Cerebellum and mental state recognition: a transcranial direct current stimulation study in healthy subjects

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Abstract

Increasing evidence from neuroimaging and clinical studies demonstrated cerebellar involvement in social cognition components, including the mentalizing process. The aim of this study was to apply transcranial direct current stimulation (tDCS) to modulate cerebellar excitability to investigate the role the cerebellum plays in mental state recognition. Forty-eight healthy subjects were randomly assigned to different groups in which anodal, cathodal, or sham tDCS (2 mA for 20 min) was delivered centring the electrode on the vermis to stimulate the posterior portion of the cerebellum. The ability to attribute mental states to others was tested before and after tDCS using a digital version of the 'Reading the Mind in the Eyes test', which includes visual perceptive and motor stimuli as control conditions. Correct response and reaction times (RT) were recorded.

Results revealed a significant reduction in RTs between the baseline and post-stimulation sessions after the cerebellar anodal tDCS only for mental state stimuli, whereas no significant effect was found in the cathodal or sham conditions or for visual perceptive and motor stimuli. Overall, our study suggests that cerebellar anodal tDCS might selectively improve mental state recognition and constitute an effective strategy to positively modulate the mentalizing process.

Introduction

Over the last decades, the cerebellum has been recognized as a critical structure not only in motor control but also in cognitive and affective functioning[1–6]. More recently, increasing evidence has extended the role of this brain region in the social cognition domain[7]. Specifically, several neuroimaging studies have shown cerebellar activation in social cognition tasks[8] and its functional connections with brain regions belonging to the ‘social brain network’ (i.e., the Default Mode Network)[9–11]. Clinical evidence in patients with cerebellar damage evidenced specific social cognition difficulties, including impairments in basic emotions and mental state recognition[3,12].

The emerging idea is that the cerebellum is a good candidate for the predictive function that allows humans to be more adaptive in social interactions[13] thanks to its neuro-anatomical connections with the limbic and associative areas[14–16]. In this view, the cerebellum might act in the social cognitive domain as a modulator and optimizer of the projection brain areas activity, in a very similar way in which it does in the motor and cognitive domains[4].

Despite the data reported above, the role played by the cerebellum in social behaviour still needs to be better defined. To address this issue, it is crucial to exclude possible confounding effects due to other methodological approaches. For instance, in functional magnetic resonance imaging (fMRI) studies, cerebellar activation during social cognition tasks is often detected together with other brain regions[8], making it difficult to test its role in specific aspects of information processing. Moreover, cerebellar damage does not often occur in isolation in patient studies[17,18], making conclusions about direct region-specific effects difficult to draw. Finally, the life changes associated with cerebellar disease onset may themselves produce alterations in social behaviour.

In this framework, brain neuro-modulation techniques, including transcranial direct current stimulation (tDCS), represent a very advantageous approach to study cerebellar involvement in specific social behaviour processes. These techniques applied to healthy individuals allow control for the influence of confounding factors, such as pharmacological treatment, concomitant damage to other cerebral brain regions, or compensatory plastic processes in cerebral regions after cerebellar damage. Moreover, the extracephalic montage in tDCS protocols allows to avoid further confounding effects[19].

Indeed, the tDCS is a non-invasive brain stimulation technique used to pass a direct current through the brain of subjects via surface electrodes fixed to the head. It allows *in vivo* manipulation of the neuronal excitability in humans in a polarity specific manner and induces functional changes in the cerebral and cerebellar cortices[19,20]. This increasingly popular neuroscientific technique offers the opportunity to investigate the behavioural consequences of regional reduction or enhancement in neuronal excitability in healthy subjects[21].

While it is most frequently applied to the cerebral cortex, tDCS was recently used to modulate cerebellar excitability. Indeed, recent modelling studies demonstrated that the electric field generated during tDCS applied over the cerebellum effectively reaches it with relatively little functional spread to neighbouring regions[22,23]. Currently, this non-invasive brain stimulation technique is gathering more insights into the contribution of the cerebellum in cognitive and affective domains[24,25]. Although a previous study by Ferrucci and colleagues[26] showed an effect of both anodal and cathodal cerebellar tDCS on the recognition of basic emotions, to the best of our knowledge, no studies have used tDCS applied over the cerebellum to investigate its role in the mentalizing process.

A fundamental aspect of social cognition is the capacity to estimate the mental state of other people to understand and predict their behaviour. This ability is known as the Theory of Mind (ToM)[27,28], or mentalizing process, and implies both lower-level automatic processes based on action recognition and “emotional contagion”[29] and a higher-level conceptual capacity to adopt the perspective of others to infer their mental state[30].

Since in mindreading the first contact between agents is the eyes[31], a task frequently used in clinical and experimental studies to investigate this ability is the Reading the Mind in the Eyes Test (RMET), which requires subjects to “tune in” to the mental state of the actor’s eye region expression at a rapid and automatic level[32].

Indeed, although very recent studies hypothesized that this task is likely to assess lower-level processes such as perceptual emotion recognition rather than genuine theory-of-mind abilities (mentalizing criteria)[33,34], the RMET remains the most used test of theory of mind also for emotional judgments.

Indeed, following the original Baron-Cohen's description, the RMET involves the first stage of the ToM: attribution of a relevant mental state (e.g., compassion), regardless its content (e.g., compassion for her mother's loss). Interestingly, in line with findings by Hoche and colleagues[35], in a recent study we found impaired performance in RMET in patients with cerebellar pathology[3].

