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Cortico-spinal tDCS in amyotrophic lateral sclerosis: A randomized, double-blind, sham-controlled trial followed by an open-label phase

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ABSTRACT

Background: Amyotrophic lateral sclerosis (ALS) is a progressive disease for which no curative treatment is currently available.
Objective: This study aimed to investigate whether cortico-spinal transcranial direct current stimulation (tDCS) could mitigate symptoms in ALS patients via a randomized, double-blind, sham-controlled trial, followed by an open-label phase.
Methods: Thirty-one participants were randomized into two groups for the initial controlled phase. At baseline (T0), Group 1 received placebo stimulation (sham tDCS), while Group 2 received cortico-spinal stimulation (real tDCS) for five days/week for two weeks (T1), with an 8-week (T2) follow-up (randomized, double-blind, sham-controlled phase). At the 24-week follow-up (T3), all participants (Groups 1 and 2) received a second treatment of anodal bilateral motor cortex and cathodal spinal stimulation (real tDCS) for five days/week for two weeks (T4). Follow-up evaluations were performed at 32-weeks (T5) and 48-weeks (T6) (open-label phase). At each time point, clinical assessment, blood sampling, and intracortical connectivity measures using transcranial magnetic stimulation (TMS) were evaluated. Additionally, we evaluated survival rates.
Results: Compared to sham stimulation, cortico-spinal tDCS significantly improved global strength, caregiver burden, and quality of life scores, which correlated with the restoration of intracortical connectivity measures. Serum neurofilament light levels decreased among patients who underwent real tDCS but not in those receiving sham tDCS. The number of completed 2-week tDCS treatments significantly influenced patient survival.
Conclusions: Cortico-spinal tDCS may represent a promising therapeutic and rehabilitative approach for patients with ALS. Further larger-scale studies are necessary to evaluate whether tDCS could potentially impact patient survival.
Clinical trial registration: NCT04293484.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a complex and progressive neurodegenerative disorder that affects both upper and lower motor neurons, resulting in a fatal prognosis. Despite ongoing research efforts, the development of effective disease-modifying therapies remains

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elusive, and patients diagnosed with ALS continue to face a median survival of three to five years from symptom onset [1]. The pathophysiology of ALS is multifaceted, involving various mechanisms such as glutamate-driven excitotoxicity, protein aggregation, mitochondrial dysfunction, and impaired axonal transport [2].

Even though drugs such as riluzole and edaravone have been shown to reduce disease progression [3,4], riluzole's efficacy is modest with an unclear impact on functional outcomes [5], while edaravone's effectiveness is limited to a specific patient population [4].

In this context, there is growing interest in exploring novel therapeutic methods to slow down clinical symptom progression in individuals diagnosed with ALS. Recent research has focused on non-invasive brain stimulation techniques, such as repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS), which have shown promising but limited results on muscle strength and neurophysiological measures in patients with ALS [6–16]. These methods aimed to modulate cortical excitability and potentially slow the progression of motor neuron degeneration. However, most studies have been limited by small sample sizes, lack of sham-controlled designs, and short follow-up periods, with stimulation limited to the motor cortex, neglecting the involvement of spinal motor neurons [6–15]. The results of most studies comparing real versus sham rTMS, have been repeatedly reviewed and analyzed elsewhere, and have been deemed insufficient to support the use of rTMS in ALS [17–20]. Nevertheless, both continuous theta burst stimulation and low frequency rTMS appear to hold promise in slowing the progression of the disease [7–14]. These methods warrant further exploration, particularly during the early stages of the disease when cortical excitability plays a significant role in its pathophysiology [20].

tDCS has the potential to mitigate motor impairment in ALS by targeting dysregulated glutamatergic and GABAergic neurotransmission, counteracting maladaptive plasticity and promoting upregulation of BDNF and other growth factors to enhance neuroplasticity and potentially slow motor pathway degeneration [21–24].

Our research group has conducted a study that demonstrated the effectiveness of a two-week treatment with cortico-spinal tDCS, which involves concurrent stimulation of both motor cortices and the spinal cord, in improving global force, caregiver burden, and quality of life scores in ALS patients [25]. Additionally, we found that this improvement was associated with the restoration of intracortical connectivity measures, as evaluated by TMS, indirectly assessing $GABA_A$ and glutamatergic-mediated circuits [26].

Despite the promising results, several unresolved issues remain that we aim to address in this study. Specifically, we seek to: (i) assess the long-term effects of multiple sessions of anodal bilateral motor cortex tDCS and cathodal spinal tDCS in patients with ALS; (ii) determine whether two rounds of tDCS treatment are more effective than a single treatment; and (iii) explore potential effects on prognostic ALS markers, such as serum neurofilament light chain (NfL) [27,28] and survival rates.

