



# The Cerebellum Modulates Attention Network Functioning: Evidence from a Cerebellar Transcranial Direct Current Stimulation and Attention Network Test Study

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## Abstract

The functional domain of the cerebellum extends beyond its traditional role in motor control. In recent years, this structure has increasingly been considered to play a crucial role even in cognitive performance and attentional processes. Attention is defined as the ability to appropriately allocate processing resources to relevant stimuli. According to the Posnerian model, three interacting networks modulate attentive processes: the alerting, orienting, and executive networks. The aim of this study was to investigate the role played by the cerebellum in the functioning of the attentive networks using the Attention Network Test (ANT). We studied the effects of transcranial direct current stimulation (tDCS), delivered over the cerebellum in cathodal, anodal, and sham sessions, on ANT parameters in healthy subjects. After anodal and sham tDCS, the efficiency of the three attention networks remained stable, and a significant reduction in reaction time (RT) following the task repetition was observed for both congruent and incongruent targets, indicating a learning effect. After cathodal stimulation, instead, while the efficiency of the alerting and orienting networks remained stable, the efficiency of the executive network was significantly reduced. Moreover, a significant reduction in RT was observed for the congruent target alone, with no difference being detected for the incongruent target, indicating that cerebellar inhibition caused an attentive executive dysfunction specifically related to the ability to process complex stimuli in which conflict signals or errors are present. These results point to a role of the cerebellum, a subcortical structure that is thought to affect error processing both directly, by making predictions of errors or behaviors related to errors, and indirectly, by managing the functioning of brain cortical areas involved in the perception of conflicting signals, in the functioning of the attentional networks, particularly the executive network.

**Keywords** Cerebellum · Attention · Attention Network Test · tDCS

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## Introduction

The cerebellum has long been associated with the preparation, the execution, and the modulation of movement [1]. However, in the last decade, numerous studies showed that the cerebellum also plays a role in higher cognitive functions [2–4].

The participation of the cerebellum in higher order brain function is likely to be mediated by extensive crossed cerebrocerebellar connections [3]. Indeed, studies on animal models demonstrated that the cerebellum receives inputs from a number of brain regions involved in cognitive functioning, including the hypothalamus, the parahippocampal gyrus, the cingulate gyrus, the superior temporal cortex, the posterior parietal cortex, and the prefrontal cortex [5–7]. These findings have recently been confirmed in humans by means of neuroimaging studies [8, 9]. Moreover, the “cerebellar cognitive revolution” was supported by observations of cerebellar activation in many functional imaging studies involving cognitive tasks [10, 11], including various aspects of executive and attentional functioning [12], as well as by findings showing that the performance of patients with cerebellar lesions is impaired in a range of perceptual [13, 14] and cognitive tasks [15, 16].

Cerebellar involvement in executive functioning was documented in isolated cerebellar lesions [17], which cause difficulties in planning, problem solving, and mental flexibility. Moreover, previous fMRI studies identified cerebellar activations during the performance of executive tasks designed to explore inhibitory control in the Stroop Color Word Test and problem solving or planning functions in the “Tower of London” test [18–20].

A link between the cerebellum and attention has also been reported [21–23]. Attentional deficits are frequently associated with the presence of morphological cerebellar abnormalities, as observed in patients with autism spectrum disorders and attention deficit hyperactivity disorder [24–26] or in cerebellar neurodegenerative disorders [22]. Moreover, previous neuropsychological reports described the role played by the cerebellum in attention, particularly in the coordination of attentional shifts [12, 27–29] and selective attention [30, 31]. More recently, the role of cerebellum in attention was demonstrated also by noninvasive neuromodulation studies [32–34].

An impairment in attentional functioning also emerged in previous psychophysiological studies following cerebellar injuries. The prolonged mismatch negativity latencies described in Moberget et al. [35] point to a deficit in the automatic attentive discrimination of the stimulus in cerebellar degenerative diseases. Moreover, delayed p300 latencies and reduced p300 amplitudes were reported following cerebellar damages [36–38]. Specifically, in a recent p300 study by our group, we observed that the functional inhibition of the cerebellum by means of transcranial direct current stimulation (tDCS) significantly reduces p300 amplitudes, thus indicating that the cerebellum is involved in the attentional

processing of the stimulus during both the orienting and the discrimination phases [39].

The tDCS is a noninvasive brain stimulation technique that is used to pass a direct current through the brain of subjects via surface electrodes fixed to the head [40]. It allows the excitability of cortical neurons to be manipulated *in vivo* in a polarity-specific manner [41]. In particular, although the after-effects of tDCS may differ between subjects [42, 43], cathodal tDCS is believed to reduce neuronal excitability, whereas anodal tDCS increases it [41, 44]. Most recently, tDCS has been used to induce plastic changes in cortical excitability and brain connectivity in human subjects [45–47]. In these experiments, tDCS appears to modulate cognitive performances thereby reducing or improving subject performance, depending on the type of stimulation applied (anodal versus cathodal) and the activation state of the targeted neuronal regions [48].

Attention is defined as the ability to appropriately allocate processing resources to relevant stimuli. Attention is not a unitary concept but one that contains multiple, separate, hitherto interacting networks, as explained in the theory by Posner and Petersen [49]: the alerting, orienting, and executive (or conflict) networks. It is known that alerting means achieving and maintaining a state of high sensitivity to incoming stimuli combined with a readiness to react. The alerting network includes the locus coeruleus and the parietal and right frontal cortex. Orienting is defined as selecting information from sensory input and shifting attention, *i.e.*, disengaging and re-engaging attention. This network contains the frontal eye fields, the superior colliculus, the temporal parietal junction, and the superior parietal cortex. The executive network comprises mechanisms for monitoring and resolving conflict between thoughts, feelings, and responses and includes the basal ganglia, anterior cingulate, and lateral ventral prefrontal cortex.