In the present study, we combined cerebellar tDCS with an ad hoc digital version of the RMET to further understand cerebellar involvement in the automatic component of the mentalizing process. Based on the possible excitatory and inhibitory influences of anodal and cathodal tDCS on cerebellar cortical excitability, herein, we hypothesized that anodal and cathodal polarity increases and reduces, respectively, optimization of the mentalizing process. We tested this hypothesis in 48 healthy individuals using a randomized, sham-controlled, double-blind, between-subjects design.

Results

No differences between groups were found with respect to age, education, or the Edinburgh Handedness Inventory (EHI)[36] as assessed by the Kruskall-Wallis Test. The results are reported in **Table 1**.

Table 1. Demographic characteristics.

Group	N.	Gender (F/M)	Age Mean (SD)	Education Mean (SD)	EHI Mean (SD)	Raven'47 Mean (SD)
Anodal	16	5/11	25.68 (6.78)	16 (2)	85.56 (11.47)	29.71 (1.88)
Cathodal	16	9/7	30.75 (11.49)	14.87 (2.06)	71.68 (49.31)	29.79 (1.06)
Sham	16	12/4	23.31 (2.86)	15.43 (1.86)	73.43 (49.14)	29.44 (1.57)
Total	48	26/22	25.71 (6.32)	15.45 (1.96)	70.43 (49.43)	29.66 (1.5)
p-value		**0.044†**	0.18*	0.38*	0.70*	0.89*

F= female; M = male; SD = standard deviation; EHI = Edinburgh Handedness Inventory.

† p-value refers to Chi-Square Test; *p-value refers to Kruskall-Wallis Test.

A lower proportion of females were assigned to the anodal group [5/16 (36%)] compared to the cathodal group [9/16 (56%)] and to the sham group [12/16 (75%)], as tested by the Chi-Square Test (see Table 1). For each group, no differences between baseline and post-stimulation sessions were detected in any of the VAS scores[37] (anxiety, mood and fatigue) (**Table 2**).

Table 2. Wilcoxon test results. The data are presented as means and standard deviation (SD).

p-values ≤ 0.005 are reported in bold. VAS = visual analogue scale; RMET = Reading the mind in the eyes test; Accuracy = number of correct responses.

		Stimulation	**Pre**	**Post**	**Z**	**p-value**
VAS scores	Anxiety	Anodal	3.1 (0.6)	2.8 (0.7)	0.396	0.692
		Cathodal	2.4 (0.45)	1.7 (0.35)	1.127	0.260
		Sham	3.8 (0.6)	2.6 (0.5)	1.490	0.136
	Mood	Anodal	7.6 (0.4)	6.3 (0.5)	0.113	0.910
		Cathodal	6.3 (0.6)	6.8 (0.6)	-0.717	0.474
		Sham	6.2 (0.7)	6.5 (0.6)	-0.282	0.777
	Fatigue	Anodal	4.2 (0.5)	3.3 (0.5)	1.150	0.250
		Cathodal	3.7 (0.9)	3.9 (0.7)	-0.245	0.806
		Sham	3.5 (0.7)	3.8 (0.6)	-0.603	0.546
RMET - Accuracy	Mental state	Anodal	25.5 (2.85)	26.7 (2.26)	-1.008	0.313
		Cathodal	25.9 (2.35)	25.7 (2.6)	0.477	0.633
		Sham	26.9 (2.58)	27.4 (2.91)	-0.570	0.568
	Visual Perception	Anodal	35 (0.82)	35.2 (0.93)	-1.035	0.300
		Cathodal	35.2 (0.6)	35.4 (1.09)	-1.403	0.160
		Sham	35 (0.89)	35 (0.89)	0	1.000
	Visuomotor	Anodal	35.5 (0.73)	35.2 (0.58)	1.31	0.188
		Cathodal	35.9 (0.34)	35.8 (0.4)	0.449	0.653
		Sham	35.8 (0.4)	35.3 (0.87)	1.92	0.054
RMET - RTs (ms)	Mental state	Anodal	3446 (697)	3007 (629)	2.546	**0.00055**
		Cathodal	3805 (649)	3460 (631)	1.470	0.0141
		Sham	3638 (532)	3414 (626)	1.149	0.25
	Visual Perception	Anodal	1009 (207)	924 (182)	1.131	0.25
		Cathodal	1178 (247)	1073 (198)	1.074	0.28

		Sham	1156 (238)	1054 (143)	1.413	0.16
	Visuomotor	Anodal	689 (93)	641 (61)	1.622	0.105
		Cathodal	768 (173)	728 (137)	0.603	0.546
		Sham	750 (99)	703 (87)	1.320	0.187

At baseline, the Kruskal–Wallis one-way analysis of variance evidenced no differences among the three groups for the correct response (MS stimuli: H = 1.39; p = 0.49; V-P stimuli: H = 0.82; p = 0.66; V-M stimuli: H = 3.48; p = 0.17) or RTs in each stimulus type (MS stimuli: H = 2.99; p = 0.22; V-P stimuli: H = 5.84; p = 0.05; V-M stimuli: H = 2.58; p = 0.27).

Moreover, no differences in number of correct responses were found between baseline and post-stimulation sessions in any group or for any stimuli, as assessed by the Wilcoxon test (see Table 2 for statistical details)

Notably, an improvement in the processing of mental state stimuli was evident after anodal stimulation, as shown by the significant decrease in RTs between the baseline and post-stimulation sessions for MS stimuli (Wilcoxon test, p = 0.00055).