In light of the aforementioned advancements and unresolved questions from previous research, our hypothesis posits that multiple sessions of anodal bilateral motor cortex tDCS combined with cathodal spinal tDCS will demonstrate enhanced therapeutic benefits in ALS patients, especially when administered in two rounds. Furthermore, we anticipate that these interventions will possibly have a measurable impact on prognostic ALS markers, such as serum neurofilament light chain (NfL) and survival rates.

2. Methods

2.1. 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), #NP2743 v2 approved 18.04.18. This trial has been registered at ClinicalTrials.gov (NCT04293484).

2.2. Participants

Thirty-one participants were recruited according to the following inclusion criteria: *i)* participants with a diagnosis of laboratory-supported probable, or definite ALS according to the El Escorial revised criteria [29], *ii)* disease duration ≤48 months, *iii)* score ≥2 at the item "swallowing" of the ALS functional rating scale-revised (ALSFRS-R), *iv)* score ≥1 at the item "walking" of the ALSFRS-R and *v)* score ≥1 at the item "respiratory insufficiency" of the ALSFRS-R.

The following exclusion criteria were applied: *i)* motor neuron diseases other than ALS, *ii)* diagnosis of dementia according to current clinical criteria, *iii)* severe head trauma in the past, *iv)* history of seizures, *v)* history of ischemic stroke or hemorrhage, *vi)* pacemaker, *vii)* metal implants in the head/neck region, *viii)* severe comorbidity or *ix)* pregnancy.

Participants who had already been given riluzole could continue to receive treatment provided that the regimen remained unchanged, but initiation after the start of the observation period was not allowed.

In designing our participant selection criteria, we were guided by the understanding of ALS's pathophysiology, the mechanisms of tDCS, and essential safety considerations. Recognizing that ALS's early to moderate stages retain a significant portion of functional motor neurons and pathways, we targeted these stages for intervention [2,3]. This approach aimed to harness the therapeutic potential of tDCS, which modulates cortical excitability and promotes neuroplasticity, to maximize the remaining functional capacity of the motor system [7,21]. As the disease progresses to its severe stages, the pronounced neuronal loss could curtail the benefits of such interventions. Additionally, we excluded patients diagnosed with dementia, given research indicating their accelerated disease progression, which could introduce biases in our survival analysis [30,31]. Safety was paramount; hence, individuals with histories of severe head trauma, seizures, or those with metal implants, pacemakers, or who were pregnant were not considered, ensuring the integrity of the tDCS procedure and minimizing potential risks [32].

Thirteen patients from our current study had also participated in our previous study conducted from September 2017 to February 2018) [25].

2.3. Study design

Participants were recruited from May 2018, with the follow-up ending in January 2022.

Participants were randomized in two groups for the first controlled phase. At baseline (T0), Group 1 received placebo stimulation (sham tDCS) while Group 2 received anodal bilateral motor cortex tDCS and cathodal spinal tDCS (real tDCS) for 5 days/week for two weeks (T1), with an 8-week (T2) and 24-week (T3) follow-up (randomized, double-blind, sham controlled phase). At the 24-week follow-up (T3), all participants (Group 1 and Group 2) received a second treatment of anodal bilateral motor cortex tDCS and cathodal spinal tDCS (real tDCS) for 5 days/week for two weeks, with a 26-week (T4), 32-week (T5), 48-week follow-up (T6) (open-label phase) (see Fig. 1A). In summary, Group 1 underwent sham stimulation followed by real stimulation (sham/real tDCS), while Group 2 underwent real stimulation both times (real/real tDCS).

In shaping our stimulation protocol, we selected a regimen of 5 days per week over two weeks, grounded in both empirical evidence and logistical considerations. Repeated tDCS sessions have been shown to cumulatively modulate cortical excitability, with a 5-day/week protocol over two weeks offering sustained neuromodulatory effects without the risk of overstimulation [22,33]. This protocol aligns with established practices in tDCS research, ensuring consistency and comparability with other studies, including our prior work [25,34,35]. Furthermore, by

