Published Ahead of Print on August 22, 2018 as 10.1212/WNL.0000000000006210

ARTICLE CLASS OF EVIDENCE

Cerebello-spinal tDCS in ataxia

A randomized, double-blind, sham-controlled, crossover trial

Alberto Benussi, MD, Valentina Dell'Era, MD, Valentina Cantoni, MS, Elisa Bonetta, MS, Roberto Grasso, PsyD, Rosa Manenti, PhD, Maria Cotelli, PhD, Alessandro Padovani, MD, PhD, and Barbara Borroni, MD

Correspondence
Dr. Borroni
bborroni@inwind.it

Neurology® 2018;00:e1-e12. doi:10.1212/WNL.0000000000006210

Abstract

Objective
To investigate whether a 2-week treatment with cerebellar anodal and spinal cathodal transcranial direct current stimulation (tDCS) could reduce symptoms in patients with neurodegenerative ataxia and could modulate cerebello-motor connectivity at the short and long terms.

Methods
We performed a double-blind, randomized, sham-controlled, crossover trial with cerebello-spinal tDCS (5 d/wk for 2 weeks) in 20 patients with neurodegenerative ataxia. Each patient underwent a clinical evaluation before and after real tDCS or sham stimulation. A follow-up evaluation was performed at 1 and 3 months with a crossover washout period of 3 months. Cerebello-motor connectivity was evaluated with transcranial magnetic stimulation at baseline and at each follow-up.

Results
Cerebello-spinal tDCS showed a significant improvement in all performance scores (Scale for the Assessment and Rating of Ataxia, International Cooperative Ataxia Rating Scale, 9-Hole Peg Test, 8-m walking time), in motor cortex excitability, and in cerebellar brain inhibition compared to sham stimulation.

Conclusions
A 2-week treatment with cerebello-spinal tDCS reduces symptoms in patients with ataxia and restores motor cortex inhibition exerted by cerebellar structures. Cerebello-spinal tDCS might represent a promising future therapeutic and rehabilitative approach in patients with neurodegenerative ataxia, still an orphan disorder of any pharmacologic intervention.

Clinical trial registration
NCT03120013.

Classification of evidence
This study provides Class II evidence that cerebello-spinal stimulation is effective and safe in cerebellar ataxia.

RELATED ARTICLE

Editorial
A novel promising therapeutic approach for patients with ataxic disorders?
Page 541

MORE ONLINE

→ **Class of Evidence**
Criteria for rating therapeutic and diagnostic studies
NPub.org/coe

From the Neurology Unit (A.B., V.D., V.C., E.B., R.G., A.P., B.B.), Department of Clinical and Experimental Sciences, University of Brescia; Department of Neuroscience, Psychology, Drug Research and Child Health (V.C.), University of Florence; and Neuropsychology Unit (R.M., M.C.), IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

ANCOVA = analysis of covariance; **BADL** = basic activities of daily living; **CBI** = cerebellar brain inhibition; **CS** = conditioning stimuli; **8 MW** = 8-m walking time; **IADL** = instrumental activities of daily living; **ICARS** = International Cooperative Ataxia Rating Scale; **ISI** = interstimulus interval; **MSA-C** = cerebellar variant of multiple system atrophy; **MEP** = motor evoked potential; **9HPT** = 9-Hole Peg Test; **rMT** = resting motor threshold; **SARA** = Scale for the Assessment and Rating of Ataxia; **SCA** = spinocerebellar ataxia; **SF-36** = Short-Form Health Survey 36; **tDCS** = transcranial direct current stimulation; **TMS** = transcranial magnetic stimulation; **TS** = target stimuli.

Neurodegenerative ataxias represent a heterogeneous group of disabling diseases characterized by limb and gait ataxia, oculomotor deficits, dysarthria, and kinetic tremor, and patients are occasionally affected by cognitive decline.[1,2]

No effective treatment is currently available for most hereditary and sporadic ataxias, and there is growing interest in finding innovative therapeutic approaches to reduce clinical symptoms.[3]

Recent studies using noninvasive cerebellar stimulation with anodal transcranial direct current stimulation (tDCS) have shown promising results in the treatment of posture, gait, and kinetic functions in patients with ataxia.[4–8] However, stimulation has been limited to cerebellar structures, somehow neglecting the involvement of the spinal cord, which is frequently compromised in most neurodegenerative ataxias.[9] Recent studies have shown that cathodal spinal tDCS may increase corticospinal excitability in healthy controls and in patients with spinal cord injury,[10–12] with modeling studies confirming that the electric field generated by tDCS can reach the spinal cord.[13]

Thus, considering the frequent involvement of both cerebellar and spinal cord structures in neurodegenerative ataxias, it has become clear that the concurrent stimulation of both structures might be synergic in reducing symptoms in this group of patients.

These observations defined the objective of this work, which was aimed at assessing the long-term effects of multiple sessions of concurrent anodal cerebellar tDCS and cathodal spinal tDCS in patients with neurodegenerative ataxia. To this end, we assessed clinical outcomes and cerebello-cerebral connectivity (cerebellar brain inhibition [CBI]) in a randomized, double-blind, sham-controlled, crossover study.

Methods

Standard protocol approvals, registrations, and patient consents

Full written informed consent was obtained from all participants according to the Declaration of Helsinki. The study protocol was approved by the local ethics committee (Brescia Hospital), No. NP1576 approved January 21, 2016. This trial has been registered at ClinicalTrials.gov (NCT03120013).

Primary research questions/classification of evidence

Our primary research question was to determine whether cerebellar anodal tDCS and spinal cathodal tDCS could reduce symptoms and modulate cerebello-cerebral connectivity in patients with ataxia at the short and long terms.

Participants

Twenty-one patients with neurodegenerative ataxia, 7 patients with spinocerebellar ataxia (SCA) type 2,[14] 6 with the cerebellar variant of multiple system atrophy (MSA-C),[15] 1 with SCA38,[16] 1 with SCA14,[17] 1 with Friedreich ataxia,[18] 1 with ataxia with oculomotor apraxia type 2,[19] and 4 with sporadic adult-onset ataxia,[20] were recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, Neurology Unit, University of Brescia, Italy, and entered the study.

The number of included patients, corrected for possible dropouts and patients in whom a reliable motor cortex could not be elicited, was assessed with a power analysis from results obtained from previous studies.[4]

Each patient fulfilled current clinical criteria and genetic traits for the specific diagnosis. All enrolled patients shared a cerebellar syndrome and, as assessed by MRI, had quantifiable cerebellar atrophy.

For each patient, a review of medical history, a semistructured neurologic examination, and a standardized assessment of cerebellar functions were carried out.

Patients were evaluated free of sedative drugs or sodium or calcium channel blockers to avoid any interaction with the presumed neuromodulatory effects of tDCS.

In addition, 10 age-matched healthy controls were recruited as a reference group for transcranial magnetic stimulation (TMS) parameters.

Study design

Patients were randomized into 2 groups; each group received anodal cerebellar tDCS and cathodal spinal tDCS (real tDCS) or sham stimulation for 5 d/wk for 2 weeks in a 1:1 ratio.

At baseline, each patient underwent a clinical evaluation, according to a standardized assessment (see Clinical Assessment below), and CBI evaluation with TMS (see CBI

Assessment) (prestimulation, T0). The same assessments were carried out after 2 weeks of either real or sham tDCS (poststimulation, T1) and at 1-month (T2) and 3-month (T3) follow-up.