A reduction of RTs between baseline and post-stimulation phases was also evident in the cathodal group but was not significant after the Bonferroni correction (Wilcoxon test, p = 0.01). No significant differences in RTs were observed between baseline and post-stimulation sessions for V-P or V-M stimuli in either the anodal or cathodal group. Again, no significant differences were found in RTs of the sham group for any stimulus type. The RMET results are detailed in **Table 2** and illustrated in **Fig 1**.

Discussion

In recent years, non-invasive brain stimulation has been increasingly used to study cerebellar involvement in motor control and cognitive functions[38]. However, only a few studies have used this approach to investigate the cerebellar role in social behaviour, focusing on basic emotion recognition by the processing of facial expressions[24,39]. In particular, Ferrucci and colleagues[26] used a standard facial emotion recognition task, focusing on basic emotions such as anger, happiness, and sadness.

The present study extends the current knowledge in this field, since it demonstrates for the first time the effect of cerebellar neuro-modulation on the mentalizing process.

Specifically, we found that the anodal tDCS delivered over the cerebellum enhanced the processes subtending the ability to recognize the mental state of another person as measured by RMET.

We observed the same tendency when the cathodal polarity was applied, even though it did not reach statistical significance after Bonferroni correction for multiple comparisons.

Interestingly, the effect on mental state processing was not correlated with a general speeding up, given that the cerebellar stimulation did not modify the elaboration speed in terms of RTs when the participants processed perceptual or visuo-motor stimuli. Moreover, in agreement with previous cerebellar neuromodulation studies[26,39], we observed no changes in anxiety, mood or fatigue after cerebellar stimulation.

Although we started from the assumption that increased and reduced cerebellar excitability (due to anodal and cathodal stimulation) could increase and reduce, respectively, optimization of the mentalizing process, we found a similar effect for the two polarity types in the present study. This observation agrees with the lack of polarity-specific tDCS-induced changes observed in cognitive/affective tasks[25,26].

One possible explanation for the lack of polarity specificity of cerebellar tDCS comes from general physiological mechanisms that have been known for years, by which a functional inhibition/disruption in any excitable tissue can be obtained with both depolarisation and hyperpolarisation[40]. For instance, classic neurophysiological experiments demonstrated that axonal conduction can be blocked, even for several hours, by depolarisation ("depolarising" block) and by hyperpolarisation ("hyperpolarising" or "anodal" block), leading to the same decreased excitability of the stimulated tissue[40].

This lack of polarity specificity could well also be applied to the cerebellum, since both hyperpolarisation and depolarisation of the Purkinje cells can lead to a block of cerebellar cortex excitability[23,41]. Furthermore, the effect on cerebellar excitability modulation could be even more complex, considering the cerebellar cortico-nuclear interactions. Specifically, Purkinje cells are well known to exert inhibition on the cerebellar nuclei, which in turn have an excitatory action on the cerebral cortex through the cerebello-thalamo-cortical pathway[14,42]. Therefore, at baseline, the Purkinje cells exert an inhibitory tone on the projection brain areas, named cerebellar brain inhibition[43–46]. Accordingly, any changes in the Purkinje cells' excitability, either positive or negative, might significantly influence the efficiency of information transmission to the projection brain areas[47], through the action exerted on the cerebellar nuclei. Therefore, in our experiment, block of the cerebellar cortex excitability might lead to a disinhibition of the cerebellar nuclei, facilitating the activity of cerebral areas involved in the mentalizing process with which the cerebellum is connected[14,16,42,48]. In this way, cerebellar tDCS improves the response efficiency to mental state stimuli.

The cerebellar role in the mentalizing process

Our study should be seen in the context of increasing interest of the scientific community in the key role of the cerebellum in social behaviour[7,49].

As reported in the introduction, the cerebellar role in the recognition of basic emotions by facial expression has been demonstrated in previous studies, in which standard facial emotion recognition task was used[26,39], focusing on basic emotions such as anger, happiness, and sadness.

Unlike the above-mentioned studies, we used the RMET as a measure of adult ‘mentalising’. Indeed, as described by Baron-Cohen et al.[32], this advanced theory of mind test involves matching terms describing complex mental states to pictures showing only the eye region. In particular, to perform the task correctly subjects have to “match the eyes in each picture to examples of eye-region expressions stored in memory and seen in the context of particular mental states to arrive at a judgement of which word the eyes most closely match”[32].

This match occurs unconsciously, rapidly and at an automatic level, then this task involves only the first stage of attribution of the theory of mind, namely attribution of the relevant mental state such as compassion, but not inferring the content of that mental state (e.g., compassion for her mother’s loss) which represents the second level.

In the present study, cerebellar tDCS-induced changes in this automatic mental state recognition process fit in well with fMRI studies showing that specific cerebellar portions are activated during mirroring and mentalizing tasks[8,50]. Our results are also in agreement with recent studies in patients with cerebellar pathology, in which impairment in mental state recognition measured by the RMET was reported[3,35]. In addition to previous studies, our results provide new evidence on the direct link between cerebellar functioning and the mentalizing process.

Interestingly, the present study adds new insights into the possible mechanisms that are implemented by the cerebellum to modulate the mentalizing process. Specifically, as reported in the introduction, we started from the idea that the cerebellum acts in the social domain in the same way in which it does in the sensorimotor on. Indeed this brain structure performs a predictive action based on forward internal models, signalizing deviations from the attended outcomes to the projection cerebral areas[4]. This operational mode is implemented by the constant modulation that the cerebellum exerts on brain regions involved in the processing of information related to specific functional domains[14].