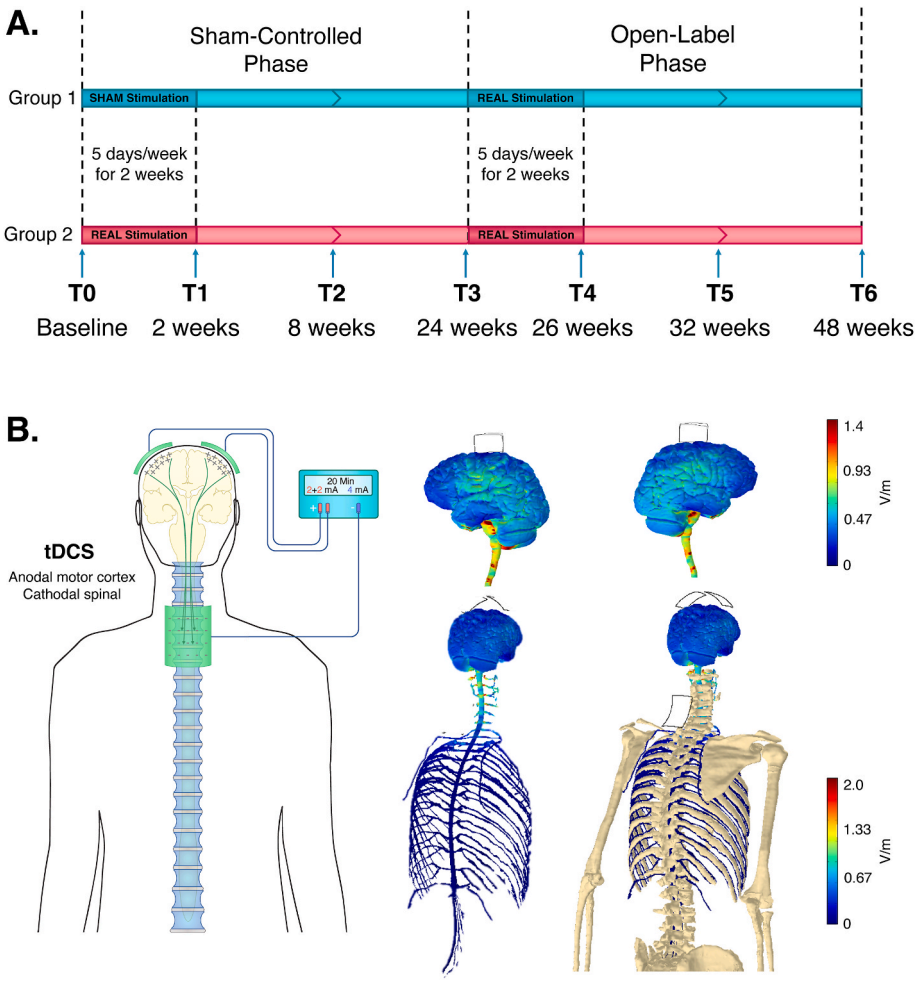

Fig. 1. A) Study design and B) computer simulation of current density distribution.
Legend. tDCS: transcranial Direct Current Stimulation.
Fig. 1B reprinted from Brain Stimulation, Vol 12, Benussi et al., Cortico-spinal tDCS in ALS: A randomized, double-blind, sham-controlled trial, Supplementary Data, Copyright (2019), with permission from Elsevier.

distributing sessions across two weeks with weekends as breaks, we aimed to optimize participant compliance and retention, particularly crucial in an ALS clinical population [36]. While no direct literature comparison exists between ten consecutive days and our chosen 5-2-5 day approach, the significance of inter-session intervals for neural homeostasis has been emphasized, suggesting potential benefits for cumulative effects [37].

At each time point (T0-T6), every patient underwent a clinical evaluation, assessment of quality of life, according to a standardized protocol (see below clinical assessment), intracortical excitability evaluation using TMS (see below TMS assessment) and blood sampling for serum NfL measurements (see below NfL assessment).

The patient and the examiners were blinded to the type of stimulation when applying tDCS (V.C.), performing clinical ratings (A.B., I.L.) and TMS protocols (V.C.). The tDCS device was previously set to real or sham stimulation by a different researcher (B.B.), who was also responsible for random allocation sequences, enrolment of participants, and assigned participants to specific interventions. A computer-assisted randomization was used to randomize subjects into groups.

2.4. Clinical assessment

Neuromuscular impairment was quantified by the five-point Medical Research Council (MRC) scale megascore (sum of MRC scores in each of the following domains: shoulder abductors, elbow flexors and extensors, wrist flexors, thumb opponent, hip flexors, knee flexors and extensors, and ankle dorsiflexors and extensors on both sides for a total of 100). Good reliability and reproducibility for manual muscle testing in patients with ALS have previously been shown [38]. Clinical status was evaluated with the ALSFRS-R [39], quality of life with the amyotrophic lateral sclerosis assessment questionnaire-40-item scale (ALSAQ-40) [40], the 5-level EuroQol-5D version (EQ-5D-5L) and the EuroQol-visual analogue scale (EQ-VAS) [41], while caregiver burden was evaluated with the caregiver burden inventory (CBI) [42].