The Attention Network Test (ANT) was developed by Fan et al. [50] to provide measures of these three functions. This computerized test combines Posner’s cuing task [51] with Eriksen’s flanker task [52] and allows the assessment of attentional dimensions of alerting, orienting, and executive function via specific reaction time (RT) patterns [50]. In fact, measuring how RT is affected by different cues (alerting and orienting cues) and different flankers and provides an assessment of the efficiency of the three attentional networks engaged in the task. When an alerting cue is presented, in fact, it mainly provides temporal information by alerting the subject to the impending arrival of the target stimulus. Comparing trials with alerting cues to those with no cues gives information about the alerting network efficiency. Similarly, orienting cues, such as spatial cues, provide spatial information that allows subjects to start orienting attention to the appropriate location while attending the target, thereby activating not only the alerting network but also the orienting network; comparing trials with spatial cues to trials with cues that do not present

spatial information provides insights regarding the orienting network efficiency. Lastly, owing to the conflicting information borne by incongruent flankers, with respect to congruent ones, comparing trials with these two different types of flankers provides a reliable measure of the executive network efficiency.

The aim of the present study was to explore the role of the cerebellum in the functioning of the attention networks. For this purpose, we transitorily modulated cerebellar activity by means of tDCS and studied the effects of the cerebellar tDCS on the ANT parameters in healthy subjects.

Given the crossed cerebro-cerebellar connections and the important role played by the right frontal-parietal areas in orienting and attentional functioning [53, 54], the left cerebellum was chosen as target for tDCS modulation.

Cerebellar output activity is known to be based mainly on an inhibitory firing toward multiple brain areas (cerebellar-brain inhibition (CBI) tone) [7]. As suggested by the results of previous experiments, the presence of cerebellar inhibition (e.g., following cathodal tDCS) may induce hyperactivity in brain areas [39, 55]. Nevertheless, this condition is associated with uncoordinated functioning in these cortical regions as well as with possible cognitive dysfunctions [39].

On the basis of these assumptions, we hypothesized that cathodal tDCS over the left cerebellum may reduce the efficiency of brain areas involved in the attentional networks and that anodal tDCS has the opposite effect. In particular, bearing in mind the great contribution of the cerebellum to executive attentive processes and focused attention, we hypothesized that the executive network will be affected to a greater extent by the effects of cerebellar tDCS modulation than the other networks.

## Methods

### Participants

Twenty-five right-handed, healthy subjects (12 male, 13 female; mean age  $26.1 \pm 2.1$  years; range 21–29 years) were enrolled in a double-blind, sham-controlled, crossover study. None of the subjects had a history of neurological or psychiatric disease or of head injury, and none reported consuming excessive amounts of alcohol or were taking any medication that affects the central nervous system. Written informed consent was obtained from all the participants prior to the experiment. The study was approved by the Local Medical Ethics Committee.

### Procedure

Each participant received anodal, cathodal, or sham tDC stimulation during three randomized tDCS sessions performed at

least 6 days apart. Both before and after tDCS stimulation, subjects reported their attention, fatigue, and perceived pain using a self-scored visual analog scale [56] and performed the ANT.

### tDCS

None of the subjects or ANT investigators, with the exception of the investigator who applied the tDCS, were aware of whether anodal, cathodal, or sham stimulation was being performed. The experimenter was present in the room at the beginning of each session so as to be able to start the tDCS system; during the stimulation phase, she was not present in the room and if any problem emerged, subjects could call her and stop tDCS procedure.

The tDCS over the left cerebellar hemisphere was applied by means of two sponge electrodes (surface area =  $25 \text{ cm}^2$ ) moistened with a saline solution. One electrode was centered over the left cerebellar cortex, 1 cm below and 4 cm lateral to theinion (corresponding approximately to the projection of cerebellar lobule VII onto the scalp). The other electrode was positioned over the left deltoid muscle [57]. The onset and offset of all the interventions (anodal, cathodal, and sham) entailed increasing and decreasing the current, respectively, in a ramp-like manner over 10 s, a method shown to achieve a good level of blinding between sessions [55, 58, 59]. The stimulation intensity was set at 2 mA and delivered over the cerebellum for 20 min using a battery-operated, constant current stimulator (BrainSTIM EMS srl, Bologna, Italy), which is similar to the intensity adopted by Ferrucci et al. [57] and is considered to be well below the tissue damage threshold [58, 60, 61]. In the sham condition, pseudo-stimulation (110  $\mu\text{A}$  over 15 ms every 550 ms) was applied instead of the stimulation current for 20 min [55].

### Paradigm: ANT

The ANT we used is the original Java version of the test, described by Fan et al. [50], that can be downloaded free of charge from the website of the Sackler Institute for Developmental Psychobiology ([www.sacklerinstitute.org/cornell/assays\\_and\\_tools/](http://www.sacklerinstitute.org/cornell/assays_and_tools/)) and provides both raw data and precalculated scores. A HP PC desktop-controlled stimulus presentation and response collection.

Participants were seated in front of a computer monitor (19 in), observed the visual stimuli (represented by cues and targets), and responded by pressing the two response mouse buttons (using the right index finger for the right mouse button and the left index finger for the left mouse button).

