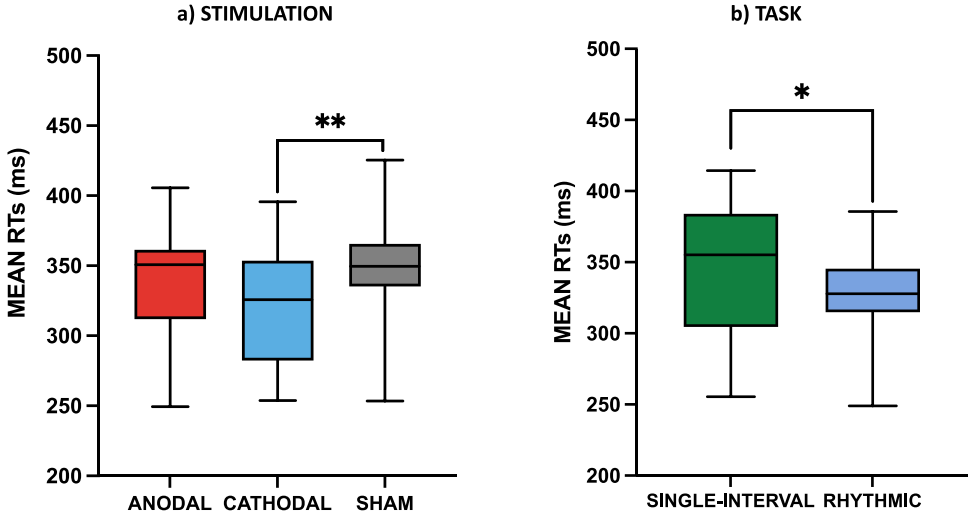

Fig. 3 Mean Reaction times (RTs) data. Results of the main effect of Stimulation (**a**) and Task (**b**). RTs were shorter in the cathodal stimulation compared to sham stimulation (a) and in the rhythmic condition compared to the single-interval one. * $p \leq 0.05$, ** $p \leq 0.01$. The values plotted are the minimum and the maximum (whiskers), the median, the 25th percentile and the 75th percentile (hinges)

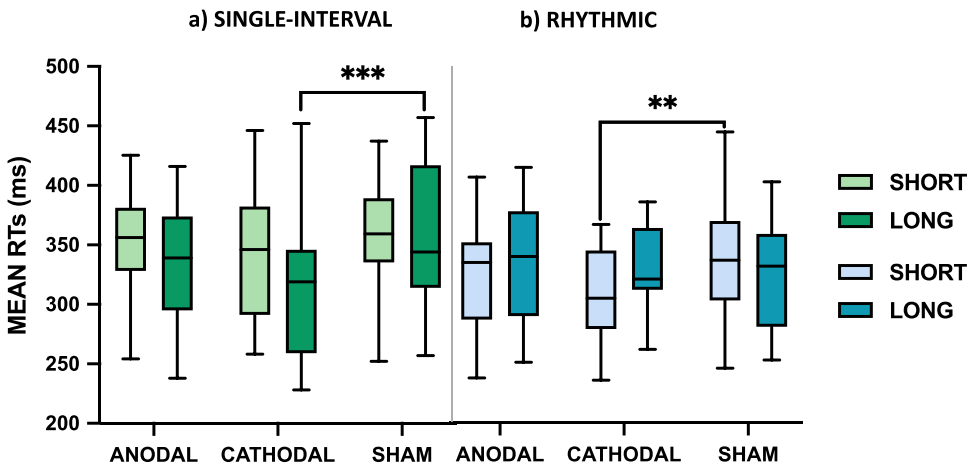

Fig. 4 Mean Reaction times (RTs) data. Results of the interaction Stimulation × Task × Duration, showing a significant reduction of RTs during the TP task selectively for cathodal tDCS in the rhythmic condition for the short trials (**b**) and for the single-interval condition for the long trials (**a**). ** $p \leq 0.01$, *** $p \leq 0.001$. The values plotted are the minimum and the maximum (whiskers), the median, the 25th percentile and the 75th percentile (hinges)

to sham (339.2 ± 14.1) (Fig. 4). Furthermore, a significant improvement of performance during the TP task was found during the cathodal tDCS, compared to sham (353.8 ± 15.3), in the single-interval block only for the long ISIs (320 ± 15.4, $p = 0.003$) (Fig. 4).