2.5. Transcranial magnetic stimulation assessment

TMS was performed with a figure-of-eight coil (each loop diameter 70 mm) connected to a Magstim Bistim^2 system (Magstim Company, Oxford, UK). 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) as previously reported [43,44]. To locate the precise representation of the target muscle on the contralateral primary motor cortex, the TMS coil was positioned approximately 4 cm laterally and 2 cm anteriorly to Cz, tangentially on the scalp with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The "hot spot" was defined as the point in which magnetic stimulation resulted in the maximum MEP amplitude with the minimum stimulator intensity [45]. To obtain

this, stimulator intensity was increased from 35 % of the maximal stimulator output (MSO) in 5 % steps until MEPs with an approximately 0.5–1 mV amplitude could be recorded. The coil was then moved in 0.5 cm steps medially, laterally, posteriorly and anteriorly while evoking 3 MEPs at each site. This was performed until the site in which the largest MEPs could be located, which was marked with a felt tip pen on the scalp to ensure constant placement of the coil throughout the experiment.

Resting motor threshold (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 [46].

SICI and ICF were studied at rest via a paired-pulse paradigm, delivered in a conditioning-test design, as previously reported. Briefly, the conditioning stimulus (CS) was set at an intensity of 70 % of the RMT, while the test stimulus (TS) was adjusted to evoke a MEP approximately 1 mV peak-to-peak in the relaxed FDI. Different inter-stimulus intervals (ISIs) between the CS and TS were employed to investigate preferentially both SICI (1, 2, 3 ms) and ICF (7, 10, 15 ms) [47,48].

Ten stimuli were delivered for each ISI and fourteen control MEPs in response to the TS alone were recorded, for each paradigm, in all participants 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 s (±10 %). Average values for SICI (1, 2, 3 ms ISI) and ICF (7, 10, 15 ms ISI) were used for analysis. All protocols were performed in a randomized order in all participants. Throughout the experiment, complete muscle relaxation was monitored by audio-visual feedback where appropriate.

2.6. Serum neurofilament light assessment

Serum was collected by venipuncture, processed and stored in aliquots at −80 °C according to standardized procedures. Serum NfL was measured using a commercial NF-light® assay (Kit# 104073, Quanterix, Lexington, MA) according to the manufacturer's instruction. The lower limit of quantitation for serum NfL was 0.174 pg/mL. Measurements were carried using an HD-X analyzer (Quanterix, Boston Massachusetts) at the same study site during one day using the same batch of reagents, and the operators were blinded to all clinical information. Quality control samples had a mean intra-assay and inter-assay coefficient of variation of less than 8 % and 20 % respectively.

2.7. Transcranial direct current stimulation

tDCS was delivered by a battery-driven constant current stimulator (HDCstim, Newronika, Milan, Italy) through saline-soaked (0.9 % NaCl) surface sponge electrodes. The anodes were placed on the scalp over the motor cortex area (corresponding to the C3–C4 locations based on the international 10–20 EEG electrode placement system) and the cathode over the spinal cervical enlargement (over C6) (see Fig. 1B). The electrodes were secured using elastic gauzes and an electroconductive gel was applied to electrodes to reduce contact impedance (<5 kΩ for all sessions).

In designing our tDCS protocol, we carefully selected the electrode montage based on both physiological and biophysical considerations. Anodal stimulation over the primary motor cortex aimed to modulate cortical excitability, especially relevant for ALS due to its characteristic motor neuron degeneration. This stimulation not only targets primary motor neurons but also influences interneuronal populations, as evidenced by its effects on ALS-impaired neural dynamics, including the modulation of SICI and ICF [49]. For the spinal region, cathodal tDCS was chosen, given its potential to modulate spinal cord excitability and influence both ascending and descending spinal pathways, a crucial aspect considering the spinal cord's role in ALS [50–53]. Biophysically, our montage was tailored to direct the current flow from the motor cortex through the corticospinal tract to the spinal region, optimizing the potential therapeutic reach to the affected motor neurons in ALS.

During real stimulation a constant current of 2 mA per each anodal electrode (4.0 × 6.5 cm^2, current density 0.077 mA/cm^2) and 4 mA per cathodal spinal electrode (5.0 × 7.5 cm^2, current density 0.107 mA/cm^2) was applied for 20 min, with a ramp-up and ramp-down time of 30 s. In the active condition, the DC stimulation was maintained for the whole duration. For the sham condition, the electrode placement was the same, but the electric current was delivered only during the ramp-up and ramp-down phases to make this condition indistinguishable from the experimental stimulation. To detect differences in the perception of the stimulation, we asked the participants whether they thought they were receiving real or sham stimulation at the end of any treatment.

2.8. Computer simulations of current density distribution

Based on the methods developed previously by our group [54], we performed computational modelling of electric field distribution for bilateral motor cortex-spinal tDCS (real tDCS).