Accordingly, in the case of RMET used in the present study, the cerebellum acts to implicitly match the external information, such as the expression of the eyes, with the internal model of eye region expression linked to previous emotional experiences to guarantee an immediate judgment about the mental state of others. When cerebellar excitability is modulated by the tDCS, the required fast and continuous exchange of information between the external stimuli and the internal model is facilitated, thus speeding up the automatic processes of mindreading.

Of note, the not normally distribution of our sample might limit the power of present discussion conclusion due to the non-parametric statistical analysis. To obviate this limit, we applied the Bonferroni correction for multiple comparisons, demonstrating that the decrease in the anodal condition is significantly larger than the decrease following cathodal or sham stimulation.

Clinical implications

The present study unveils new scenarios on the possible application of cerebellar tDCS, not only in patients with cerebellar damage but also in those clinical conditions in which this brain structure is implicated. Indeed, as extensively reported in the literature, the cerebellum has been described as involved in the pathogenesis of psychiatric disorders (i.e., schizophrenia) and neurodevelopmental conditions (i.e., autism spectrum disorders) characterized by social behaviour difficulties[51,52]. Specifically, structural and functional cerebellar alterations have been reported in such conditions[51,53–55]. In line with this, knowing more about the cerebellar tDCS-induced changes on mental state recognition ability would aid in developing new therapeutic protocols in these patient populations[56].

The primary strength of this approach is that it does not generate discomfort and can be easily combined with cognitive-behavioural therapy in those pathological conditions that present with pharmacological treatment resistance[57]. All in all, non-invasive cerebellar stimulation may represent a promising strategy for improving residual cerebellar circuit functioning and as a complement tool for rehabilitation protocols in patients with cerebellar dysfunction[58]. However, we have to take into account that the after-effects of tDCS over the cerebellum are highly variable among individuals. Additionally, the stimulation effects could be different depending on whether a behaviour is tested during (on-line effects) or after (off-line effects) the stimulation session.

These aspects highlight the need to better understand the individual factors that determine the efficacy of this technique (e.g., the baseline neural excitability, cognitive capacity, or personality traits) and to test this cerebellar tDCS protocol in a larger and more homogeneous sample.

Therefore, studies employing non-invasive brain stimulation with functional imaging[59] and EEG data could be considered to better understand the effect of cerebellar stimulation on the brain networks in which it participates.

Conclusions

The present study demonstrates that the anodal cerebellar tDCS enhances the processes subtending the ability to recognize the mental state of another person and that the cerebellum directly contributes in the first stage of mental state recognition. Our findings reinforce and extend current knowledge on the role of cerebellum in the mentalizing process, and add new insights about the possible application of cerebellar tDCS in the social cognition domain. In this view the cerebellum could be considered a promising target of non-invasive neuro-stimulation in various impairments of social cognition reported in both neurological and psychiatric disorders associated with cerebellar dysfunction. We believe that these aspects are crucial in clinical practice, and we are confident that our results have clinical and translational potential in terms of treatment implementation.

Methods

Participants

Forty-eight healthy right-handed volunteers with no history of neurological or psychiatric conditions participated in the study (demographic characteristics are reported in **Table 1**). Handedness was confirmed using the Edinburgh Handedness Inventory (EHI)[36].

None of the participants were taking medications or illicit drugs that can affect the central nervous system, and their intellectual level as assessed by Raven's 47 progressive matrices was in the normal range (score below the cut-off value of 18.96)[60].

A double blinded, randomized, placebo-controlled study was conducted, and each participant was randomly assigned to different groups in which anodal, cathodal or sham tDCS was delivered over the cerebellum. All groups were well-matched with respect to age, education and intellectual level as reported in **Table 1**.

A digital version of the RMET[61] and three self-evaluation 'Visual Analogue Scale' (VAS)[37] for mood, anxiety and fatigue were administered before and 35 minutes after the end of the tDCS stimulation over the cerebellum.

All participants signed an informed consent in accordance with the Declaration of Helsinki, and the experimental procedures were approved by the Ethical Committee of the IRCCS Santa Lucia Foundation of Rome (Prot. CE/PROG.570).

Cerebellar tDCS protocol

The real stimulation was delivered by a DC stimulator (Eldith, NeuroConn, Germany) connected to a pair of saline-soaked sponge electrodes (6x7), at 2 mA intensity (current density = 0.06 mA/cm2) for 20 min[26]. The active electrode was centred over the cerebellum (median line, 2 cm below the inion) and the reference electrode over the right deltoid muscle. The extra-cephalic montage was used to avoid any confounding effects due to the action of the opposite polarity electrode on other brain areas[62]. Stimulation was applied with anodal or cathodal polarity, referring to the electrode placed on the cerebellum or in a placebo mode (sham). In sham conditions, the electrodes were placed as for real stimulation, but the stimulator was turned on for 10 to 15 s. During the stimulation session, the participants were not involved in specific cognitively demanding activities and the investigator was present throughout the experimental session to check for adverse effects or any technical issue. Details about the tDCS protocol and montage are also reported in **Fig 2**.

At the end of every tDCS session, participants completed an ad hoc questionnaire to test for possible adverse effects, including headache, nausea, and impaired balance.

Behavioural tasks

Reading the Mind in the Eyes Test (RMET) – digital version

The RMET consists of 36 photographs of the eye-region of different actors (19 men and 17 women) illustrating an emotionally charged or neutral mental state (MS - stimuli). As in the original version by Baron-Cohen and colleagues[32], the subject is required to choose from 4 words (displayed below the photograph) the one that best describes what the person in the photos is thinking or feeling.