No other main effects or interactions were significant in the ANOVA (all $p > 0.05$).

Supplemental Experiment

Reaction Times

No discomfort or adverse effects during tDCS were reported or noticed in any of the participants. The RM ANOVA on mean RTs revealed a significant main effect of Duration [$F_{(1,7)} = 8.50$; $p = 0.02$; $p\eta^2 = 0.59$] and a significant three-way Stimulation × Task × Duration interaction [$F_{(1,7)} = 11.04$; $p = 0.01$; $p\eta^2 = 0.61$]. Post hoc analysis of the main effect revealed that RTs were shorter in the short trials compared to the long ones (245.1 ± 8.5, 252.8 ± 7.4). Most importantly, the post hoc analysis of the interaction showed, like the main experiment, a different improvement in TP task performance, during the cathodal tDCS, according to the temporal features of the block and the target interval. Specifically, RTs were significantly decreased, in the cathodal tDCS session, in the single interval only for the long ISIs compared to the sham one (248.0 ± 9.10, 265.90 ± 9.4, $p = 0.04$). In the rhythmic block, we found a trend for a decrease of the RTs, specifically in the short ISIs trials, in the active session of stimulation compared to the sham one (225 ± 5.1, 242 ± 14.3, $p = 0.08$) (Fig. 5). No other main effects or interactions were significant in the ANOVA (all $p > 0.05$).

Comparison Between Main and Supplemental Experiments

The mixed ANOVA on the mean RTs index revealed a significant Task × Duration interaction [$F_{(1,21)} = 23.35$; $p < 0.001$; $p\eta^2 = 0.55$]. Post hoc analysis of the interaction revealed that in the rhythmic condition, the RTs were significantly decreased in the short trials compared to the long ones (Main experiment: short, − 7.66 ± 2.48, long, − 3.14 ± 3.05; Supplemental experiment: short, − 5.02 ± 4.01, long, − 1.44 ± 5.18, $p = 0.014$). In the single-interval condition, RTs were significantly decreased in the long trials compared to the short ones (Main experiment: long, − 11.09 ± 2.10, short, − 6.30 ± 1.93; Supplemental experiment: long, − 6.41 ± 2.70, short, 0.9 ± 1.7, $p < 0.01$) (Fig. 6). No other main effects or interactions with the Experiment were significant in the ANOVA (all $p > 0.05$), showing the same direction of effect in both experiments.

Discussion

In this study, we investigated the role of the cerebellum in the temporal prediction ability in two temporal contexts (rhythmic and single-interval), with different time intervals between the events (short, 600 ms and long, 900 ms). Specifically, we explored whether modulation of cerebellum excitability, via tDCS, influences temporal prediction. To achieve this goal, anodal, cathodal, and sham tDCS were used to perturb the cerebellar activity in healthy subjects during the temporal prediction task. We hypothesized that the cerebellum may play a different role in temporal prediction depending on the temporal context (rhythmic and single-interval).

First, we found that cathodal tDCS resulted in reduced RTs in the temporal prediction task compared to sham and anodal tDCS conditions, suggesting that the inhibition of the cerebellar activity improves the ability to predict the occurrence of the target within a temporal context. Cerebellar tDCS has been proven to modulate the cerebellar excitability by altering the activity of the Purkinje cells [48, 49, 60, 61, 63]. A study by Galea and co-workers