The written instructions were read aloud to the participants and emphasized the importance of quick and accurate responses. The experimenter was present in the testing room at the beginning of each session so as to be able to start the

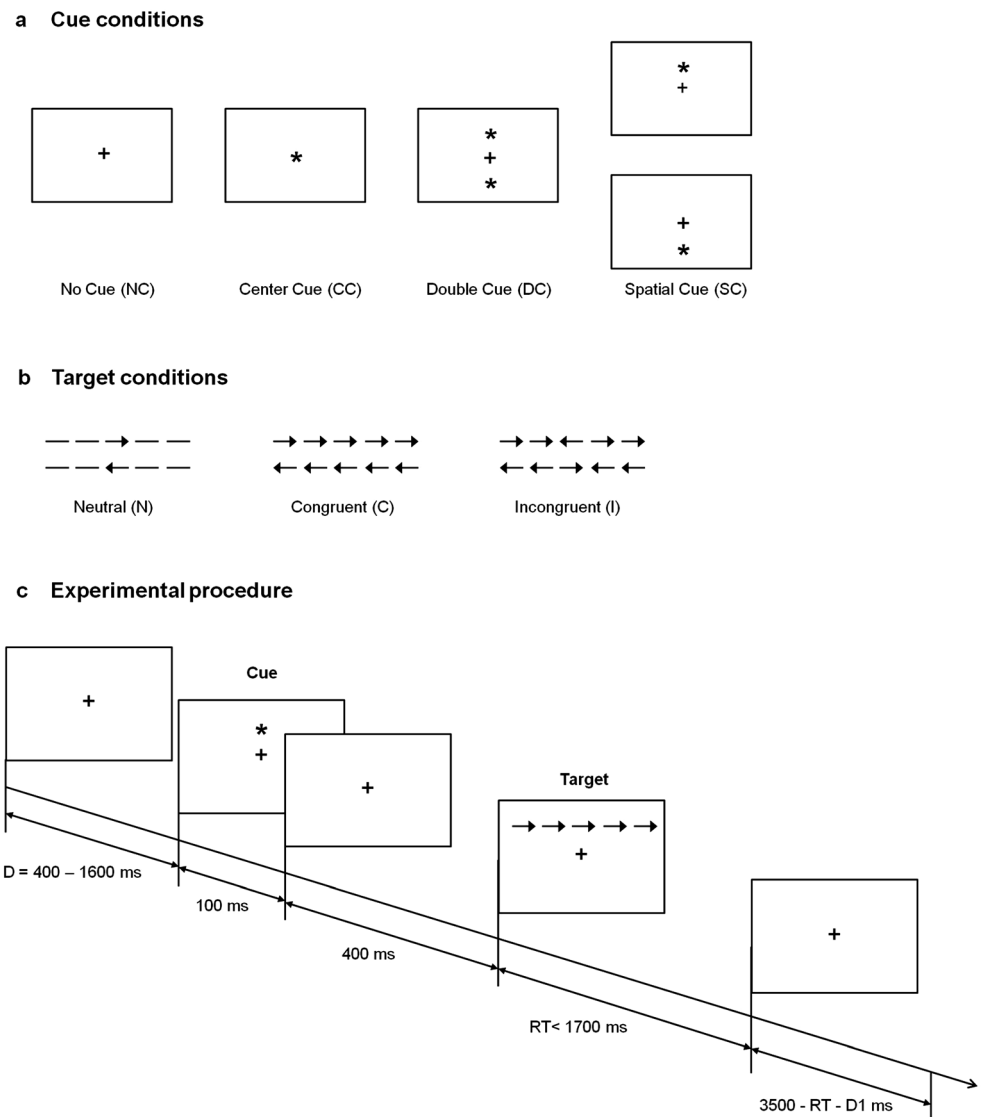
computer program, read the instructions, and answer any questions. Feedback regarding performance accuracy was given only during a practice block. Prompts to begin the next block appeared after the practice trials (a prompt to click CONTINUE to start the test appeared on the monitor), thus giving the participant a short break and allowing the experimenter to ascertain whether participants understood the instructions and could move from practice to test phase. Additional practice trials were given to participants who did not initially understand the instructions or who had difficulty with the task.

The task requires participants to determine whether a central target arrow points left or right by responding as quickly and as accurately as possible by pressing either a button for the left-pointing arrow or another button for the right-pointing arrow. The central target arrow may or may not be preceded by a warning cue, consisting of an asterisk (cue condition). Depending on the presence and position of the asterisk, there

are four cue conditions: (1) “no cue” (NC), in which no asterisk precedes the target, and only a central cross, which represents the fixation point, is present on the screen; (2) “double cue” (DC), in which the asterisk appears simultaneously both above and below the fixation point; (3) “center cue” (CC), in which the asterisk appears in the center of the screen, replacing the fixation point, and indicates, like the DC, that the target arrow is about to appear, though without providing any information on its location; and (4) “spatial cue” (SC), in which the asterisk appears either above or below the fixation point, indicating that the arrow is about to appear and exactly where it will appear (Fig. 1a).

Moreover, the central target arrow always appears above or below the fixation point and may or may not be accompanied by flankers, which are represented by two arrows on either side (target conditions). There are three target conditions divided according to the presence and type of the flankers: (1) “neutral” condition (N), in which the central target arrow is

**Fig. 1** Description of the Attention Network Test. The figure illustrates the four possible cue conditions (a), the six possible target conditions (b), and an example of a trial with spatial cue (SC)-congruent target (c) combination (correct response being right) with the time course



not surrounded by flankers; (2) “congruent” condition (C), in which the five arrows (the four flankers plus the central target arrow) all point in the same direction; and (3) “incongruent” condition (I), in which the four flanker arrows point in the direction opposite to that of the target (Fig. 1b).

The test consists of a 24-trial practice block, during which the subject receives a visual online feedback, at the end of each trial, on whether each response was correct (the word CORRECT appears in black type at the center of the screen when the subject pushes the correct button related to the direction of the central arrow, within 1700 ms after its presentation) or incorrect (the word INCORRECT appears in red type at the center of the screen when the subject pushes the opposite button or when the time response exceeds 1700 ms), and three experimental blocks with no feedback.