The whole-body model we utilized was sourced from the Virtual Family dataset [55]. This model was pivotal in discerning the current flow across the cortex and spine. The process of creating MRI-based forward models, while retaining the resolution of the input data for transcranial stimulation, was grounded in earlier research by our team [54]. We employed the "Duke" model, representing a 34-year-old male, and identified 15 distinct tissue compartments using the software Simpleware-Synopsys Ltd., USA. To ensure a continuous cerebrospinal fluid (CSF), we made modifications using a blend of morphological filters and manual adjustments. The electrodes and gel used for stimulation were integrated as CAD models, mirroring the precise geometry and positioning employed in our experiments: two anodal electrodes placed bilaterally over the motor cortex and a cathodal electrode over the C6 spinous process. Given that our montage was not anticipated to influence current flow beneath the torso, we truncated the whole-body model at that level. Subsequently, we generated volumetric meshes from these data and transitioned them to a solver for finite element analysis using COMSOL Multiphysics, USA. Each compartment was assigned specific electrical properties with representative isotropic values in (S/m): skin: 0.465; bone: 0.01; CSF: 1.65; gray matter: 0.276; white matter: 0.126; muscle: 0.35; intestines: 0.164; heart: 0.381; cartilage: 1.01; liver: 0.221; kidney: 0.403; tongue: 0.255; air: 1×10^{-15}; gel: 0.3; and electrode: 5.9×10^{7}. The boundary conditions, mirroring the tDCS dose used in our experiments, were set (anode 1: 2 mA; anode 2: 2 mA and cathode: ground), while all other external surfaces were insulated. After solving the standard Laplacian equation, we assessed the induced electric field (EF) magnitudes in the cortex [54]. As shown in Fig. 1B, the current effectively reaches the motor cortex, brainstem and cervical spinal cord.

2.9. Outcome measures

The primary endpoint was defined as a significant change from baseline in *a)* muscle strength (MRC scale megascore).

The secondary endpoints were defined as significant changes from baseline in the *b)* ALSFRS-R score, *c)* ALSAQ-40 scale, *d)* EQ-5D-5L scale, *e)* EQ-VAS, *f)* CBI, *g)* SICI, and *h)* ICF.

The exploratory endpoints were defined as *a)* significant changes from baseline in serum NfL levels and *b)* significant difference in survival rates according to the number of completed 2-week treatments with real tDCS.

2.10. Statistical analyses

We used a power analysis to determine the number of included participants, corrected for possible dropouts and participants in whom a reliable MEP could not be elicited, based on preliminary results obtained from previously published work, with power (1-β = 0.80) and α = 0.05

[25].

Considering the unneglectable number of drop-out patients that characterize most trials in ALS with the extensive follow-up (approximately 1 year), we performed an intention-to-treat analysis to reduce any bias due to the possible unbalanced drop-out rates between treatment groups. Clinical, neurophysiological and biological endpoints were analyzed in the full-analysis set, defined as all randomly assigned participants who completed the two-weeks tDCS treatment and had at least one efficacy assessment post baseline.

Cohen's Kappa was run to determine if there was agreement between the type of sensation perceived and the type of stimulation received.

To assess the effect of tDCS treatment on clinical scores and neurophysiological measures over time, we used mixed effect models [56] with TIME as within-subject factor, TREATMENT (real/real stimulation vs sham/real stimulation) as between-subject factor, and subject indices as random effects assuming Missing at Random (MAR) for the missing repeated measures [57]. Difference from baseline values (delta values) of each score were used as outcomes, to reduce possible effects of baseline characteristics on clinical score changes over time. Moreover, we separately evaluated effects of TIME, TREATMENT and TIME × TREATMENT effects in the randomized, double-blind phase and in the open-label phase.

As exploratory analysis, Spearman rank-order correlations were used to assess associations between the improvement in global MRC scores and neurophysiological parameters.

Survival was calculated as time from symptom onset to time of death from any cause (outcome = 1) or censoring date (outcome = 0). Information on the current status at censoring date was collected by reports from the regional Health Service or from a telephone interview. Survival analysis was carried out by means of a Cox proportional-hazard regression analysis; hazard ratios (HR) are provided with their respective 95 % confidence intervals (CIs). To avoid overfitting in the model, variables were chosen based on previous findings and clinical constraints. Therefore, we included region of symptoms onset (bulbar vs limbs), age, baseline ALSFRS-R, baseline total MRC score, riluzole treatment (yes vs no), edaravone treatment (yes vs no) and number of completed 2-week tDCS treatments (0 vs 1 vs 2).

Figures with missing data computed by linear interpolation are provided as Supplementary Figs. 1–3.

Statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY) and R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

2.11. Data availability

All data, including outcome measure results, study protocol and statistical analysis plan, will be shared through ClinicalTrials.gov via public access (https://clinicaltrials.gov/ct2/show/NCT04293484).

3. Results

3.1. Participants

Thirty-one participants were initially enrolled and randomized into Group 1 and 2 to receive sham (n = 15) or real stimulation (n = 16), respectively, in the initial controlled phase, which was followed by an open-label phase after 24-weeks follow-up. Demographic and clinical characteristics of included participants at baseline are reported in Table 1.

Over the 48-week follow-up, fifteen participants dropped-out from the study: six in Group 1 (one worsening of general clinical conditions after T1, two respiratory failures after T2, one worsening of general clinical conditions after T4, one respiratory failure after T4, and one respiratory failure after T5) and nine in Group 2 (two worsening of general clinical conditions after T1, one respiratory failure after T2, two worsening of general clinical conditions after T3, one respiratory failure after T4, one respiratory failure after T5, two worsening of general clinical conditions after T5); no treatment related adverse events were observed (see Fig. 2).

Table 1
Demographic and clinical characteristics of included patients.

	Group 1 (sham/real tDCS)	Group 2 (real/real tDCS)	*p* value[a]
Patients, n	15	16	–
Sex, % female	33.3	25.0	0.704
Age, years	62.8 ± 11.5	57.4 ± 9.1	0.175
Disease duration, years	1.6 ± 1.2	2.3 ± 1.7	0.119
Education, years	10.3 ± 3.3	11.8 ± 4.3	0.318
Bulbar onset, %	20.0	25.0	1.000
Riluzole, %	93.3	87.5	1.000
Edaravone, %	13.3	6.3	0.600
Total MRC score	84.6 ± 13.9	80.1 ± 12.4	0.232
ALSAQ-40	38.1 ± 21.7	42.3 ± 20.1	0.599
ALSFRS-R	35.2 ± 7.1	33.6 ± 6.2	0.318
EQ-5D-5L	11.9 ± 4.0	12.8 ± 3.3	0.520
EQ-VAS	53.7 ± 22.1	44.4 ± 13.3	0.129
CBI	14.9 ± 14.1	22.6 ± 17.0	0.072
RMT	58.2 ± 13.6	58.9 ± 11.5	0.661
SICI	0.64 ± 0.23	0.74 ± 0.29	0.400
ICF	1.41 ± 0.28	1.63 ± 0.35	0.133

tDCS = transcranial direct current stimulation; MRC = strength evaluated with the Medical Research Council megascore; ALSAQ-40 = amyotrophic lateral sclerosis assessment questionnaire-40-item scale; ALSFRS-R = amyotrophic lateral sclerosis functional rating scale-revised; EQ-5D-5L = 5-level EuroQol-5D version; EQ-VAS = EuroQol-visual analogue scale; CBI = caregiver burden inventory; RMT = resting motor threshold expressed as % of the maximum stimulator output; SICI = mean short interval intracortical inhibition (1, 2, 3 ms); ICF = mean intracortical facilitation (7, 10, 15 ms); results are expressed as mean ± standard deviation unless otherwise specified.

[a] Differences were assessed by Fisher's exact test or Mann-Whitney *U* test where appropriate.

There was no statistically significant association between the type of stimulation and participants' perception, as assessed by Cohen's Kappa ($\kappa = 0.05$, $p = 0.779$), suggesting that real tDCS could not be distinguished from sham stimulation.

3.2. Clinical assessment

In the initial randomized, double-blind phase (T0-T3), we observed a

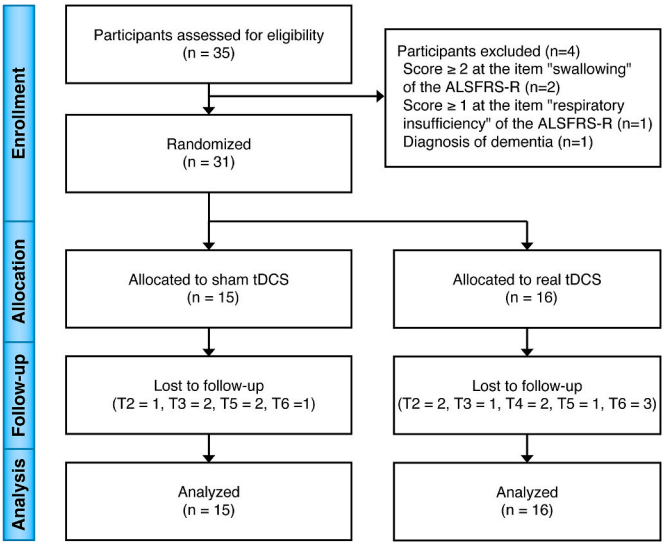

Fig. 2. Flowchart of study participants.
Legend. tDCS: transcranial direct current stimulation.

significant main effect of TREATMENT for delta scores across times of MRC, ALSAQ-40, EQ-5D-5L, EQ-VAS, CBI (all $p < 0.050$), but not for ALSFRS-R ($p = 0.948$).