After a washout period of 3 months after the last visit (i.e., T3), each patient received the opposite treatment (crossover phase) and underwent the same standardized assessment as in the first phase, at baseline, at 2 weeks after stimulation, at 1 month, and at 3 months (figure 1).

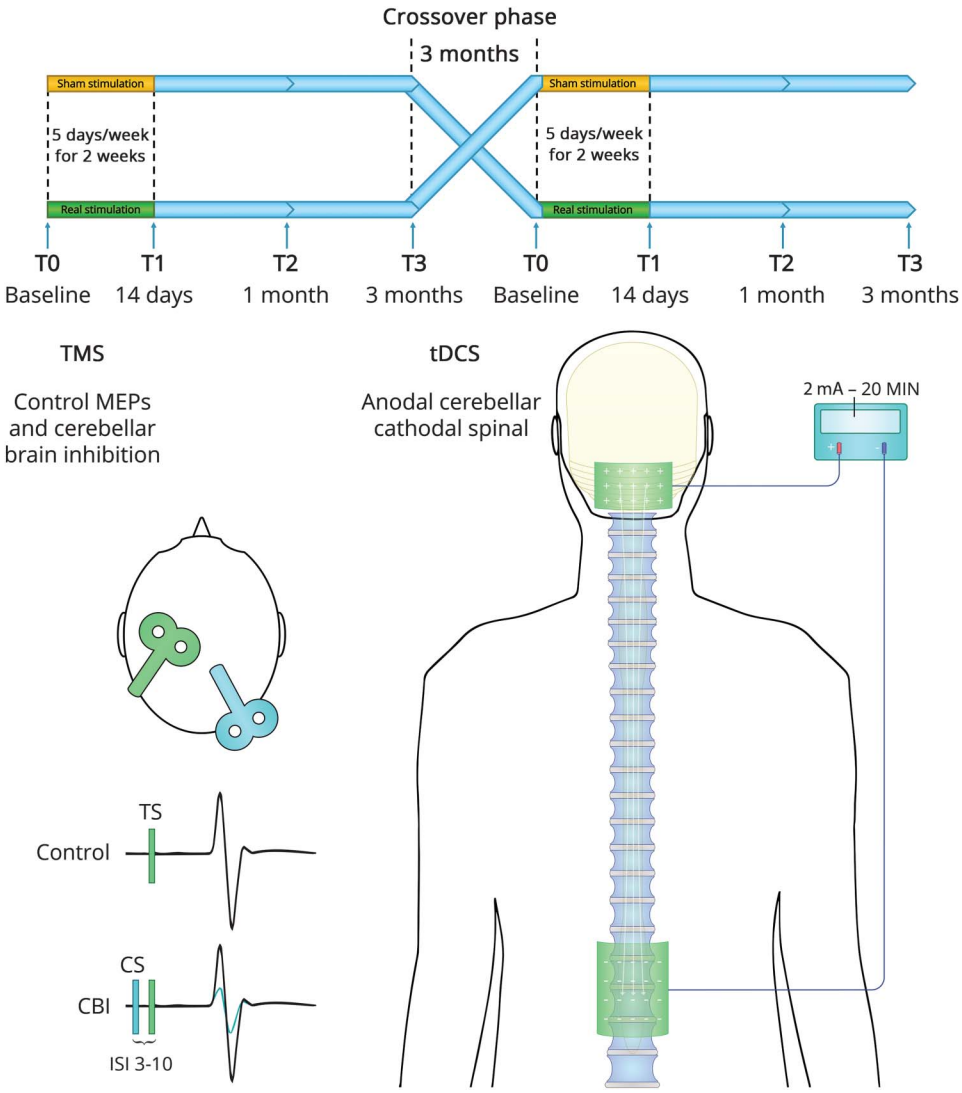
Seven principal investigators were involved: 1 (A.B.) performing the clinical evaluation, 1 (V.C.) performing CBI at baseline and at follow-up, and 4 (V.D., E.B., R.G., R.M.) performing tDCS. The patient and the examiners performing clinical ratings and TMS protocols were blinded to the type of stimulation; 1 investigator (B.B.) was responsible for random allocation sequences, enrollment of participants, and assignment of participants to specific interventions.

Clinical assessment

At each time point, the Scale for the Assessment and Rating of Ataxia (SARA)[21] and the International Cooperative Ataxia Rating Scale (ICARS)[22] were used to evaluate cerebellar deficits.

SARA consists of 8 items, including gait, stance, sitting, speech disturbance, finger chase, nose-finger test, fast alternating hand movements, and heel-shin slide. The higher the score is, the worse the patient's performance is. ICARS is a semiquantitative 100-point scale consisting of 19 items divided into 4 weighted subscores, namely posture and gait disturbances, limb kinetic function, speech disorder, and oculomotor deficits.

Figure 1 Study design



CBI = cerebellar brain inhibition; CS = conditioning stimulus; ISI = interstimulus interval; MEP = motor evoked potential; tDCS = transcranial direct current stimulation; TMS = transcranial magnetic stimulation; TS = test stimulus.

To evaluate finger dexterity and upper limb coordination, 4 timed trials of the 9-Hole Peg Test (9HPT)[23] were performed separately for each hand. The 9HPT is a commonly used test to assess finger dexterity: the patient picks the pegs 1 at time and puts them in 9 holes on a peg board until all holes are filled and then removes them 1 at a time as quickly as possible. The total time to complete the task is recorded for each trial and for each separate hand (dominant and nondominant).

To assess gait speed, we performed, 4 times for each session, the 8-m walking time (8 MW),[24] defined as the time needed to walk 8 m as quickly as possible but safely with any device but without the help of another person or wall.

Finally, the Italian version of the Short-Form Health Survey 36 (SF-36), an interview-administered self-reported scale consisting of 36 scaled scores assessing 8 subdomains (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, mental health, communication, psychosocial and energy), was used to assess changes in the patient's quality of life.[25]

Cerebellar brain inhibition

Two Magstim TMS stimulators connected with two 70-mm figure-of-8 coils (Magstim Company, Oxford, UK) were used to evaluate CBI. The current waveform for the magnetic stimuli had a monophasic configuration, with a rise time of 100 microseconds and decaying back to zero in 800 microseconds.

Surface Ag/AgCl electrodes positioned in a belly-tendon montage on the right first interosseous muscles were used to record motor evoked potentials (MEPs) with a Biopac MP-150 EMG (BIOPAC Systems Inc, Santa Barbara, CA), as previously reported.[26]

The stimulation coil was positioned with the handle directed 45° laterally and posteriorly to the sagittal plane, over the region corresponding to the primary motor cortex (hand area), contralateral to the target first dorsal interosseous FDI. The area where TMS consistently yielded the largest MEP at 120% of the resting motor threshold (rMT) was defined as the motor hotspot and was labeled on the scalp with a marking pen to guarantee a steady placement of the coil throughout the session.[27]

rMT was obtained with the minimum stimulus intensity required to evoke MEPs with an amplitude of at least 50 μV in 50% of 10 continuous trails. Visually checking the absence of EMG activity at high-gain amplification ensured complete muscle relaxation throughout trials.[28]

CBI was assessed with previously described techniques.[29–31] Briefly, the second coil was used to deliver the conditioning stimuli (CS), which was placed over the contralateral cerebellar hemisphere[32] (1 cm inferior and 3 cm right to the inion), a site corresponding to the posterior and superior lobules of the lateral cerebellum.[33] For cerebellar stimulation, the handle was positioned upward with the coil placed tangentially to the skull (figure 1). The cerebellar CS intensities were set at 90% rMT obtained in the ipsilateral motor cortex.[31] CS preceded the target stimuli (TS) by different interstimulus intervals (ISIs) ranging from 3 to 10 milliseconds (3, 5, 10 milliseconds). There were 4 conditions corresponding to the 3 different ISIs and the TS alone. Ten responses were collected for each different ISI and 15 for the TS alone in a pseudorandomized sequence. The amplitude of the conditioning MEPs was expressed as a ratio of the mean unconditioned response. The intertrial interval was set at 5 seconds (±10%).