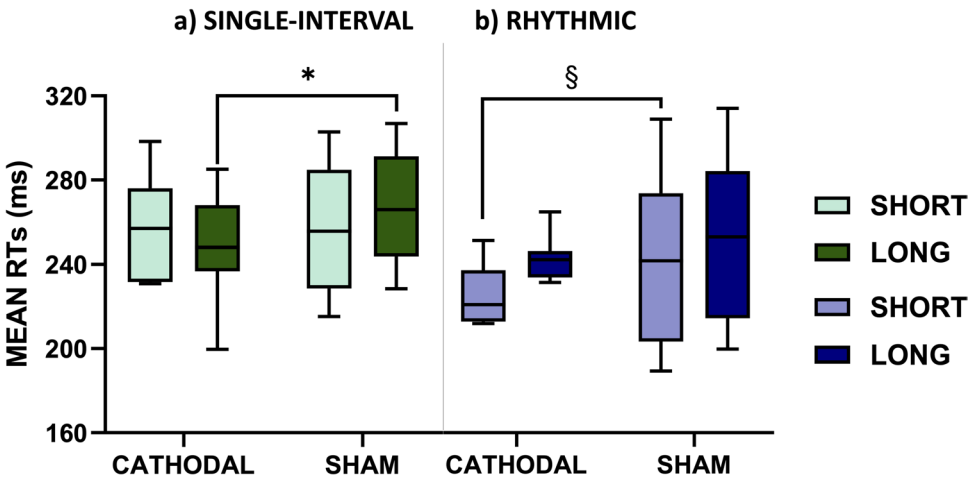

Fig. 5 Mean Reaction times (RTs) data. Results of the interaction Stimulation × Task × Duration, showing a significant reduction of RTs during the TP task selectively for cathodal tDCS in the rhythmic condition for the short trials (**b**) and for the single-interval condition for the long trials (**a**). * $p \leq 0.05$, § $p \leq 0.08$. The values plotted are the minimum and the maximum (whiskers), the median, the 25th percentile and the 75th percentile (hinges)

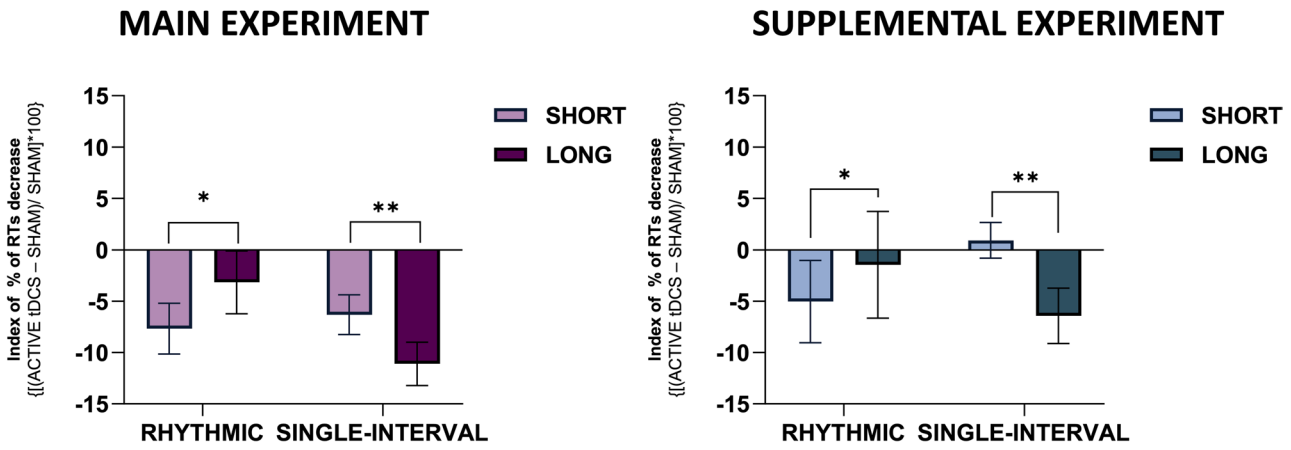

Fig. 6 Mean reaction time index data. Results of the interaction Task × Duration, showing a significant reduction of RTs during the TP task in the rhythmic condition for the short trials with respect to long and a significant reduction of RTs in the single-interval condition in the long trials respect to the short, in both the main and the supplemental experiments. *$p \leq 0.05$, ** $p \leq 0.01$. Error bars represent the mean standard error

[49] demonstrated that cathodal cerebellar tDCS decreased cerebellar brain inhibition (CBI), whereas anodal tDCS increased it. CBI is a reliable index of the activation of cerebellar Purkinje cells and the subsequent inhibition of dentate–thalamo–cortical pathway [64]. In the literature, some studies have shown that cathodal tDCS improved task performance across cognitive and motor domains, while others have shown the opposite or no effect (for reviews, see [33, 65]).