Considering all time points, for both the randomized, double-blind, placebo-controlled phase and the open-label phase (T0-T6), we observed a significant TIME × TREATMENT interaction for total MRC scores ($p < 0.001$), but not for ALSAQ-40 ALSFRS-R, EQ-5D-5L, EQ-VAS or CBI (all $p > 0.05$). We observed a significant main effect of TREATMENT for delta measures across time of total MRC scores, ALSAQ-40, EQ-VAS, CBI (all $p < 0.050$), but not for ALSFRS-R or EQ-5D-5L ($p > 0.050$) (see Table 2 and Fig. 3).

3.3. Intracortical connectivity

Nineteen participants (nine in Group 1 and ten in Group 2) underwent a TMS paired-pulse protocol, while in twelve participants MEPs could not be reliably evoked and were thus excluded from TMS analysis. In the initial randomized, double-blind phase (T0-T3), we observed a significant main effect of TREATMENT for RMT, SICI and ICF (all $p < 0.050$). Considering all time points, for both the randomized, double-blind, placebo-controlled phase and the open-label phase (T0-T6), we observed a significant interaction of TIME × TREATMENT for RMT, SICI, and ICF (all $p < 0.050$), and a significant main effect of TREATMENT for SICI and ICF (all $p < 0.050$) but not for RMT ($p > 0.05$) (see Table 2 and Fig. 4).

3.4. Serum NfL measures

Serum NfL measurements were performed on twenty-seven participants (15 in Group 1 and 12 in Group 2). In the initial randomized, double-blind phase (T0-T3), no significant main effect of treatment was observed (p = 0.596). However, considering all time points for both the randomized, double-blind, placebo-controlled phase and the open-label phase (T0-T6), a significant interaction between TIME × TREATMENT was observed in serum NfL measures ($p = 0.018$) (Fig. 5).

3.5. Survival analysis

Survival analysis was available for all participants. Overall, at the censoring date (April 6th, 2023) 15 deaths occurred in the whole sample. The multivariate Cox regression analysis showed a significant association between survival and bulbar onset of symptoms (HR 4.99 95% CI 1.06–23.4, $p = 0.042$), baseline total MRC scores (HR 0.94 95%CI 0.89–0.99, $p = 0.021$) and the number of 2-week tDCS treatments (HR 0.29 95%CI 0.11–0.75, $p = 0.011$), suggesting that the number of completed tDCS treatments significantly reduced the risk of death and increased survival (see Fig. 6).

4. Discussion

Our study presents several encouraging findings regarding the therapeutic potential of cortico-spinal tDCS for patients with ALS, comprehensively encompassing highly relevant aspects of ALS: global muscle strength, patient-rated quality of life, caregiver burden and survival.

These independent measures provide a more holistic view of the impact of tDCS treatment on the lives of patients and their caregivers. Global muscle strength is a valid indicator of disease progression in ALS [58], while quality of life ratings capture subjective experiences that extend beyond physical impairment and functional limitations [59]. Furthermore, caregiver burden is influenced by the patient's behavioral and physical impairments, and reduced caregiver well-being can negatively impact the patient's well-being. These improvements suggest that cortico-spinal tDCS could offer a valuable non-pharmacological intervention for alleviating the debilitating symptoms of ALS and enhancing patients' overall well-being [60].

In addition to the clinically relevant outcomes, our study emphasizes the potential modulatory effect of tDCS on intracortical connectivity measures, such as SICI and ICF. In ALS, cortical hyperexcitability has emerged as a key factor contributing to the degeneration of motor neurons [24,61–69]. TMS has been instrumental in elucidating this

Table 2
Clinical and neurophysiological parameters (average Δ±SD compared to T0).