In the present study, we used a computerized version of the RMET in which the original photos (MS stimuli) were randomly administered together with two kinds of control stimuli to evaluate visual perception (V-P) (trials = 36) and visual motor (V-M) (trials =36) factors. All the stimuli were presented on a PC screen (size: 38 cm × 21.6 cm; 17 inches) and in the Italian language[63].

In our experiment, subjects sat comfortably approximately 60 cm in front of the PC monitor and used the C, V, B, and N keys of the keyboard to respond. In particular, the left-hand middle finger and index were positioned on the C and V keys, respectively, and the right-hand index and middle fingers on B and N keys, respectively.

In the MS trials, 4 adjectives appear in a four-cell grid placed under each photo, and the subject is required to press the key corresponding to the position in which the chosen adjective appears. In the V-P trials, the words ‘man’ and ‘woman’ appear in two of the four grid cells placed under each photo, and the subject is required to judge the actor’s gender from the eyes’ region, pressing the key corresponding to the position in which the chosen adjective appeared in the grid. For the V-M trials, a black dot appears in one of the four grid cells, and the subject is required to press the key corresponding to the position in which the dot appears.

The three types of stimuli were randomly administered with an inter-trial interval of 6 seconds and were preannounced by a cross appearing in the middle of the screen for 500 ms. Overall, the administration of the digital RMET test takes about 10 minutes.

The subject had to respond as quickly and accurately as possible and did not receive error feedback.

The task was administered, before and 35 minutes after the end of the stimulation session, using a PC with Presentation software, and the correct response (accuracy) and reaction time (RT) in milliseconds were recorded. Trial structure and examples of stimuli are reported in **Fig 2**.

Visual Analogue Scale (VAS)

The VAS[37]-consists of a horizontal line, 100 mm in length, anchored at each end by a word descriptor, and the subject is required to mark on the line the point they felt best represented how they perceived their current state. The VAS score is calculated by measuring the distance from the left-hand end of the line to the point that the subject marks in millimetres.

We used VAS for anxiety (0 mm, no anxiety and 100 mm, the worst anxiety), mood (0 mm, the worst mood and 100 mm, the best mood) and fatigue (0 mm, no fatigue and 100 mm, the highest level of fatigue).

Statistical analysis

The Shapiro-Wilk Test was used to assess our sample distribution, which results not normally distributed (see **Table 3** for details). Therefore, non-parametric analysis was performed.

Table 3. Shapiro-Wilk results.

	Group	Age	Education	EHI	Raven'47
Shapiro-Wilk W	Anodal	0.639	0.792	0.928	0.876
	Cathodal	0.841	0.788	0.645	0.929
	Sham	0.955	0.801	0.515	0.877
	Total	0.758	0.795	0.531	0.892
Shapiro-Wilk p	Anodal	< .001	0.002	0.226	0.034
	Cathodal	0.010	0.002	< .001	0.239
	Sham	0.579	0.003	< .001	0.035
	Total	< .001	< .001	< .001	< .001

EHI = Edinburgh Handedness Inventory.

The Kruskal-Wallis one-way analysis of variance with group (anodal N=16, cathodal N=16, sham N=16) as the independent variable was used to test differences in age, education, Raven[60] and EHI[36] scores. The Chi Square test (N F/M= 26/22) was used to evaluate the association between group and gender.

To exclude significant differences in the performance among the three groups at baseline, the Kruskal–Wallis one-way analysis of variance with group as an independent variable was used to compare the accuracy and RT for each stimulus type (MS, V-P, V-M). Statistical significance was considered at $p < 0.05$, and direct comparisons between groups were performed applying the Bonferroni post hoc correction if necessary.

The Wilcoxon test for matched pairs (dependent samples) was used to assess differences in VAS[37] scores (anxiety, mood and fatigue) and in accuracy and RT for the different stimuli of the RMET[32] before and after tDCS, separately for each group. A p-value ≤ 0.005, as corrected for 9 multiple comparisons by the Bonferroni, was considered significant. Statistical analyses were performed using SPSS for Windows (version 21.0, Armonk, NY: IBM Corp. Released 2012).

Declarations

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Authors' contributions

Conceptualization, Methodology, Original draft preparation: S. C.; Data curation: M. L. and G. F.; Statistical analyses: A.M.; Supervision, Reviewing and Editing: M. L.

Conflicts of interest/Competing interests: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval: Approval of the experimental protocols was obtained from the local ethical committee of the IRCCS Santa Lucia Foundation of Rome (Prot. CE/PROG.570).

Consent to participate: Written informed consent was obtained from all participants before starting the study.

Consent for publication: Information is anonymized and the submission does not include images that may identify the person.

Availability of data and material: The datasets used and analyzed during the current study available from the corresponding author (Dr. Silvia Clausi) on reasonable request.