Transcranial direct current stimulation

tDCS was delivered by a battery-driven constant current stimulator through a pair of saline-soaked (0.9% NaCl) surface sponge electrodes (7×5 cm^2, current density 0.057 mA/cm^2 for the anodal cerebellar electrode; 8×6 cm^2, current density 0.042 mA/cm^2 for the cathodal spinal electrode). The anode was placed on the scalp over the cerebellum area (2 cm under the inion), and the cathode was placed over the spinal lumbar enlargement (2 cm under T11) (figure 1). The electrodes were secured with elastic gauzes, and an electroconductive gel was applied to electrodes to reduce contact impedance (<5 kΩ for all sessions).

During anodal stimulation, a constant current of 2 mA was applied for 20 minutes, as suggested by recently published consensus recommendations[34,35] and on the basis of computation modeling studies.[36–38]

For the sham condition, the electrode placement was the same, but the electric current was ramped down 5 seconds after the beginning of the stimulation to make this condition indistinguishable from the experimental stimulation. To detect differences in the perception of the stimulation, we asked the patients whether they thought they were receiving real or sham stimulation at the end of the 2-week treatment.

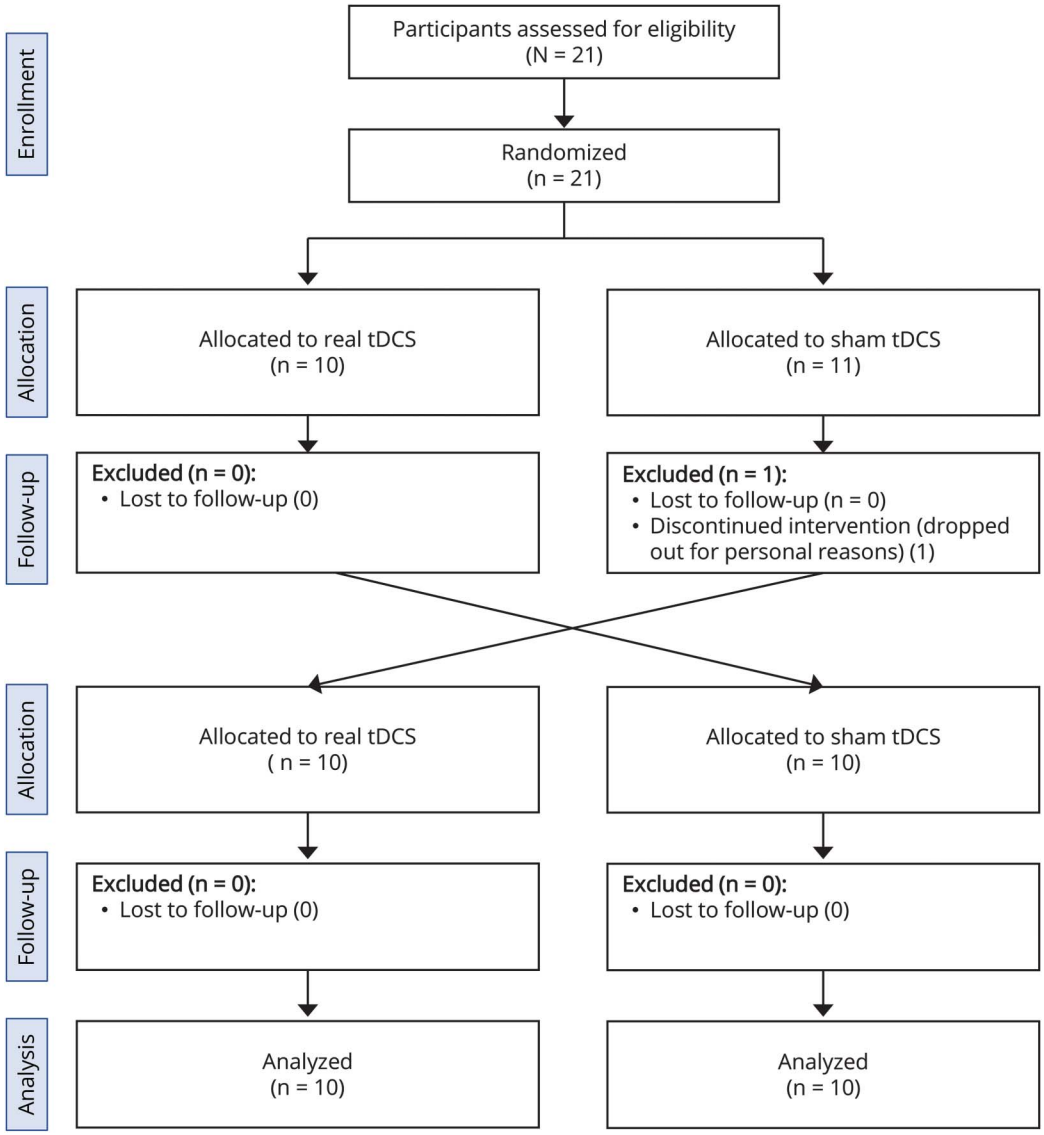
Statistical analyses

To assess the effect of tDCS treatment on clinical scores over time, we used a 2-way repeated-measure analysis of covariance (ANCOVA) with time (T0, T1, T2, and T3) and treatment (sham vs real stimulation) as within-participant factors and the sequence in which stimulation was performed (real-sham vs sham-real) as covariates.

To assess the effect of tDCS treatment on CBI, we used a 3-way repeated-measures ANCOVA with time (T0, T1, T2, and T3), ISI (3, 5, 10 milliseconds) and treatment (sham vs real stimulation) as within-participant factors and the sequence in which stimulation was performed (real-sham vs sham-real) as the covariate.

When a significant main effect was reached, post hoc tests with Bonferroni correction for multiple comparisons were conducted

Figure 2 Flowchart of study patients



tDCS = transcranial direct current stimulation.

to analyze group differences at respective ISIs or time points. The Mauchly test was used to test for assumption of sphericity, while Greenhouse-Geisser epsilon determination was used to correct in case of sphericity violation.

Spearman rank-order correlations were used to assess associations between the improvement in functional scores, neurophysiologic parameters, and demographic or clinical characteristics.

Statistical analyses were performed with SPSS version 21 (SPSS, Inc, Chicago, IL).

Data availability

All data, including outcome measure results, study protocol, and statistical analysis plan, will be shared through ClinicalTrials.gov via public access (clinicaltrials.gov/ct2/show/NCT03120013).

Results

Participants

Twenty-one patients were enrolled and randomized to receive sham or real stimulation first in a 1:1 ratio, with crossover treatment after a 3-month washout period after the last evaluation (T3) 6 months after baseline (T0). One patient with MSA-C dropped out from the study for personal reasons during the first round (sham stimulation) and was not considered in the present analysis (figure 2; for individual disease group randomizations, table e-1, links.lww.com/WNL/A675). Demographic characteristics of included patients are reported in table 1.