Cathodal stimulation to the cerebellum has improved task performance on verbal working memory [37] and inhibition tasks [66, 67], but has also resulted in performance decrements [44, 68] and increased response variability [69]. It should also be noted that there is literature demonstrating no effects of stimulation on cognitive task domains including working memory [70, 71], and inhibition [70]. When cathodal tDCS improved cognitive functions, it has been proposed that cerebellar inhibition was capable of facilitating prefrontal cortex activity [37]. Connections between the cerebellum and prefrontal regions of the cortex are via the inhibitory Purkinje cells of the cerebellar cortex. Thus, cathodal stimulation is expected to inhibit this inhibitory output from the cerebellum to the prefrontal cortex, making the latter region (typically associated with working memory functions and attention) more active, which can partly explain the facilitatory effects of tDCS on cognition. Our findings go along with these results, showing that cerebellar cathodal tDCS induces an improvement in temporal prediction performance. Indeed, prefrontal cortex is critical for temporal prediction functions, also by engaging working memory and attention processes [2]. No effects were observed following sham or anodal tDCS, suggesting that such modulation couldn't be attributed to a non-specific manipulation effect.

In addition, to control the effect of individual baseline variables, such as anxiety or evaluation apprehension, over reaction time performance, we administered the STAI questionnaire to the participants before each experimental session. The STAI score results reveal no difference between the three sessions of stimulation suggesting that the improvement observed following cathodal tDCS could not be explained by a general modulation of anxiety and therefore arousal in such session of stimulation.

Second, we found that reduced RTs in the temporal prediction task during cathodal cerebellar tDCS were specifically observed in the single-interval context for long trials (900 ms) and in the rhythmic context for short trials (600 ms). This finding was unexpected. Indeed, both the durations tested in the main experiment of this study fall within the range typically associated with cerebellum activity [72] and past work such as that by Breska and colleagues (2018) showed similar effects for stimuli in this range [24] and we a priori expected to find the same modulation of task performance only in the single-interval condition but no differences between the interval durations. These findings instead suggest that the cerebellum is particularly active (and susceptible to tDCS modulation in healthy subjects) in processing rhythmic information at short sub-second time intervals and memory-based information at long sub-second time intervals. We performed a supplemental experiment to strengthen our unexpected findings related to differences between interval conditions. In the supplemental experiment, we explored the role of cathodal tDCS in modulating cerebellar activity during the same temporal prediction task adopted in the main experiment, involving both rhythmic and single-interval conditions, but with different time intervals: a short time interval of 500 ms (instead of 600 ms

adopted in the main experiment) and a long time interval of 1000 ms (instead of 900 ms). If consistent with the results of the main experiment, we expect to find a Stimulation × Task × Duration interaction with tDCS-induced task performance modulation for the rhythmic condition at 500 ms and for the single-interval condition at 1000 ms. The results of the supplemental experiment confirmed this hypothesis. Indeed, we found a different improvement in temporal prediction task performance, during the cathodal tDCS, according to the temporal features of the block and the target interval. Specifically, RTs were significantly decreased, in the cathodal tDCS session, in the single interval only for the long ISIs (1000 ms). In the rhythmic block, we found a consistent decrease of the RTs, specifically in the short ISIs trials (500 ms), in the active session of stimulation compared to the sham one. It is noteworthy to underlie that there was a difference in raw reaction times between the two experiments, with RT in the main experiments longer with respect to supplemental experiment. Such difference could be attributable to different inter-stimulus intervals adopted in the two experiments (Main experiment: 600 and 900 ms, Supplemental experiment: 500 and 1000 ms). We can speculate that the greater difference between the two intervals in the supplemental with respect to main experiment (500 ms and 300 ms in the supplemental and main experiment respectively) makes interstimulus intervals more easily distinguishable in the supplemental experiment, improving the temporal expectancy of the target and response time. Indeed, the discrepancy between the experiments may align with the literature indicating that temporal expectation modulates our perception and attention, thereby influencing motor readiness to stimuli [2]. Furthermore, the use of different intervals (in the sub-second range) between the two experiments allowed us to conclude that such contextual modulation of the cerebellum in the domain of temporal prediction might be an absolute phenomenon in the sub-second range and not a relative one.