Code availability: Not applicable

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References

1. Argyropoulos, G. P. D. *et al.* The Cerebellar Cognitive Affective/Schmahmann Syndrome: a Task Force Paper. Cerebellum **19**, 102–125 (2020).
2. Clausi, S. *et al.* The role of the cerebellum in unconscious and conscious processing of emotions: A review. Appl Sci **7**, (2017).
3. Clausi, S. *et al.* The cerebellar predictions for social interactions: theory of mind abilities in patients with degenerative cerebellar atrophy. Front Cell Neurosci **12**, (2019).
4. Ito, M. Control of mental activities by internal models in the cerebellum. Nat Rev Neurosci **9**, 304–313 (2008).
5. Schmahmann, J. D. & Caplan, D. Cognition, emotion and the cerebellum. Brain **129**, 290–292 (2006).
6. Tedesco, A. M. *et al.* The cerebellar cognitive profile. Brain **134**, 3672–3686 (2011).
7. Van Overwalle, F. *et al.* Consensus Paper: Cerebellum and Social Cognition. Cerebellum **19**, 833–868 (2020).
8. Van Overwalle, F., Baetens, K., Mariën, P. & Vandekerckhove, M. Social cognition and the cerebellum: A meta-analysis of over 350 fMRI studies. Neuroimage **86**, 554–572 (2014).
9. Olivito, G. *et al.* Functional changes of mentalizing network in SCA2 patients: novel insights into understanding the social cerebellum. Cerebellum **19**, 235–242 (2020).

10. Van Overwalle, F., D’aes, T. & Mariën, P. Social cognition and the cerebellum: A meta-analytic connectivity analysis. Hum Brain Mapp **36**, 5137–5154 (2015).
11. Van Overwalle, F. & Mariën, P. Functional connectivity between the cerebrum and cerebellum in social cognition: A multi-study analysis. Neuroimage **124**, 248–255 (2016).
12. Sokolov, A. A. The cerebellum in social cognition. Front Cell Neurosci **12**, (2018).
13. Sokolov, A. A., Miall, R. C. & Ivry, R. B. The cerebellum: adaptive prediction for movement and cognition. Trends Cogn Sci **21**, 313–332 (2017).
14. Middleton, F. A. & Strick, P. L. Cerebellar projections to the prefrontal cortex of the primate. J Neurosci **21**, 700–712 (2001).
15. Ramnani, N. The primate cortico-cerebellar system: anatomy and function. Nat Rev Neurosci **7**, 511–522 (2006).
16. Schmahmann, J. D. & Pandya, D. N. The cerebrocerebellar system. Int Rev Neurobiol **41**, 31–60 (1997).
17. Clausi, S. *et al.* Quantification of gray matter changes in the cerebral cortex after isolated cerebellar damage: a voxel-based morphometry study. Neuroscience **162**, 827–35 (2009).
18. Michela Lupo *et al.* Cerebello-cortical alterations linked to cognitive and social problems in patients with spastic paraplegia Type 7: A Preliminary Study. Front Neurol **11**, (2020).
19. Grimaldi, G. *et al.* Non-invasive cerebellar stimulation-a consensus paper. Cerebellum **13**, 121–138 (2014).
20. Nitsche, M. A. *et al.* Modulation of cortical excitability by weak direct current stimulation – technical, safety and functional aspects. Suppl Clin Neurophysiol **56**, 255–276 (2003).
21. Rahman, A. *et al.* Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. J Physiol **591**, 2563–2578 (2013).
22. Ferrucci, R. *et al.* Modulating human procedural learning by cerebellar transcranial direct current stimulation. Cerebellum **12**, 485–492 (2013).
23. Parazzini, M. *et al.* Modelling the electric field and the current density generated by cerebellar transcranial DC stimulation in humans. Clin Neurophysiol **125**, 577–584 (2014).
24. Ferrucci, R. & Priori, A. Transcranial cerebellar direct current stimulation (tcDCS): Motor control, cognition, learning and emotions. Neuroimage **85**, 918–923 (2014).
25. Oldrati, V. & Schutter, D. J. L. G. Targeting the human cerebellum with transcranial direct current stimulation to modulate behaviour: a Meta-Analysis. Cerebellum **17**, 228–236 (2018).
26. Ferrucci, R. *et al.* Cerebellum and processing of negative facial emotions: cerebellar transcranial DC stimulation specifically enhances the emotional recognition of facial anger and sadness. Cogn Emot **26**, 786–799 (2012).
27. Brothers, L. & Ring, B. A neuroethological framework for the representation of mind. J Cogn Neurosci **4**, 107–118 (1992).

28. Premack, D. & Woodruff, G. Does the chimpanzee have a theory of mind? Behav. Brain Sci. **1**, 515–526 (1978).
29. Meltzoff, A. N. & Moore, M. K. Imitation of facial and manual gestures by human neonates. Science (80-.). **198**, 74–78 (1977).
30. Coricelli, G. Two-levels of mental states attribution: from automaticity to voluntariness. Neuropsychologia **43**, 294–300 (2005).
31. Maurer, D. Infants' perception of facedness. in *Social perception in infants* (eds. T, F. & N, F.) 73–100 (Ablex, 1985).
32. Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y. & Plumb, I. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. J Child Psychol Psychiatry Allied Discipl **42**, 241–251 (2001).
33. Oakley, B. F., Brewer, R., Bird, G. & Catmur, C. Theory of mind is not theory of emotion: A cautionary note on the Reading the Mind in the Eyes Test. J Abn Psychol **125**, 818–823 (2016).
34. Francois, Q. & Rossetti, Y. What do theory-of-mind tasks actually measure? Theory and practice. Persp Psychol Sci **15**, 384–396 (2020).
35. Hoche, F., Guell, X., Sherman, J. C., Vangel, M. G. & Schmahmann, J. D. Cerebellar Contribution to Social Cognition. Cerebellum **15**, 732–743 (2016).
36. Oldfield, R. C. The assessment and analysis of handedness: The Edinburgh Inventory. Neuropsychologia **9**, 97–113 (1971).
37. Gift, A. G. Visual analogue scales: measurement of a subjective phenomena. Nurs Res **38**, 286–288 (1989).
38. Van Dun, K., Bodranghien, F., Manto, M. & Mariën, P. Targeting the cerebellum by noninvasive neurostimulation: a Review. Cerebellum **16**, 695–741 (2017).
39. Schutter, D. J. L. G., Enter, D. & Hoppenbrouwers, S. S. High-frequency repetitive transcranial magnetic stimulation to the cerebellum and implicit processing of happy facial expressions. J Psychiatry Neurosci **34**, 60–65 (2009).
40. Lorente de No, R. A study in nerve physiology. Studies from the Rockefeller Institute for Medical Research 1–548 (1947).
41. Rampersad, S. M., Janssen, A. M. & F, L. Simulating transcranial direct current stimulation with a detailed anisotropic human head model. IEEE Trans Neural Syst Rehabil Eng **22**, 441–52 (2014).
42. Kelly, R. M. & Strick, P. L. Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. J Neurosci **23**, 8432–8444 (2003).
43. Daskalakis, Z. J. *et al.* Exploring the connectivity between the cerebellum and motor cortex in humans. J Physiol **557**, 689–700 (2004).
44. Pinto, A. D. & Chen, R. Suppression of the motor cortex by magnetic stimulation of the cerebellum. Exp Brain Res **140**, 505–510 (2001).