Table 1 Demographic and clinical characteristics of included patients

	All patients	SCA	MSA-C	Other ataxias
Patients, n	20	9	5	6
Age, y	54.6 ± 14.5	47.8 ± 12.6	68.0 ± 8.2	53.5 ± 15.1
Age at onset, y	41.7 ± 19.5	29.7 ± 9.4	63.8 ± 10.0	41.2 ± 21.9
Disease duration, y	12.9 ± 12.6	18.1 ± 11.6	4.2 ± 3.1	12.3 ± 16.0
Female, %	50.0	30.0	75.0	66.7
BADL lost	1.7 ± 2.3	1.4 ± 2.2	2.2 ± 2.4	1.7 ± 2.7
IADL lost	3.2 ± 2.6	2.0 ± 2.3	4.6 ± 2.5	3.7 ± 2.7
MMSE score	27.2 ± 3.1	28.4 ± 2.2	26.5 ± 1.6	25.8 ± 4.6
rMT, %	41.6 ± 6.2	45.6 ± 5.1	39.2 ± 6.6	37.6 ± 3.8

Abbreviations: BADL = basic activities of daily living; IADL = instrumental activities of daily living; MMSE = Mini-Mental State Examination; MSA-C = cerebellar variant of multiple system atrophy; rMT = resting motor threshold; SCA = spinocerebellar ataxia.
Results are expressed as mean ± SD.

Patients were recruited from December 2016 to April 2017, with the follow-up ending in December 2017.

Regarding the differences in the patients' perception of the stimulation, there was no statistically significant association between type of stimulation and perception, as assessed by the Fisher exact test ($p = 0.205$), suggesting that real tDCS could not be distinguished from sham stimulation.

Clinical assessment

Baseline SARA scores, ICARS scores, 8 MW, and 9HPT are reported in table 2 (for detailed precrossover and postcrossover measures for each group, table e-2, links.lww.com/WNL/A675).

Repeated-measures ANCOVA performed on SARA scores revealed a significant time × treatment interaction ($F_{3,54} = 38.54$, $p < 0.001$, partial $\eta^2 = 0.68$). The main effect of treatment showed a significant difference between real and sham stimulation at T1, T2, and T3 (all $p < 0.001$) but not at baseline (T0) ($p = 0.422$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.001$) but not in the sham tDCS group (all $p > 0.05$).

For ICARS scores, there was a statistically significant time × treatment interaction ($F_{3,54} = 32.11$, $p < 0.001$, partial $\eta^2 = 0.64$). The main effect of treatment showed a significant difference between real and sham tDCS at T1, T2, and T3 (all $p < 0.001$) but not at baseline (T0) ($p = 0.613$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.001$) but not in the sham tDCS group (all $p > 0.050$) (figure 2B).

The individual assessment of the 4 weighted subscores of the ICARS scale showed that there was a statistically significant time × treatment interaction in the posture and gait ($F_{3,54} = 4.08$, $p = 0.011$, partial $\eta^2 = 0.19$) and in the kinetic limb coordination ($F_{3,54} = 70.16$, $p < 0.001$, partial $\eta^2 = 0.70$) subscores, while there was not a statistically significant time × treatment interaction in the dysarthria ($F_{3,54} = 1.46$, $p = 0.235$, partial $\eta^2 = 0.08$) and in the oculomotor movement ($F_{3,54} = 1.00$, $p = 1.00$, partial $\eta^2 = 0.00$) subscores.

For the posture and gait subscores, the main effect of treatment showed a significant difference between real and sham tDCS at T1, T2, and T3 (all $p < 0.001$) but not at baseline (T0) ($p = 0.705$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.001$) but not in the sham tDCS group (all $p > 0.050$). For the limb coordination subscores, the main effect of treatment showed a significant difference between real and sham tDCS at T1, T2, and T3 (all $p < 0.001$) but not at baseline (T0) ($p = 0.567$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.001$) but not in the sham tDCS group (all $p > 0.050$).

Regarding 9HPT, we observed a significant time × treatment interaction in both the dominant ($F_{3,48} = 7.36$, $p < 0.001$, partial $\eta^2 = 0.32$) and nondominant ($F_{3,45} = 3.94$, $p = 0.014$, partial $\eta^2 = 0.208$) hand. The main effect of treatment showed a significant difference between real and sham tDCS at T1, T2, and T3 (all $p < 0.010$) in both the dominant and nondominant hand but not at baseline (T0) (dominant $p = 0.670$, nondominant $p = 0.926$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.050$) but not in the sham tDCS group (all $p > 0.050$) (figure 3, C and D).

Furthermore, a significant time × treatment interaction was also found in the 8 MW ($F_{3,27} = 5.12$, $p = 0.006$, partial $\eta^2 = 0.34$). The main effect of treatment showed a significant difference between real and sham tDCS at T1, T2, and T3 (all $p < 0.050$)

Table 2 Clinical and neurophysiologic parameters of included patients

	T0	T1	T2	T3
Real tDCS				
SARA score	20.2 ± 7.3	15.8 ± 7.6[a]	15.1 ± 7.7[a]	16.1 ± 7.9[a]
ICARS score	53.0 ± 18.6	43.0 ± 19.6[a]	41.3 ± 19.6[a]	44.2 ± 19.6[a]
9HPT-D score, s	53.0 ± 24.3	46.2 ± 21.3[a]	47.1 ± 21.9[a]	49.5 ± 20.5[a]
9HPT-nD score, s	56.1 ± 21.5	50.2 ± 20.0[a]	50.8 ± 19.1[a]	52.9 ± 19.5[a]
8 MW score, s	9.4 ± 3.4	8.0 ± 2.7[a]	7.8 ± 2.7[a]	8.4 ± 2.8[a]
SF-36 score	54.7 ± 17.5	66.5 ± 17.5[a]	66.7 ± 16.8[a]	61.1 ± 18.8[a]
TMS-rMT, %	41.2 ± 7.2	44.0 ± 8.2[a]	44.3 ± 10.4	41.4 ± 7.9[a]
TMS-CBI, %	0.94 ± 0.12	0.54 ± 0.13[a]	0.65 ± 0.14[a]	0.74 ± 0.11[a]
Sham tDCS				
SARA score	19.9 ± 7.3	19.7 ± 7.5	19.7 ± 7.3	19.9 ± 7.6
ICARS score	52.7 ± 19.4	52.1 ± 19.7	52.5 ± 19.2	52.4 ± 19.4
9HPT-D score, s	52.2 ± 25.9	53.4 ± 23.8	55.8 ± 28.1	54.2 ± 26.2
9HPT-nD score, s	56.2 ± 22.1	56.1 ± 20.7	58.8 ± 22.6	58.9 ± 25.3
8 MW score, s	9.3 ± 3.3	9.0 ± 3.9	9.9 ± 5.0	9.7 ± 4.1
SF-36 score	55.3 ± 19.5	57.9 ± 18.5	60.1 ± 17.8	56.3 ± 18.8
TMS-rMT, %	41.4 ± 7.5	41.5 ± 7.7	41.4 ± 7.9	41.3 ± 7.6
TMS-CBI, %	0.88 ± 0.12	0.91 ± 0.12	0.95 ± 0.13	0.90 ± 0.12

Abbreviations: 8 MW = 8-m walking time; ICARS = International Cooperative Ataxia Rating Scale; 9HPT-D = 9-Hole Peg Test, dominant hand; 9HPT-nD = 9-Hole Peg Test, nondominant hand; rMT = resting motor threshold; SARA = Scale for the Assessment and Rating of Ataxia; SF-36 = Short-Form Health Survey 36; tDCS = transcranial direct current stimulation; TMS-CBI = mean transcranial magnetic stimulation–cerebellar brain inhibition (interstimulus interval 5 milliseconds) expressed as percent of mean motor evoked potential (MEP) amplitude related to the control MEP.
Clinical assessment and neurophysiologic parameters at baseline and after sham stimulation or real tDCS at baseline (T0), 2 weeks (T1), 1 month (T2), and 3 months (T3) after stimulation. Results are expressed as mean ± SD.
[a] Significant difference from baseline (T0) (Bonferroni correction for multiple comparisons).

but not at baseline (T0) ($p = 0.414$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.050$) but not in the sham tDCS group (all $p > 0.050$) (figure 3E).