These findings support our idea that the cerebellum is dynamically involved in temporal prediction task in both rhythmic and single-interval based predictions, depending on time interval durations.

Furthermore, our results on RT index, where performance under cerebellar cathodal tDCS was normalized with respect to performance under sham stimulation, to minimize inter-individual variability and placebo effects expectable under sham stimulation, confirmed the results obtained with raw data, with normalized RTs that were significantly different between intervals (short vs long), in both contexts (rhythmic and single-interval).

We adopted a temporal prediction task (see [24]) that stressed the distinction between two types of temporal prediction and different durations of time intervals between the events. In the rhythmic-based prediction, temporal anticipation of the timing target relies on endogenous features of the stimuli stream, characterized by regular inter-stimulus intervals. In this case, the target timing was fully predictable. Conversely, in the single-interval based prediction, temporal estimation of the target relied on the encoding of individual intervals between stimuli, facilitating the formation of a memory template. Both predictive mechanisms were deemed essential for preparing behavioral responses [73] and indeed in the paradigm adopted here, temporal prediction ability was tested by measuring reaction time, i.e., by testing the ability to exploit temporal information in fasten motor response.

Our findings appear to contradict the existing body of evidence, and our a-priori hypothesis, that the cerebellum has a unitary role in single-interval prediction [24]. Indeed, previous neuroimaging studies [74, 75] and neuropsychological investigations [24, 76] have reported a double dissociation in patients with cerebellar degeneration between rhythmic and singe-interval timing perception and estimation. Breska and Ivry [24], using a similar task to that adopted in our study, have pointed out that patients with cerebellar degeneration were compromised in forming temporal expectation based on remembered temporal association (single-interval predictions) but not in temporal prediction embedded in a rhythmic context. Differently, our results suggest that the cerebellum could be dynamically involved in two types of predictions: one based on rhythmic information and the other based on remembered temporal associations, depending on the time interval between the events (short interval for the rhythmic based prediction and long interval for the single-interval based prediction). Noteworthy, the study by Breska and Ivry (2018) [24] focused on patients with spinocerebellar ataxia, with an intrinsic heterogeneity in terms of the extent of the pathology on the cortical tissue [77].

To the best of our knowledge, this is the first study showing the role of the cerebellum in exploiting rhythmic temporal information supplied at short sub-second intervals in making implicit temporal prediction. However, our results on the role of the cerebellum in implicit temporal prediction in the sub-second domain may be explained considering the cerebellum's role in explicit rhythmic timing tasks in the same temporal domain and electrophysiological data on the predictive function of the cerebellum [78].

In healthy subjects, the role of the cerebellum in rhythmic timing has been highlighted in different studies. Particularly, activation of the cerebellum has been shown in tasks based on the use of rhythmic temporal information, when temporal information is used in order to represent precise temporal durations through a sustained or a periodic motor act (explicit motor timing task) [15, 74, 79–82]. Noteworthy, in explicit rhythmic timing tasks, the cerebellum has been proven to be particularly active in the sub-second domain [15, 83, 84]. As an example, perturbing cerebellar activity

by means of rTMS interfered selectively when subjects were required to tap their index finger following an auditory cue at a high frequency (2 Hz, corresponding to an interstimulus interval of 500 ms), but not at a low frequency (1 Hz, corresponding to an interstimulus interval of 1000 ms) [82]. Taken together, these works indicate that the cerebellum provides a representation of the precise timing of salient events, determining the onset and offset of movements or the duration of a stimulus mainly in short intervals lasting hundreds of milliseconds [14].

