Title of the study: Rehabilitative Trial With Cerebello-Spinal tDCS in Neurodegenerative Ataxia

NCT number: NCT03120013

Date: January 21, 2016

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), #NP1576 approved 01.21.16. 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 improve symptoms and modulate cerebello-cerebral connectivity in patients with ataxia, at short and long-term.

Participants

Twenty-one patients with neurodegenerative ataxia, respectively seven patients with spinocerebellar ataxia (SCA) type 2,[1] six with the cerebellar variant of multiple system atrophy (MSA-C),[2] one with SCA38,[3] one with SCA14,[4] one with Friedreich's ataxia,[5] one with Ataxia with Oculomotor Apraxia (AOA) type 2,[6] four with sporadic adult-onset ataxia,[7] were recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, Neurology Unit, University of Brescia, Italy and entered the study. One patient with MSA-C dropped out from the study during the first round (sham stimulation) and was not considered in the present analysis.

The number of included patients, corrected for possible drop-outs and patients in which a reliable motor cortex could not be elicited, was assessed using a power analysis, from results obtained from previous studies.[8]

Each patient fulfilled current clinical criteria and genetic trait 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 past medical history, a semi-structured neurological examination and a standardized assessment of cerebellar functions was 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, ten age-matched healthy control subjects were recruited as reference group for TMS parameters.

Study design

Patients were randomized into two groups: each group received anodal cerebellar tDCS and cathodal spinal tDCS (real tDCS) or sham stimulation for 5 days/week for 2 weeks, in a 1:1 ratio respectively.

At baseline, each patient underwent a clinical evaluation, according to a standardized assessment (see below, clinical assessment), and CBI evaluation using Transcranial Magnetic Stimulation (TMS) (see below, CBI assessment) (pre-stimulation, T0). The same assessments were carried out after two-weeks of either real or sham tDCS (post-stimulation, T1), at one-month (T2) and at three-months follow-up (T3).

After a wash-out period of three months after the last visit (i.e., T3), each patient received the opposite treatment (cross-over phase), and underwent the same standarized assessment as in the first phase, at baseline, at two-weeks post-stimulation, at one-month and at three-months (see **Fig 1**).

Six principal investigators were involved: one performing the clinical evaluation (A.B.), one performing CBI at baseline and at follow-up (V.C.) and four performing tDCS (V.D., E.B., R.G., R.M.). The patient and the examiners performing clinical ratings and TMS protocols were blinded to the type of stimulation.

Clinical assessment

At each time-point, the Scale for the Assessment and Rating of Ataxia (SARA)[9] and the International Cooperative Ataxia Rating Scale (ICARS)[10] were employed to evaluate cerebellar deficits.

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

To evaluate finger dexterity and upper limb coordination, four timed trials of the 9-hole peg test (9HPT)[11] were performed separately for each hand. The 9HPT is a commonly used test to assess finger dexterity: the patient picks the pegs one at time and puts them in nine holes on a peg board until all holes are filled and then removes them one 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 non-dominant).

To assess gait speed, we performed, four times for each session, the 8-meter walking time (8MW),[12] defined as the time needed to walk 8 meters "as quickly as possible but safely", with any device but without 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.[13]

Cerebellar Brain Inhibition (CBI)

TMS was performed with two figure-of-eight coils (each loop diameter 70 mm) connected to two Magstim stimulators (Magstim Company, Oxford, UK). The magnetic stimuli had a monophasic

current waveform (rise time of 100 μs, decaying back to zero over 800 μs). Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle (FDI) through surface Ag/AgCl electrodes placed in a belly-tendon montage and acquired using a Biopac MP-150 electromyograph (BIOPAC Systems Inc., Santa Barbara, CA, USA).

The TMS coil was held tangentially over the scalp region corresponding to the primary hand motor area contralateral to the target muscle, with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The motor hot spot was defined as the location where TMS consistently produced the largest MEP size at 120% of the resting motor threshold (rMT) in the target muscle and was marked with a felt tip pen on the scalp to ensure constant placement of the coil throughout the experiment.

rMT was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 50 μV in 5 out of 10 consecutive trails during complete muscle relaxation, which was controlled by visually checking the absence of EMG activity at high-gain amplification [14].

CBI was assessed using previously described techniques [15-17]. Briefly, the second coil was used to deliver the conditioning stimuli (CS) which was placed over the contralateral cerebellar hemisphere [18] (1 cm inferior and 3 cm right to the inion), a site corresponding to the posterior and superior lobules of the lateral cerebellum [19]. For cerebellar stimulation, the handle was positioned upward with the coil placed tangentially to the skull (see **Fig. 1**). The cerebellar CS intensities were set at 90% rMT obtained in the ipsilateral motor cortex [17]. CS preceded the target stimuli (TS) by different interstimulus intervals (ISIs) ranging from 3 to 10 ms (3, 5, 10 ms). There were four conditions, corresponding to the three different ISI and the TS alone. Ten responses were collected for each different ISI and fifteen 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 inter trial interval was set at 5 sec (±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 0.041 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 over the spinal lumbar enlargement (2 cm under T11) (see **Fig. 1**). The electrodes were secured using 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[20,21] and on the basis of computation modeling studies.[22,23]

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 two-weeks' treatment.

Statistical Analyses

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

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

When a significant main effect was reached, *post hoc* tests with Bonferroni correction for multiple comparisons were conducted to analyze group-differences at respective ISIs or time points. Mauchly's test was used to test for assumption of sphericity, while Greenhouse–Geisser epsilon determination was used to correct in case of sphericity violation.

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

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

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