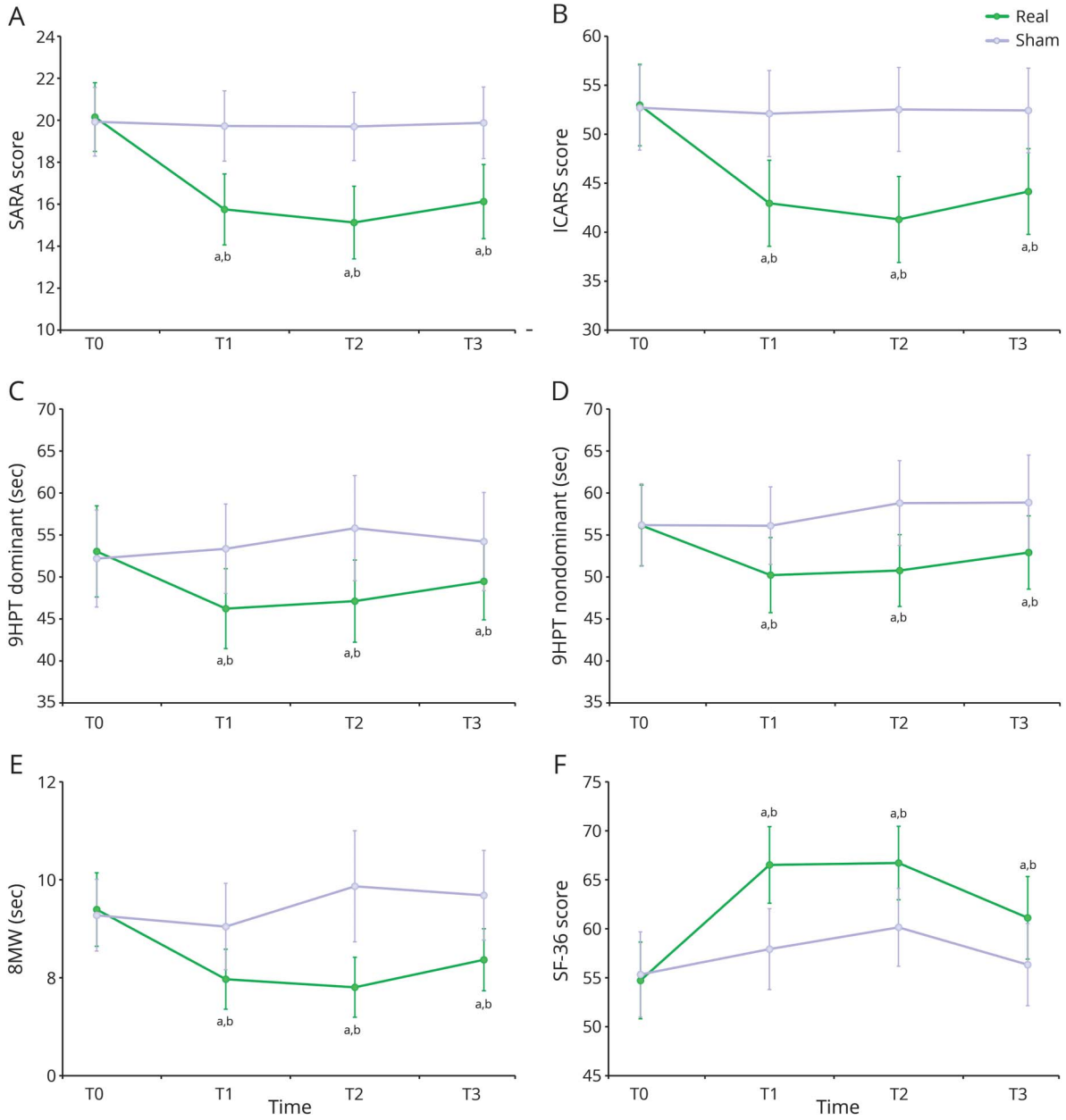
A Spearman rank-order correlation was run to assess the relationship between percentage of average change in functional scores (ICARS and SARA) after the real tDCS trial (T1, T2, and T3) and demographic or clinical characteristics. There was a negative correlation between SARA score at baseline and average change in SARA ($r_s = -0.67$, $p = 0.001$) and ICARS score at baseline and average change in ICARS ($r_s = -0.77$, $p < 0.001$), underlying how patients who showed a greater improvement were less affected clinically. Furthermore, there was a negative association with the number of basic activities of daily living (BADL) and instrumental activities of daily living (IADL) lost and the percentage of improvement in SARA and ICARS scores (BADL and SARA: $r_s = -0.50$, $p = 0.026$; BADL and ICARS: $r_s = -0.61$, $p = 0.004$; IADL and SARA: $r_s = -0.70$, $p = 0.001$; IADL and ICARS: $r_s = -0.70$, $p = 0.001$), further confirming the above statement.

There was no significant association between the percentage of improvement in SARA or ICARS and sex, age at evaluation, age at disease onset, duration of disease, and disease subtype.

When patients with SCA (n = 9) and MSA-C (n = 5) were considered separately, comparable results were found on clinical scores (SCA: SARA $F_{3,21} = 17.36$, $p < 0.001$, partial $\eta^2 = 0.71$; and ICARS $F_{3,21} = 26.23$, $p < 0.001$, partial $\eta^2 = 0.79$; MSA-C: SARA $F_{3,9} = 8.38$, $p = 0.006$, partial $\eta^2 = 0.74$ and ICARS $F_{3,9} = 5.46$, $p = 0.001$, partial $\eta^2 = 0.65$) (for individual precrossover and postcrossover measures for both patients with SCA and those with MSA-C, table e-2, links.lww.com/WNL/A675).

We observed a significant time × treatment interaction in the SF-36 total scores ($F_{3,54} = 2.89$, $p = 0.043$, partial $\eta^2 = 0.132$). The main effect of treatment showed a significant difference between real and sham tDCS at T1, T2, and T3 (all $p < 0.050$) but not at baseline (T0) ($p = 0.775$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.050$) but

Figure 3 Clinical measures of included patients at different time points



(A) Scale for the Assessment and Rating of Ataxia (SARA), (B) International Cooperative Ataxia Rating Scale (ICARS), 9-Hole Peg Test (9HPT) for the (C) dominant and (D) nondominant hand, (E) 8-m walking time (8 MW), and (F) Short-Form 36 (SF-36) scores, before sham and after sham, and real transcranial direct current stimulation at different time points (T0 = baseline, T1 = after 2-week treatment, T2 = at 1-month follow-up, T3 = at 3-month follow-up). Error bars represent standard errors. [a] Significant difference from baseline (T0). [b] Significant difference compared to sham stimulation (Bonferroni correction for multiple comparisons).

not in the sham tDCS group (all $p > 0.050$) (figure 3F). There was also a significant interaction in the physical functioning subscore ($F_{3,54} = 13.40$, $p < 0.001$, partial $\eta^2 = 0.414$), in the vitality and energy subscore ($F_{3,54} = 6.37$, $p = 0.001$, partial $\eta^2 = 0.251$), and in the general health perception subscore ($F_{3,54} = 8.21$, $p < 0.001$, partial $\eta^2 = 0.302$) but not in the other subscores.