Each of the three experimental blocks consists of 48 trials repeated twice so that the entire experiment comprised a total of 288 trials. Each trial includes the following possible combinations: 4 cue types  $\times$  2 target locations (above/below)  $\times$  2 target directions (right/left)  $\times$  3 flanker conditions. Each trial is composed of a first fixation that varies randomly between 400 and 1600 ms. Next, a cue is presented for 100 ms (or, in the NC condition, a second fixation lasting 100 ms). Four hundred milliseconds after offset of the cue, a target (the central arrow and occasionally the flankers) is presented, remains until the subject responds, but disappears after 1700 ms if no response is given. The last post-target fixation period varies according to the RT and the first fixation (it lasts 3500 ms minus the reaction time (RT) minus the duration of the first fixation) (Fig. 1c). The task lasts approximately 20 min.

The efficiency of the three attentional networks is assessed by measuring how RT is affected by alerting cues (no cue versus double cue), spatial orienting cues (center cue versus spatial cue), and flankers (congruent versus incongruent). In particular, when the double cue is presented, it tends to hold the subject’s attention on two potential target locations (below or above the fixation point), but mainly provides temporal information and, therefore, recruits the alerting network. In addition, both center and spatial cues are alerting cues, but only the spatial cue provides spatial information thereby activating not only the alerting network but also the orienting network. Lastly, the executive network is required to make a greater effort when responding to incongruent flanker target, which carries conflicting information, than to the congruent flanker target.

## Statistical Analysis

The analyses were performed using the SPSS statistical package (version 24).

Data are expressed as the mean ( $\pm$  1 standard deviation) for continuous variables and as proportions for categorical

variables. The Shapiro-Wilk test was applied to assess the normal distribution of the data.

For the calculation of RTs, an initial data reduction was conducted to exclude trials with incorrect responses, trials with a RT  $<$  100 or  $>$  1700 ms, and trials with a RT that exceeded the subject’s mean RT  $\pm$  2SD. After this skimming procedure, the mean number of trials excluded was 39/7200 (288 trials each subjects) (0.5%) for anodal pre-tDCS session, 27/7200 (0.4%) for anodal post-tDCS session, 52/7200 (0.7%) for cathodal pre-tDCS session, 28/7200 (0.4%) for cathodal post-tDCS session, 35/7200 (0.5%) for sham pre-tDCS session, and 43/7200 (0.6%) for sham post-tDCS session.

As the efficiency of the three attentional networks is obtained by measuring how RT is affected by alerting and spatial orienting cues as well as by flankers, the subtraction method was applied as follows, in accordance with Fan et al.: for the alerting network efficiency: mean RT<sub>NC</sub> trials ( $n = 72$ ) – mean RT<sub>DC</sub> trials ( $n = 72$ ); for the orienting network efficiency: mean RT<sub>CC</sub> trials ( $n = 72$ ) – mean RT<sub>SC</sub> trials ( $n = 72$ ); for the executive network efficiency: mean RT<sub>I</sub> trials ( $n = 96$ ) – mean RT<sub>C</sub> trials ( $n = 96$ ). For both the alerting and orienting effects, higher subtraction scores indicate greater efficiency; by contrast, the more efficient the executive network is, the lower the subtraction score. The overall accuracy was calculated as the percentage of correct responses.

The main ANT outcome measures (i.e., the efficiency of the alerting, orienting, and executive networks, the mean overall RT) were analyzed separately by means of ANOVA for repeated measures, with experimental “condition” (cathodal, anodal, sham) and “timing” (pre-tDCS and post-tDCS) as the within-subject factors. When required, a post hoc correction according to Bonferroni was then applied. Degrees of freedom were adjusted, when necessary, by using the Greenhouse-Geisser epsilon coefficient for possible violations of the sphericity assumption, and corrected  $p$  values are reported; the original degrees of freedom are reported together with their correction factor epsilon. Effect sizes were measured by calculating the partial eta squared ( $\eta^2_p$ ). For the interpretation of magnitude of the effect size provided by partial eta squared, we refer to the following guideline: = 0.01 small effect, = 0.06 medium effect, and = 0.14 large effect [62].

Regarding the error rate, data were not normally distributed, probably because of the ceiling effect; thus, no further analyses were conducted.

Moreover, in order to evaluate whether the tDCS stimulation exerted any effect on a specific target, cue, or cue-target combination, an ANOVA for repeated measures was conducted, with “cue” (NC, DC, CC, SC), “target” (C, I, N), timing (pre-tDCS and post-tDCS), and experimental condition (cathodal, anodal, sham) as the within-subject factors.

**Table 1** Psychological measures at pre-tDCS and post-tDCS evaluation

	Attention			Fatigue			Pain		
	Pre-tDCS	Post-tDCS	<i>p</i>	Pre-tDCS	Post-tDCS	<i>p</i>	Pre-tDCS	Post-tDCS	<i>p</i>
Cathodal	9.1 ± 0.9 (9.4)	9.1 ± 1.0 (9.3)	0.75	9.2 ± 0.8 (9.3)	9.4 ± 0.8 (9.7)	0.24	9.5 ± 0.9 (10)	9.4 ± 0.7 (9.7)	0.25
Anodal	9.3 ± 1.0 (10)	9.2 ± 1.2 (10)	0.21	9.2 ± 1.1 (9.7)	9.0 ± 1.4 (9.0)	0.11	9.7 ± 0.5 (10)	9.7 ± 0.7 (10)	0.95
Sham	9.4 ± 0.7 (10)	9.4 ± 0.8 (10)	0.68	9.4 ± 0.7 (10)	9.4 ± 0.8 (10)	0.80	9.7 ± 0.6 (10)	9.6 ± 0.6 (10)	0.73

Values are displayed as mean ± SD (median) and depict the subject's choice in a visual analog scale in which 1 represents poorest attention, maximal fatigue, and maximal pain and 10 represents maximal attention, least fatigue, and least pain. Significance level was set at  $p \leq 0.05$

Attention, fatigue, and perceived pain pre-tDCS and post-tDCS were analyzed separately for each condition (cathodal, anodal, sham) by means of Wilcoxon signed-rank test. A post hoc correction according to Bonferroni was applied if necessary.