Relative to the predictive function of the cerebellum, physiological evidence from animal studies suggests that a dichotomous separation between basal ganglia and cerebellum between rhythmic and single-based timing perception and estimation is too simplistic and both the subcortical neural structures are involved in both tasks [85]. In a nice review, Tanaka and co-workers [85] report a number of studies showing electrophysiological recordings of both cerebellar neurons and basal ganglia neurons (striatum or caudate) during a variety of timing tasks requiring prediction of periodic event timing or synchronized movements with periodic preparatory activity. Furthermore, a recent EEG study [73] showed that both rhythmic streams of stimuli and memory-based prediction have been associated with an entrainment of low-frequency oscillations that are usually retrievable in response selection processes [86–88]. Thus, to what extent rhythmic and single-interval predictive abilities rely on distinct or overlapping neural mechanisms is still a matter of debate. Taken all together, the above-report evidence suggests that in the rhythmic context, the involvement of the cerebellum could reflect the integration of timing of events with the motor response, in the sub-second domain, providing an accurate temporal estimation for the motor anticipation of the response [14, 85].

The second main finding of our study was the one related to faster RTs in the single-based temporal prediction task, only for long intervals, and following cathodal cerebellar tDCS. It has been demonstrated that remembered-based predictions, as the single-interval adopted here, particularly with long intervals between the two events, are based on cognitive functions, such as attention and memory [12, 89]. For instance, a study by Cravo and colleagues [12] demonstrated that the encoding of temporal associations (with long inter-stimulus intervals, 800 or 2000 ms) in long-term memory facilitated the temporal anticipation of target stimuli. Positron emission tomography (PET) evidence has also shown the involvement of the cerebellum in episodic memory recall, supporting its role in memory retrieval [90]. Long interval trials, due to their extended duration, demand accurate temporal estimation based on the memory encoding and retrieval of temporal features of target interval. Related to single-based prediction, improvements in temporal prediction in the context of stimuli with longer intervals may be explained by a facilitation of cerebellar cognitive functions. Indeed, a facilitation in cognitive performance has been brought to light by studies showing that cathodal cerebellar tDCS would counteract the inhibitory output from the cerebellum to the prefrontal cortex, facilitating information processing by the cortex [37, 49]. Our data further strengthen the hypothesis that in the single-interval context, particularly in long trials, the cerebellum could play a crucial role in integrating the representation of time interval stored in memory with the elapsed time, thereby providing accurate temporal prediction.

Conclusions

Our findings provide novel insight into the causal involvement of the cerebellum in temporal prediction. Additionally, we expand upon previous neuropsychological evidence, further supporting the existence of context-dependent functioning in neural mechanisms underlying temporal prediction. To clarify the role of the cerebellum in temporal prediction has significance not only for this perceptual-cognitive function per se but also to make a step forward in the comprehension of physiopathology of cerebellar disturbances. Indeed, cerebellar damage does not cause loss of movement but instead leads to clear and consistent abnormalities in movement that can include a lack of coordination, increased variability, tremor, and poor accuracy. An altered predictive control can potentially explain these effects of cerebellar damage on many movement types [78]. Thus, to better understand the role of the cerebellum in temporal prediction may be helpful in designing novel rehabilitative strategies in patients affected by cerebellar damage.

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Data Availability Data are available upon request.

Declarations

Ethics Approval The experimental protocol was approved by the ethics committee at the University of Genoa and was carried out in agreement with legal requirements and international norms stated in the adjourned declaration of Helsinki (2001).

Competing Interests S. Terranova, A. Botta, G. Bonassi, M. Putzolu M, C. Cosentino, S. Mezzarobba, E. Ravizzotti has nothing to declare.

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