No significant differences were observed between T0 baseline scores in patients who were first allocated to real tDCS and then to sham tDCS, possibly underlying that the effects were completely abolished after a 3-month crossover phase (table e-2, links.lww.com/WNL/A675).

Cerebellar brain inhibition

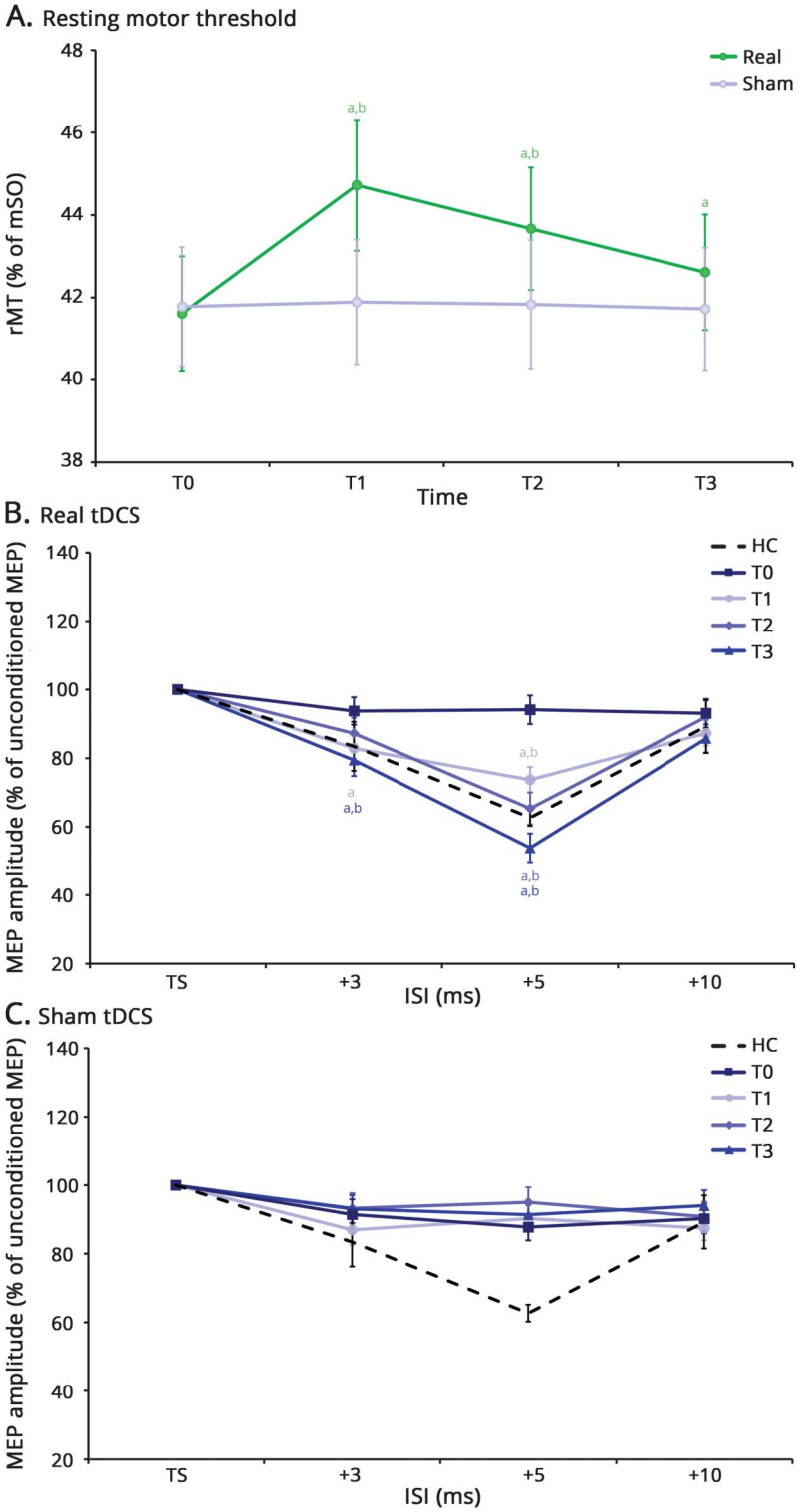
Eighteen patients and 10 age-matched healthy controls underwent CBI assessment with a TMS paired-pulse protocol, while 2 patients could not maintain a constant muscle relaxation and thus were excluded from TMS analysis.

There was a significant increase in average rMT in the real stimulation group with a significant time × treatment

interaction ($F_{3,48} = 9.25$, $p < 0.001$, partial $\eta^2 = 0.37$). The main effect of treatment showed a significant difference between real and sham tDCS at T1 and T2 (all $p < 0.001$) but not at baseline (T0) ($p = 0.454$) or T3 ($p = 0.053$), while the main effect of time showed a significant difference in the real tDCS group at T1, T2, and T3 compared to baseline (T0) (all $p < 0.050$) but not in the sham tDCS group (all $p > 0.050$) (figure 4A).

Repeated-measures ANCOVA performed on CBI measures revealed a statistically significant 3-way interaction between

Figure 4 Neurophysiological measures of included patients at different time points



(A) Resting motor threshold (rMT) and (B) cerebellar brain inhibition assessed by transcranial magnetic stimulation (TMS) in the (B) real and (C) sham stimulation groups at different interstimulus intervals (ISIs) and at different time points (T0 = baseline, T1 = after 2-week treatment, T2 = at 1-month follow-up, T3 = at 3-month follow-up). Data are plotted as a ratio to the unconditioned motor evoked potential (MEP) amplitude. Error bars represent standard errors. HC = healthy control; tDCS = transcranial direct current stimulation; mSO = maximal Stimulator Output. [a] Significant difference from baseline (T0); [b] Significant difference compared to sham stimulation (Bonferroni correction for multiple comparisons).

time (T0, T1, T2, T3), ISI (3, 5, and 10 milliseconds), and group (sham vs real) ($F_{6,96}$ = 5.691, p < 0.001, partial η^2 = 0.26). There was a statistically significant simple 2-way time × ISI interaction for the real tDCS group ($F_{6,96}$ = 7.58, p < 0.001) but not for sham stimulation ($F_{6,96}$ = 1.27, p = 0.28, partial η^2 = 0.07). There was a statistically significant simple main effect of time for the real tDCS group at ISI of 5 milliseconds (p < 0.001) but not for ISI of 3 or 10 milliseconds (p > 0.05) or for sham stimulation at all ISIs (3, 5, and 10 milliseconds) (p > 0.05). There was a statistically significant simple main effect of ISI for the real tDCS group at time T1, T2, and T3 (p < 0.001) but not at T0 or for sham stimulation at all time points (T0, T1, T2, T3) (p > 0.050) (figure 4, B and C).

A significant correlation was observed between the percentage of improvement in SARA and ICARS scores and the restoration of CBI (SARA: r_s = 0.595, p = 0.009; ICARS: r_s = 0.496, p = 0.036).

Discussion

In the present work, we observed a significant improvement in clinical scores and in neurophysiologic measure of motor cortex excitability and cerebellar-cerebral connectivity after a 2-week treatment with anodal cerebellar tDCS and cathodal spinal tDCS in patients with neurodegenerative ataxia. Patients who were less affected clinically and functionally showed the greatest improvement in clinical scores, which outlasted the stimulation interval for at least 3 months.