The significance level was set at  $p \leq 0.05$ .

## Results

All the subjects completed the three tDCS sessions without encountering any difficulties. No significant differences emerged in the subjects' self-reported ratings of attention, fatigue, and perceived pain before and after tDCS stimulation across the three sessions (Table 1). For each session, all the measures were completed within 30 min of the cessation of tDCS.

## Main Outcome Measures

### Network Efficiency

As regards the alerting network efficiency, ANOVA revealed a main effect of the timing factor ( $F_{(1,24)} = 6.7$ ,  $p = 0.02$ ,  $\eta^2_p = 0.22$ ), with higher values after stimulation. ANOVA did not reveal a main effect of the condition factor ( $F_{(2,48)} = 1.1$ ,  $p =$

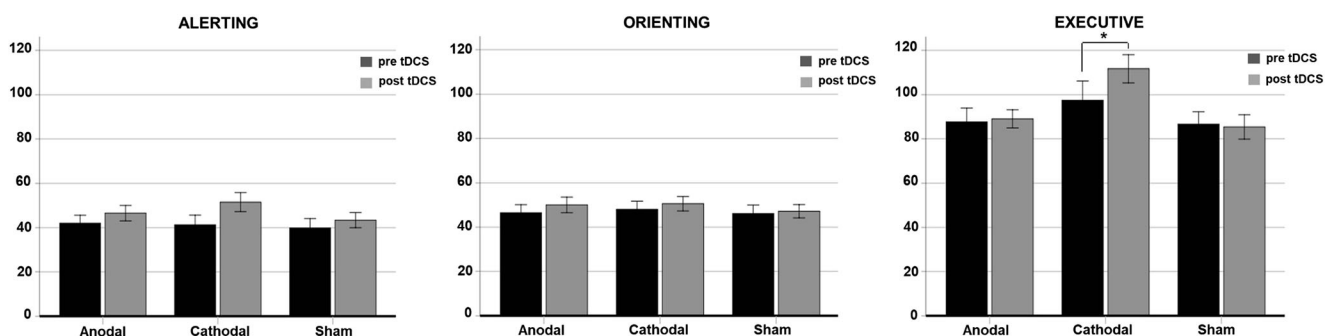
0.34,  $\eta^2_p = 0.04$ ). The condition × timing interaction was not significant ( $F_{(2,48)} = 0.84$ ,  $p = 0.44$ ,  $\eta^2_p = 0.03$ ).

As regards the orienting network efficiency, ANOVA did not reveal any main effect of either the timing factor or the condition factor (respectively,  $F_{(1,24)} = 1.4$ ,  $p = 0.25$ ,  $\eta^2_p = 0.05$ ;  $F_{(2,48)} = 0.3$ ,  $p = 0.73$ ,  $\eta^2_p = 0.01$ ). The condition × timing interaction was not significant ( $F_{(2,48)} = 0.1$ ,  $p = 0.90$ ,  $\eta^2_p = 0.004$ ).

As regards the executive network efficiency, ANOVA revealed a main effect of the condition factor ( $F_{(2,48)} = 5.5$ ,  $p = 0.007$ ,  $\eta^2_p = 0.19$ ), while it did not reveal a main effect of the timing factor ( $F_{(1,24)} = 3.5$ ,  $p = 0.07$ ,  $\eta^2_p = 0.13$ ). Moreover, a significant condition × timing interaction did emerge ( $F_{(2,48)} = 3.8$ ,  $p = 0.03$ ,  $\eta^2_p = 0.13$ ). After Bonferroni's correction, a significant difference emerged for the cathodal stimulation alone, with a worse post-tDCS than pre-tDCS network efficiency being detected (subjects displayed significantly higher values after cathodal tDCS: pre-tDCS  $97.4 \pm 44$ ; post-tDCS  $111.7 \pm 31.6$ ;  $p = 0.02$ ), while no difference emerged for the anodal or sham conditions (see Fig. 2).

### Accuracy

For descriptive purposes, Table 2 displays accuracy rates for each experimental condition.



**Fig. 2** Attention networks' efficiency calculated by means of the subtraction method by Fan et al. (for the alerting network efficiency: mean RT<sub>NC</sub> trials - mean RT<sub>DC</sub> trials; for the orienting network efficiency: mean RT<sub>CC</sub> trials - mean RT<sub>SC</sub> trials; for the executive

network efficiency: mean RT<sub>I</sub> trials - mean RT<sub>C</sub> trials) is shown for anodal, cathodal, and sham conditions both in pre-tDCS and post-tDCS. Error bars indicate ± 1 SE. \* $p < 0.05$ , after Bonferroni correction for multiple comparisons