45. Ugawa, Y. *et al.* Modulation of motor cortical excitability by electrical stimulation over the cerebellum in man. J Physiol **441**, 57–72 (1991).
46. Ugawa, Y., Uesaka, Y., Terao, Y., Hanajima, R. & Kanazawa, I. Magnetic stimulation over the cerebellum in humans. Ann Neurol **37**, 703–713 (1995).
47. Shah, B., Nguyen, T. T. & Madhavan, S. Polarity independent effects of cerebellar tDCS on short term ankle visuomotor learning. Brain Stimul **6**, 966–968 (2013).
48. Schmahmann, J. D. Dysmetria of thought: clinical consequences of cerebellar dysfunction on cognition and affect. Trends Cogn Sci **2**, 362–371 (1998).
49. Van Overwalle, F., Manto, M., Leggio, M. & Delgado-García, J. M. The sequencing process generated by the cerebellum crucially contributes to social interactions. Med Hypotheses **128**, 33–42 (2019).
50. Heleven, E., Dun, K. & Van Overwalle, F. The posterior cerebellum is involved in constructing social action sequences: An fMRI study. Sci Rep **9**, (2019).
51. Ding, Y. *et al.* Cerebellar structural and functional abnormalities in first-episode and drug-naive patients with schizophrenia: A meta-analysis. Psy Res Neuroima **283**, 24–33 (2019).
52. Fatemi, S. H. *et al.* Consensus paper: pathological role of the cerebellum in autism. Cerebellum **11**, 777–807 (2012).
53. Wang, S. S.-H., Kloth, A. D. & Badura, A. The cerebellum, sensitive periods, and autism. Neuron **83**, 518–532 (2014).
54. Olivito, G. *et al.* Resting-state functional connectivity changes between dentate nucleus and cortical social brain regions in autism spectrum disorders. Cerebellum **6**, 283–292 (2017).
55. Olivito, G. *et al.* Lobular patterns of cerebellar resting-state connectivity in adults with autism spectrum disorder. Eur J Neurosci **47**, 729–735 (2018).
56. Stoodley, C. J. *et al.* Altered cerebellar connectivity in autism and cerebellar-mediated rescue of autism-related behaviors in mice. Nat Neurosci **20**, 1744–1751 (2017).
57. Antal, A. *et al.* Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol **128**, 1774–1809 (2017).
58. Ferrucci, R. *et al.* Cerebellar transcranial direct current stimulation (tDCS), leaves virtual navigation performance unchanged. Front Neurosci **13**, (2019).
59. Rice, L. C., D'Mello, A. M. & Stoodley, C. J. Differential behavioral and neural effects of regional cerebellar tDCS. Neuroscience **462**, 288–302 (2021).
60. Raven, J. C. Progressive Matrices. Sets A, Ab, B: Board and Book Form. (1947).
61. Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y. & Plumb, I. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. J Child Psychol Psychiatry Allied Discipl **42**, 241–251.
62. Ferrucci, R. *et al.* Cerebellar transcranial direct current stimulation impairs the practice-dependent proficiency increase in working memory. J Cogn Neurosci **20**, 1687–1697 (2008).

63. Vellante, M. *et al.* The “Reading the Mind in the Eyes” test: Systematic review of psychometric properties and a validation study in Italy. Cogn Neurpsych **18**, 326–354 (2013).