Previous studies have already identified temporary functional improvement after a single session of cerebellar tDCS in patients with ataxia,[2,39–41] and 1 study reported long-lasting clinical effects after repeated sessions of anodal cerebellar tDCS.[4,5]

In the present work, we corroborated and extended previous preliminary data, and we carried out a randomized, double-blind, sham-controlled, crossover, first-in-humans trial by using combined anodal cerebellar and cathodal spinal tDCS. This clinical trial sheds further light on the possibility of treating patients with neurodegenerative ataxia, which mostly remains an orphan of disease-modifying therapies. Compared to previous studies on the application of tDCS in cerebellar ataxias, we have increased the strength of our observations by using a crossover design and implementing the concurrent stimulation of the spinal cord. An important aspect that should be assessed in future studies is whether the concurrent stimulation of the spinal cord is synergic in improving functional outcome measure compared to cerebellar-only stimulation. A previous study implementing a 2-week treatment with cerebellar-only anodal tDCS has shown a significant long-term improvement in SARA and ICARS scores but not in 9HPT, 8 MW, or quality-of-life scores.[5] However, several differences distinguish these studies; thus, comparisons should be interpreted with caution.

No significant placebo effect was observed in clinical outcome measures, possibly resulting from a complex response expectancy effect in which the conditional response was frequently not consciously mediated, while for other scales such as quality-of-life scores (SF-36) in which the conditional response corresponded to the actual patients' outcomes, the improvement in outcome scores was substantially increased. Moreover, response expectancy mechanisms tend to explain placebo effect at the short term but not the long term.[42]

tDCS has proved to be effective, noninvasive, and easily applicable with long-lasting effects, with the possibility of implementing repeated stimulations over time to extend the duration of its effects.[38]

The physiopathologic mechanisms underlying the effects of noninvasive stimulation of the cerebellar cortex and of the spinal cord are still not completely understood. Cerebellar tDCS seems to exert its effects by inducing an excitatory tone on Purkinje cells and changing the pattern of activity in the deep cerebellar output nuclei, with anodal tDCS increasing the excitability of the cerebellar cortex,[43,44] enhancing the physiologic inhibitory tone over the primary motor cortex through the inhibition of the dentate nucleus, which has an excitatory effect on the ventrolateral motor thalamus and eventually on the motor cortex.[32,45,46]

On the other hand, transcutaneous spinal tDCS has been shown to influence the ascending and descending spinal pathways and spinal reflex excitability, with increasing evidence that it can induce prolonged functional neuroplastic changes.[47] Cathodal spinal tDCS has been shown to improve gait training in chronic stroke patients[48] and to improve functional outcomes, decreasing spasticity in chronic spinal cord injury.[11]

At the molecular level, mechanisms of action of tDCS could involve the modulation of ionic gradients in the extracellular space; inactivation or activation of specific cellular processes, including gene expression, protein synthesis, and channel or pump regulation; and receptor or neurotransmitter modulation.[44] Furthermore, multiple sessions seem to have a cumulative effect and are needed to induce reliable and long-lasting aftereffects, possibly mediated by the modulation of neuronal plasticity.[49]

The neurophysiologic evaluations performed with TMS in this study showed a sustained restoration of CBI in patients treated with tDCS compared to patients who underwent sham stimulation. This was further corroborated by the significant decrease in the motor threshold only after real stimulation.

On the basis of these results, the observed clinical improvement seems to effectively correlate with an increase excitability of the cerebellar cortex and therefore of the cerebello-thalamo-cortical connections, as demonstrated by an increase in CBI.

The milder the disease stage was, the greater the observed clinical improvement was, suggesting that tDCS should be delivered at an early stage of disease to be more effective.

We acknowledge that the present study has some limitations. Neurodegenerative cerebellar ataxias are considerably uncommon, and our group of patients was relatively small and heterogeneous, so clear-cut associations need to be made with caution. Studies on repetition rate, session duration, and number of sessions have not been performed for cerebellar tDCS,[41] and the optimal repetition rate and ISI still have to be determined. Finally, the effect of tDCS on cognitive functions was not objectively assessed in this study.

Another important aspect that was not evaluated in this study was the effect of tDCS on orthostatic hypotension, particularly in patients with MSA-C, considering the prominent involvement of autonomic pathways in this disease,[15] bearing in mind the possible effects of spinal tDCS on the intermediolateral gray columns of the spinal cord. Furthermore, Unified Parkinson's Disease Rating Scale scores should be assessed in future clinical trials in patients with extrapyramidal syndromes treated with cerebellar tDCS.

In the light of limited pharmacologic and nonpharmacologic treatment options for patients with neurodegenerative ataxia, on the basis of the results of this study, a 2-week treatment with cerebello-spinal tDCS could be considered a potentially promising tool for future rehabilitative approaches.[50]

Author contributions

A.B. and B.B. made substantial contributions to the conception and design of the study. A.B. and B.B. did the literature searches. A.B. and B.B. did the statistical analysis and drafted the figures. A.B. and B.B. drafted the initial version of the manuscript. All authors contributed to data acquisition and data interpretation, revised the manuscript critically for important intellectual content, approved the final, submitted version of the manuscript, and agreed to be accountable for all aspects of the work.

Study funding

This Study was supported by AIRAlzh ONLUS-COOP Italia to V.C.

Disclosure

A. Benussi, V. Dell'Era, V. Cantoni, E. Bonetta, R. Grasso, R. Manenti, and M. Cotelli report no disclosures relevant to the manuscript. A. Padovani is consultant for and served on the scientific advisory board of GE Healthcare, Eli-Lilly, and Actelion Ltd Pharmaceuticals and received speaker honoraria from Nutricia, PIAM, Lansgstone Technology, GE Healthcare, Lilly, UCB Pharma, and Chiesi Pharmaceuticals. B. Borroni reports no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Received February 5, 2018. Accepted in final form June 6, 2018.