**Table 2** Mean RT and accuracy for each experimental condition

	Target	Cue type							
		No cue		Double cue		Center cue		Spatial cue	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean RT (ms) (SD)									
Anodal	Congruent	589 (99)	558 (68)	534 (84)	521 (60)	545 (100)	523 (71)	487 (64)	480 (63)
	Incongruent	658 (101)	647 (78)	638 (84)	610 (66)	634 (75)	627 (76)	578 (88)	555 (67)
	Neutral	524 (63)	526 (60)	472 (73)	459 (54)	471 (72)	459 (50)	443 (62)	423 (38)
Cathodal	Congruent	578 (80)	574 (68)	551 (73)	529 (56)	546 (75)	525 (56)	502 (70)	493 (57)
	Incongruent	681 (93)	684 (75)	637 (88)	640 (70)	660 (92)	667 (73)	589 (90)	578 (72)
	Neutral	532 (58)	531 (56)	479 (58)	465 (40)	484 (74)	467 (48)	453 (62)	440 (40)
Sham	Congruent	556 (68)	542 (59)	518 (63)	509 (62)	521 (70)	518 (72)	478 (57)	470 (54)
	Incongruent	637 (82)	637 (70)	614 (67)	605 (65)	615 (78)	602 (69)	554 (73)	536 (65)
	Neutral	513 (50)	511 (46)	455 (44)	444 (42)	448 (42)	447 (41)	422 (38)	418 (32)
% Accuracy (SD)									
Anodal	Congruent	1.00 (0.01)	1.00 –	1.00 –	1.00 –	1.00 (0.01)	1.00 –	1.00 (0.01)	1.00 –
	Incongruent	0.97 (0.08)	0.99 (0.02)	0.98 (0.02)	0.99 (0.01)	0.98 (0.03)	0.98 (0.03)	1.00 (0.01)	0.99 (0.02)
	Neutral	1.00 –	1.00 (0.01)	0.99 (0.02)	1.00 –	1.00 (0.01)	1.00 –	1.00 (0.01)	1.00 –
Cathodal	Congruent	1.00 (0.02)	1.00 –	0.99 (0.01)	1.00 (0.01)	0.99 (0.02)	1.00 –	0.99 (0.02)	1.00 –
	Incongruent	0.97 (0.09)	0.97 (0.08)	0.96 (0.08)	0.99 (0.02)	0.99 (0.03)	0.98 (0.08)	0.99 (0.01)	1.00 (0.01)
	Neutral	1.00 (0.01)	1.00 –	1.00 (0.02)	1.00 –	0.99 (0.02)	1.00 (0.01)	0.99 (0.02)	1.00 –
Sham	Congruent	1.00 (0.01)	1.00 –	1.00 –	1.00 (0.01)	1.00 –	1.00 (0.01)	1.00 –	1.00 –
	Incongruent	0.98 (0.03)	0.97 (0.05)	0.99 (0.02)	0.99 (0.02)	0.99 (0.02)	0.99 (0.02)	0.99 (0.02)	0.99 (0.01)
	Neutral	0.99 (0.02)	1.00 (0.01)	1.00 –	1.00 (0.01)	1.00 (0.01)	1.00 (0.01)	1.00 (0.01)	0.99 (0.01)

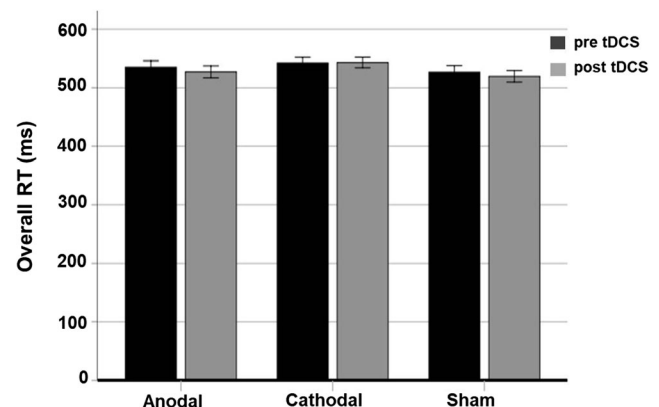
## RT

**Mean Overall RT** As regards the mean overall RT, ANOVA did not reveal a main effect of the timing factor ( $F_{(1,24)} = 2.3$ ,  $p = 0.14$ ,  $\eta_p^2 = 0.09$ ); it revealed a main effect of the condition factor ( $F_{(2,48)} = 3.5$ ,  $p = 0.039$ ,  $\eta_p^2 = 0.13$ ). The condition  $\times$  timing interaction was not significant ( $F_{(2,48)} = 0.8$ ,  $p = 0.45$ ,  $\eta_p^2 = 0.3$ ) (see Fig. 3).

**Additional RT Results** Table 2 displays mean RT in each trial combinations for each experimental session.

As expected, a significant main effect of the cue factor was observed ( $F_{(3,72)} = 357.6$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.94$ ), with the RT being significantly faster for the SC condition than for either the CC or DC conditions and significantly slower for the NC condition. A significant main effect of the target factor was also observed ( $F_{(2,48)} = 220.4$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.9$ ), with significantly faster RT for the C than for the I target. Moreover, a significant main effect of the timing factor was observed ( $F_{(1,24)} = 5.3$ ,  $p = 0.03$ ,  $\eta_p^2 = 0.18$ ), with a significantly shorter RT being detected after tDCS than before tDCS, as well as of the condition factor ( $F_{(2,48)} = 10.3$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.3$ ); after Bonferroni's correction, a significant difference was observed for cathodal stimulation compared with the sham condition

( $p = 0.001$ ), with longer RTs. No significant interactions emerged with the exception of the cue  $\times$  target interaction ( $F_{(6,144)} = 20.8$ ;  $p < 0.001$ ;  $\eta_p^2 = 0.46$ ), condition  $\times$  target interaction ( $F_{(4,96)} = 4.6$ ;  $p = 0.002$ ;  $\eta_p^2 = 0.16$ ), condition  $\times$  cue  $\times$  target interaction ( $F_{(12,228)} = 3.01$ ;  $p < 0.001$ ;  $\eta_p^2 = 0.11$ ), and timing  $\times$  cue  $\times$  target interaction ( $F_{(6,144)} = 2.5$ ;  $p = 0.02$ ;  $\eta_p^2 = 0.1$ ). Moreover, a significant condition  $\times$  timing  $\times$  target interaction emerged ( $F_{(4,96)} = 3.7$ ;  $p = 0.01$ ;  $\eta_p^2 = 0.13$ ); after