Figures

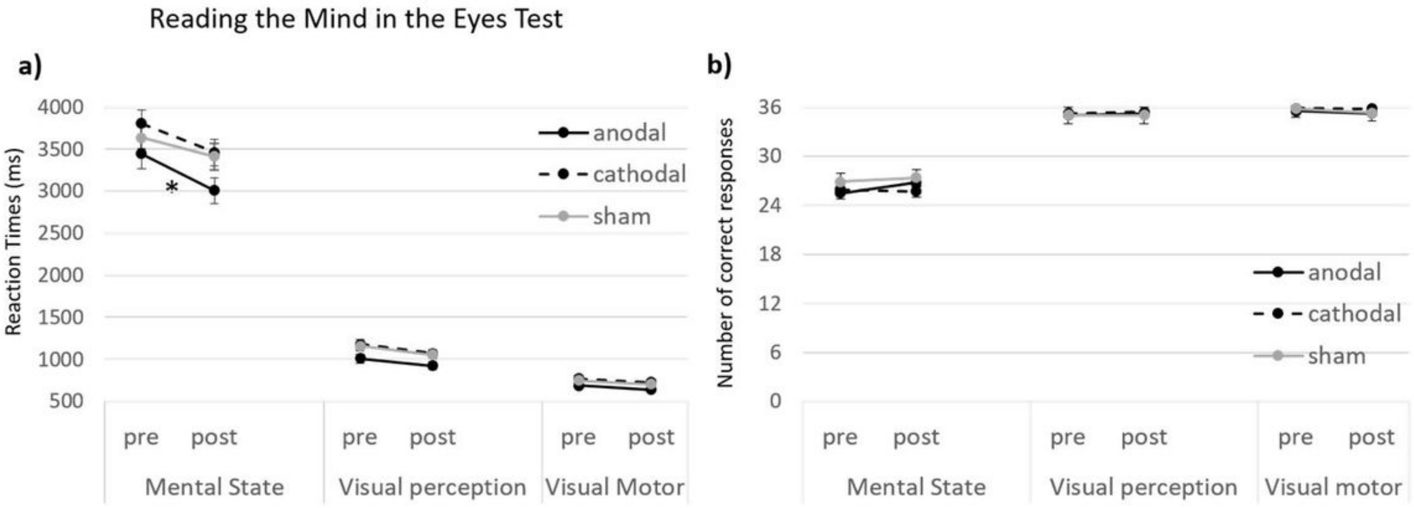

Figure 1

Reading the Mind in the Eyes Test results.

Graphs show the mean reaction times (in milliseconds) (a) and the number of correct responses (b) for each group and stimuli before and after tDCS stimulation. Standard Error is also reported.

* < 0.005.

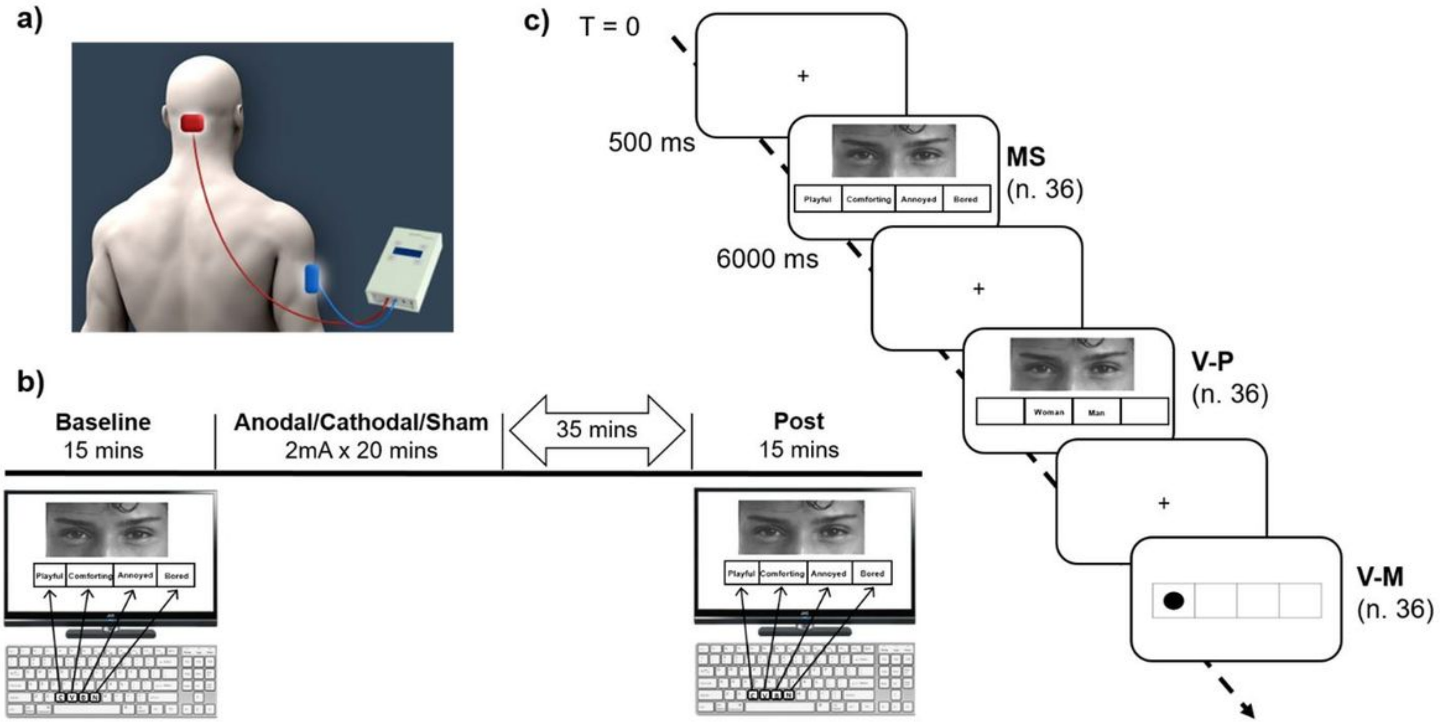

Figure 2

tDCS montage, experimental protocol and stimuli examples.

a) Extra-cephalic montage: the active electrode is centered on the cerebellar vermis and the reference electrode is placed on the right deltoid.

b) tDCS protocol and experimental procedure.

c) Example stimuli of the digital Reading the Mind in the Eyes Test. MS = Mental State stimuli; V-P = visual perception; V-M = visual motor.