References

1. Marmolino D, Manto M. Past, present and future therapeutics for cerebellar ataxias. Curr Neuropharmacol 2010;8:41–61.
2. Olivito G, Lupo M, Iacobacci C, et al. Structural cerebellar correlates of cognitive functions in spinocerebellar ataxia type 2. J Neurol 2018;129:1–10.
3. Manes M, Alberici A, Di Gregorio E, et al. Docosahexaenoic acid is a beneficial replacement treatment for spinocerebellar ataxia 38. Ann Neurol 2017;82:615–621.
4. Benussi A, Koch G, Cotelli M, Padovani A, Borroni B. Cerebellar transcranial direct current stimulation in patients with ataxia: a double-blind, randomized, sham-controlled study. Mov Disord 2015;30:1701–1705.
5. Benussi A, Dell'Era V, Cotelli MS, et al. Long term clinical and neurophysiological effects of cerebellar transcranial direct current stimulation in patients with neurodegenerative ataxia. Brain Stimul 2017;10:242–250.
6. van Dun K, Manto M. Non-invasive cerebellar stimulation: moving towards clinical applications for cerebellar and extra-cerebellar disorders. Cerebellum 2017;48:1–5.
7. van Dun K, Bodranghien F, Manto M, Mariën P. Targeting the cerebellum by non-invasive neurostimulation: a review. Cerebellum 2016;16:695–741.
8. Grecco LAC, Oliveira CS, Duarte NA, Lima VLCC, Zanon N, Fregni F. Cerebellar transcranial direct current stimulation in children with ataxic cerebral palsy: a sham-controlled, crossover, pilot study. Dev Neurorehabil 2017;20:142–148.
9. Seidel K, Siswanto S, Brunt ERP, Dunnen den W, Korf HW, Rüb U. Brain pathology of spinocerebellar ataxias. Acta Neuropathol 2012;124:1–21.
10. Bocci T, Barloscio D, Vergari M, et al. Spinal direct current stimulation modulates short intracortical inhibition. Neuromodulation 2015;18:686–693.
11. Powell ES, Carrico C, Raithatha R, Salyers E, Ward A, Sawaki L. Transvertebral direct current stimulation paired with locomotor training in chronic spinal cord injury: a case study. NeuroRehabilitation 2016;38:27–35.
12. Bocci T, Marceglia S, Vergari M, et al. Transcutaneous spinal direct current stimulation modulates human corticospinal system excitability. J Neurophysiol 2015;114:440–446.
13. Fiocchi S, Ravazzani P, Priori A, Parazzini M. Cerebellar and spinal direct current stimulation in children: computational modeling of the induced electric field. Front Hum Neurosci 2016;10:306.
14. Pulst SM, Nechiporuk A, Nechiporuk T, et al. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 1996;14:269–276.
15. Gilman S, Wenning GK, Low PA, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 2008;71:670–676.
16. Di Gregorio E, Borroni B, Giorgio E, et al. ELOVL5 mutations cause spinocerebellar ataxia 38. Am J Hum Genet 2014;95:209–217.
17. Yamashita I, Sasaki H, Yabe I, et al. A novel locus for dominant cerebellar ataxia (SCA14) maps to a 10.2-cM interval flanked by D19S206 and D19S605 on chromosome 19q13.4-qter. Ann Neurol 2000;48:156–163.
18. Filla A, De Michele G, Coppola G, et al. Accuracy of clinical diagnostic criteria for Friedreich's ataxia. Mov Disord 2000;15:1255–1258.
19. Moreira M-C, Klur S, Watanabe M, et al. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nat Genet 2004;36:225–227.
20. Abele M, Bürk K, Schöls L, et al. The aetiology of sporadic adult-onset ataxia. Brain 2002;125:961–968.
21. Yabe I, Matsushima M, Soma H, Basri R, Sasaki H. Usefulness of the Scale for Assessment and Rating of Ataxia (SARA). J Neurol Sci 2008;266:164–166.
22. Trouillas P, Takayanagi T, Hallett M, et al. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. J Neurol Sci 1997;145:205–211.
23. Mathiowetz V, Weber K, Kashman N, Volland G. Adult norms for the Nine Hole Peg Test of finger dexterity. Am J Occup Ther 1985;5:24–38.
24. Schmitz-Hübsch T, Giunti P, Stephenson DA, et al. SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. Neurology 2008;71:486–492.
25. Ware JE, Sherbourne CD. The MOS 36-Item Short-Form Health Survey (SF-36), I: conceptual framework and item selection. Med Care 1992;30:473–483.
26. Benussi A, Di Lorenzo F, Dell'Era V, et al. Transcranial magnetic stimulation distinguishes Alzheimer disease from frontotemporal dementia. Neurology 2017;89:665–672.
27. Benussi A, Cosseddu M, Filareto I, et al. Impaired long-term potentiation-like cortical plasticity in presymptomatic genetic frontotemporal dementia. Ann Neurol 2016;80:472–476.
28. Rossini PM, Barker AT, Berardelli A, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application: report of an IFCN committee. Electroencephalogr Clin Neurophysiol 1994;91:79–92.
29. Koch G, Porcacchia P, Ponzo V, et al. Effects of two weeks of cerebellar theta burst stimulation in cervical dystonia patients. Brain Stimul 2014;7:564–572.
30. Brusa L, Ponzo V, Mastropasqua C, et al. Theta burst stimulation modulates cerebellar-cortical connectivity in patients with progressive supranuclear palsy. Brain Stimul 2014;7:29–35.
31. Carrillo F, Palomar FJ, Conde V, et al. Study of cerebello-thalamocortical pathway by transcranial magnetic stimulation in Parkinson's disease. Brain Stimul 2013;6:582–589.
32. Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. Ann Neurol 1995;37:703–713.
33. Del Olmo MF, Cheeran B, Koch G, Rothwell JC. Role of the cerebellum in externally paced rhythmic finger movements. J Neurophysiol 2007;98:145–152.
34. Grimaldi G, Argyropoulos GP, Boehringer A, et al. Non-invasive cerebellar stimulation–a consensus paper. Cerebellum 2014;13:121–138.
35. Antal A, Alekseichuk I, Bikson M, et al. Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol 2017;128:1774–1809.

36. Parazzini M, Rossi E, Ferrucci R, et al. Computational model of cerebellar transcranial direct current stimulation. Conf Proc IEEE Eng Med Biol Soc 2013;2013:237–240.
37. Parazzini M, Fiocchi S, Liorni I, et al. Modeling the current density generated by transcutaneous spinal direct current stimulation (tsDCS). Clin Neurophysiol 2014; 125:2260–2270.
38. Lefaucheur JP, Antal A, Ayache SS, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol 2017;128:56–92.
39. Grimaldi G, Manto M. Anodal transcranial direct current stimulation (tDCS) decreases the amplitudes of long-latency stretch reflexes in cerebellar ataxia. Ann Biomed Eng 2013;41:2437–2447.
40. Grimaldi G, Oulad Ben Taib N, Manto M, Bodranghien F. Marked reduction of cerebellar deficits in upper limbs following transcranial cerebello-cerebral DC stimulation: tremor reduction and re-programming of the timing of antagonist commands. Front Syst Neurosci 2014;8:9.
41. van Dun K, Bodranghien FCAA, Mariën P, Manto MU. tDCS of the cerebellum: where do we stand in 2016? Technical issues and critical review of the literature. Front Hum Neurosci 2016;10:208.
42. Hyland ME. Motivation and placebos: do different mechanisms occur in different contexts? Philos Trans R Soc Lond B Biol Sci 2011;366:1828–1837.
43. Galea JM, Jayaram G, Ajagbe L, Celnik P. Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. J Neurosci 2009;29:9115–9122.
44. Grimaldi G, Argyropoulos GP, Bastian A, et al. Cerebellar transcranial direct current stimulation (ctDCS): a novel approach to understanding cerebellar function in health and disease. Neuroscientist 2016;22:83–97.
45. Ugawa Y, Hanajima R, Kanazawa I. Motor cortex inhibition in patients with ataxia. Electroencephalogr Clin Neurophysiol 1994;93:225–229.
46. Daskalakis ZJ, Paradiso GO, Christensen BK, Fitzgerald PB, Gunraj C, Chen R. Exploring the connectivity between the cerebellum and motor cortex in humans. J Physiol 2004;557:689–700.
47. Nardone R, Höller Y, Taylor A, et al. Noninvasive spinal cord stimulation: technical aspects and therapeutic applications. Neuromodulation 2015;18:580–591.
48. Picelli A, Chemello E, Castellazzi P, et al. Combined effects of transcranial direct current stimulation (tDCS) and transcutaneous spinal direct current stimulation (tsDCS) on robot-assisted gait training in patients with chronic stroke: a pilot, double blind, randomized controlled trial. Restor Neurol Neurosci 2015;33: 357–368.
49. Brunoni AR, Nitsche MA, Bolognini N, et al. Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stimul 2012;5: 175–195.
50. Block HJ, Celnik P. Can cerebellar transcranial direct current stimulation become a valuable neurorehabilitation intervention? Expert Rev Neurother 2012; 12:1275–1277.