**Fig. 3** Mean overall RTs for anodal, cathodal, and sham conditions both in pre-tDCS and post-tDCS. Error bars indicate  $\pm 1$  SE

Bonferroni's correction, a significantly reduced RT was observed after anodal and sham stimulation for both congruent (anodal:  $p = 0.02$ ; sham:  $p = 0.04$ ) and incongruent targets (anodal:  $p = 0.04$ ; sham:  $p = 0.09$ ), whereas after cathodal stimulation, a significant reduction was observed for congruent targets alone ( $p = 0.06$ ), while no difference emerged for incongruent targets ( $p = 0.95$ ) (see Fig. 4).

## Discussion

The aim of this study was to explore the role of the cerebellum in the functioning of the attentional networks by evaluating the effects of the cerebellar tDCS on behavioral parameters assessed during the ANT task.

The efficiency scores of the alerting and orienting networks are stable before and after the ANT task repetition regardless of the type of cerebellar tDCS stimulation. The executive index also remains stable after anodal and sham stimulation. Cathodal stimulation results in an increase of the executive efficiency score, which points to an impairment in this specific attentive system.

The executive efficiency score numerically measures the ability of the executive system to check, compare, and solve conflicts [50]. The executive network score, as measured by the ANT, is based on the response times to stimuli of varying congruence, it being expressed in particular as the difference between the response times to incongruent and congruent target stimuli [50]. In general, the incongruous stimulus, which bears conflicting information, requires a greater effort to identify correct responses, thereby resulting in longer reaction times than those required for the congruent stimulus. Lower subtraction scores thus indicate greater efficiency. Similarly, higher subtraction scores suggest a reduced efficiency of the executive network, and this may derive either from a selective increasing in response times to incongruent stimulus or a reduction in response times to congruent stimulus or to both situations simultaneously.

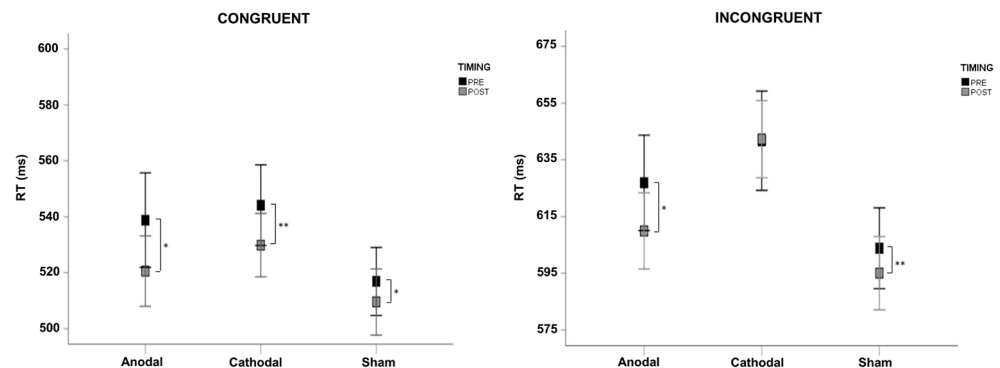
It is worthy of note that the analysis of the RTs for different targets following the repetition of the task and according to the

type of cerebellar stimulation performed revealed that anodal and sham stimulation significantly reduce RTs to congruent and incongruent stimuli following the task repetition. These findings point to a learning effect that is to be expected following exposure to repeated stimuli [63, 64]. Since the extent of this reduction is the same as that observed for the two different stimuli (congruent and incongruent), it did not affect the functioning of the overall executive network after task repetition. Following cathodal stimulation, a reduction is observed in RTs for the congruent stimuli but not for incongruent stimuli. On the basis of these data, we may assume that the increase in the executive network subtraction score (that reflects lower network efficiency) observed after cathodal cerebellar tDCS is mainly due to an inability to discriminate and correctly learn the incongruous stimulus. These findings thus indicate that cerebellar inhibition causes an attentive executive dysfunction specifically related to the ability to process complex stimuli in which conflict signals or errors are present.

Error processing requires the ability to identify a mismatch between previously stored information and assumes the ability to recognize salient differences between events, which in turn activates an orienting response [65, 66]. Error stimuli are relatively infrequent and unexpected and lead to rapid cognitive and behavioral adaptation [66]. Moreover, the perception of error is a discriminative act that requires the involvement of multiple cortical associative regions such as the cingulate cortices, the lateral prefrontal cortex, and the inferior parietal cortex [66]. In this regard, EEG-fMRI studies showed that the mid-cingulate cortex is the main generator of error-related negativity, a fronto-central negative voltage component related to error processing [67–69]. Moreover, recent fMRI studies reported an abnormal error-related activity in the anterior cingulate cortex (ACC) [70–72]. The lateral prefrontal cortex has been implicated in maintaining and updating representations, goals, and contextual information and in exerting top-down control in mutual interactions with the ACC for the monitoring of conflicting information [49, 73–75].

The cerebellum is a subcortical structure that it is thought indirectly affecting cognitive functioning and probably even

**Fig. 4** RTs raw data related to congruent and incongruent target both pre-tDCS and post-tDCS in anodal, cathodal, and sham sessions. Error bars indicate  $\pm 1$  SE. \* $p < 0.05$ ; \*\* $p < 0.1$  after Bonferroni correction





error processing [3, 76]. This structure projects to both motor and associative brain areas via the thalamus, with the prefrontal cortices appearing to be the main cognitive hubs that interact with the cerebellum [4, 77, 78]. In particular, it is believed that the cerebellum as a general coordinator may regulate the activation and inhibition levels of these cortical areas thus controlling the speed, timing, and appropriateness of cognitive processes [3].

However, the cerebellum is also believed to directly regulate error processing by performing specific sensory predictions according to a forward model [3, 76]. The sensory predictions generated by a forward model can be used to coordinate motor output, thereby providing a means of anticipating the effects of a motor act and of updating the motor system [79, 80]. Error signals are essential for sensorimotor control insofar as they allow rapid adjustments in motor output. They are also essential for learning, for refining future sensory predictions, and for reducing the prediction error signal in subsequent movements. The role played by the cerebellum is likely related to the evaluation of temporal patterns, i.e., not of the stimulus alone, but even between the stimulus and its related response [3]. Specifically, in an ANT task, the cerebellum may help to generate a temporally constrained expectancy between a congruent target stimulus and a motor response. When an incongruous stimulus appears, the role of the cerebellum is likely to exploit these conflicting signals to improve future predictions and/or produce online changes in behavior in response to the error.

We believe that the effect of cerebellar transient inhibition with cathodal tDCS that we observed is rather cognitive than purely motor, even if it exerts its results through modified RTs. In fact, it is selectively produced in incongruent trials alone, whereas the RTs to congruent trials seem to be relatively preserved, indicating that the cognitive discrimination of error is implicated above all. This hypothesis is in line with the results of a previous study, in which we demonstrated that a cerebellar inhibition significantly reduced the P300 amplitudes for both the target and novel stimuli during a task that did not require a motor response, thus suggesting that the attentional processing of the stimulus was perturbed by this specific stimulation [39].

We believe that the executive dysfunction that emerged in our subjects may, first of all, have depended directly on a possible reduction of the inhibitory output of the cerebellum to the cerebrum induced by cathodal tDCS [81], which prevented error prediction. Secondly, cerebellar inhibition function indirectly also induced a specific functional alteration of the brain regions involved in error perception, particularly in the prefrontal cortices. Indeed, we believe that by inhibiting the cerebellum, cathodal tDCS stimulation reduces the CBI [81] and, as we hypothesized in a recent study [39], its control over the prefrontal areas, thereby rendering them transiently hyperactive and uncoordinated. These two conditions (both

direct cerebellar inhibition and cortical dysfunction) meant that a selective difficulty was encountered in attentive executive processing, particularly in the discrimination of conflicting stimuli, causing longer response times to these specific stimuli when the subject had to repeat the ANT task.

To date, the exploration of attentional processes via tDCS has been carried out only by a few studies. It has been shown that anodal tDCS applied on parietal cortices improved covert spatial orienting [82] and significantly speeded up motor responses during attentional orienting task [83]. Moreover, enhanced object detection after anodal tDCS but not after sham tDCS on right inferior frontal cortex was found to be associated with increased alerting network functioning [84] while anodal tDCS of the right parietal cortex enhanced spatial reorienting during an ANT task [85]. On the other hand, Moos et al. [86] demonstrated that cathodal stimulation applied over the right intraparietal sulcus significantly enhanced top-down attentional control. As regards studies exploring the cerebellar role in cognitive functions, Pope and Miall [55] reported that the cerebellar cathodal stimulation facilitated cognitive performances especially when cognitive tasks become difficult. Similarly, a recent study reported that the combination of a neurophysiological pre-conditioning induced by cathodal cerebellar tDCS with a cognitive conditioning stimulation facilitated spatial attention [33].

On the contrary, our recent study showed that cerebellar cathodal stimulation altered attentional processing of the acoustic stimulus specifically reducing P3 amplitudes during a P300 novelty task.

In contrast to our initial hypothesis, anodal stimulation did not yield significant behavioral effects and the ANT parameters obtained following the anodal session were comparable to those in the basal condition and in the sham session. In fact, the possible excitatory effect of anodal stimulation [41] should have induced a motor performance enhancement with a reduction in reaction times and an improvement in the efficiency of attention networks. Nevertheless, our subjects, as healthy controls, had no cognitive deficits and performed to the utmost of their ability in the ANT task, even during the baseline evaluation. Thus, any excitation in cerebellar activity could not have improved further their attentive and motor performances, already optimal in the baseline time. On the contrary, deterioration in network efficiency or single motor performance would have emerged more easily.

In a recent meta-analysis, Jacobson et al. [42] attempted to verify the assumption that anodal (or excitatory) tDCS versus cathodal (or inhibitory) tDCS leads to respective improvement versus impairment in performance. These findings were confirmed in only a part of the studies while negative or null effects were not included thus creating a bias of publication toward positive effects in the literature. For these reasons, it is still difficult to determine a true effect size for tDCS.

Moreover, as suggested by several studies with cognitive tasks, the common assumption about the inhibiting effect of cathodal stimulation has been found less frequently, while anodal stimulation more consistently increased cortical excitability [42, 87]. In this sense, our data are not in line with the previous reports. However, it is rather difficult to find certain and repeatable effects of tDCS above all in cognitive studies [87, 88]. In fact, the effects might depend not only on the applied tDCS intensity, duration, and timing stimulation [89–93] but also on task characteristics, the site of application, and the excitability status of the underlying cortical tissue [87].

To conclude, these data show that the cerebellum underlies the functioning of attentional networks, particularly the executive attention network. The cerebellum affects error processing both directly, by making predictions of errors or behaviors related to errors, and indirectly, by managing the functioning of the cortical areas involved in the perception of conflicting signals.

### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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