Primary outcome		Randomized double-blind phase			Open-label phase			*p* values		
		T1	T2	T3	T4	T5	T6	TIME	TREAT	TIME × TREAT
Total MRC score	Group 1	−0.5 ± 1.2	−2.8 ± 2.2	−6.6 ± 2.6	−5.1 ± 2.6	−6.9 ± 3.5	−11.3 ± 5.4	**<0.001**	**<0.001**	**<0.001**
	Group 2	2.0 ± 2.3	1.5 ± 3.2	0.9 ± 3.2	0.3 ± 3.2	−1.2 ± 3.3	−2.9 ± 4.4			
Secondary outcomes										
ALSAQ-40	Group 1	0.5 ± 2.5	6.4 ± 5.9	11.8 ± 5.9	5.2 ± 6.4	4.2 ± 9.6	9.6 ± 11.3	**<0.001**	**<0.001**	0.232
	Group 2	−6.8 ± 8.4	−6.9 ± 9.6	−3.0 ± 8.2	−6.2 ± 9.3	−5.1 ± 9.0	−4.3 ± 9.0			
ALSFRS-R	Group 1	0.3 ± 1.2	−1.3 ± 2.6	−3.6 ± 3.4	−3.0 ± 3.4	−4.0 ± 2.3	−6.2 ± 2.4	**<0.001**	0.859	0.672
	Group 2	1.1 ± 1.1	0.0 ± 1.6	−2.5 ± 2.9	−0.7 ± 1.7	−1.8 ± 2.2	−3.4 ± 2.2			
EQ-5D-5L	Group 1	0.4 ± 1.2	0.8 ± 1.7	1.5 ± 2.6	0.7 ± 3.3	0.8 ± 3.6	0.8 ± 3.9	**0.015**	0.073	0.281
	Group 2	−1.1 ± 2.4	−0.9 ± 1.7	−0.7 ± 2.3	−1.1 ± 2.2	−0.1±2-7	0.4 ± 3.1			
EQ-VAS	Group 1	0.0 ± 5.3	−3.2 ± 5.0	−7.1 ± 7.8	−0.4 ± 10.8	0.0 ± 9.4	−4.4 ± 11.3	0.349	**0.001**	0.934
	Group 2	10.3 ± 8.1	7.9 ± 6.1	6.6 ± 6.6	11.4 ± 10.3	7.5 ± 7.5	5.0 ± 9.6			
CBI	Group 1	2.3 ± 3.4	3.9 ± 5.8	6.9 ± 9.2	6.6 ± 9.8	8.9 ± 9.0	10.7 ± 9.7	**<0.001**	**<0.001**	0.332
	Group 2	−5.0 ± 3.6	−3.8 ± 3.1	0.3 ± 3.0	−1.7 ± 4.5	0.3 ± 5.6	2.7 ± 7.8			
RMT	Group 1	0.4 ± 1.4	4.8 ± 6.8	2.3 ± 2.3	−4.7 ± 4.2	1.4 ± 0.9	2.0 ± 4.0	0.103	0.161	**0.030**
	Group 2	−4.7 ± 1.6	4.4 ± 3.5	4.9 ± 4.3	−3.7 ± 1.5	3.3 ± 4.3	1.0 ± 2.0			
SICI	Group 1	0.03 ± 0.05	0.06 ± 0.10	0.04 ± 0.10	−0.31 ± 0.09	0.04 ± 0.04	0.11 ± 0.12	0.111	**<0.001**	**0.005**
	Group 2	−0.37 ± 0.19	0.07 ± 0.23	0.16 ± 0.19	−0.21 ± 0.14	0.12 ± 0.10	−0.04 ± 0.05			
ICF	Group 1	0.03 ± 0.10	0.02 ± 0.09	0.08 ± 0.10	−0.49 ± 0.26	0.11 ± 0.03	0.28 ± 0.38	**0.005**	**0.006**	**<0.001**
	Group 2	−0.40 ± 0.18	0.07 ± 0.15	0.18 ± 0.15	−0.28 ± 0.11	0.02 ± 0.10	0.10 ± 0.16			

Average Δ±SD compared to T0 (baseline) of clinical assessments and neurophysiological parameters at T1 (after 2-week treatment of randomized sham (Group 1) or real (Group 2) transcranial Direct Current Stimulation (tDCS), at T2 (8-week follow-up), T3 (24-week follow-up), T4 (after 2-week open-label real tDCS treatment), T5 (32-week follow-up), T6 (48-week follow-up).

tDCS = transcranial direct current stimulation; MRC = strength evaluated with the Medical Research Council megascore; ALSAQ-40 = amyotrophic lateral sclerosis assessment questionnaire-40-item scale; ALSFRS-R = amyotrophic lateral sclerosis functional rating scale-revised; EQ-5D-5L = 5-level EuroQol-5D version; EQ-VAS = EuroQol-visual analogue scale; CBI = caregiver burden inventory; RMT = resting motor threshold expressed as % of the maximum stimulator output; SICI = mean short interval intracortical inhibition (1, 2, 3 ms); ICF = mean intracortical facilitation (7, 10, 15 ms); results are expressed as mean ± standard deviation; TREAT = treatment.

Significant *p* values are reported in bold.

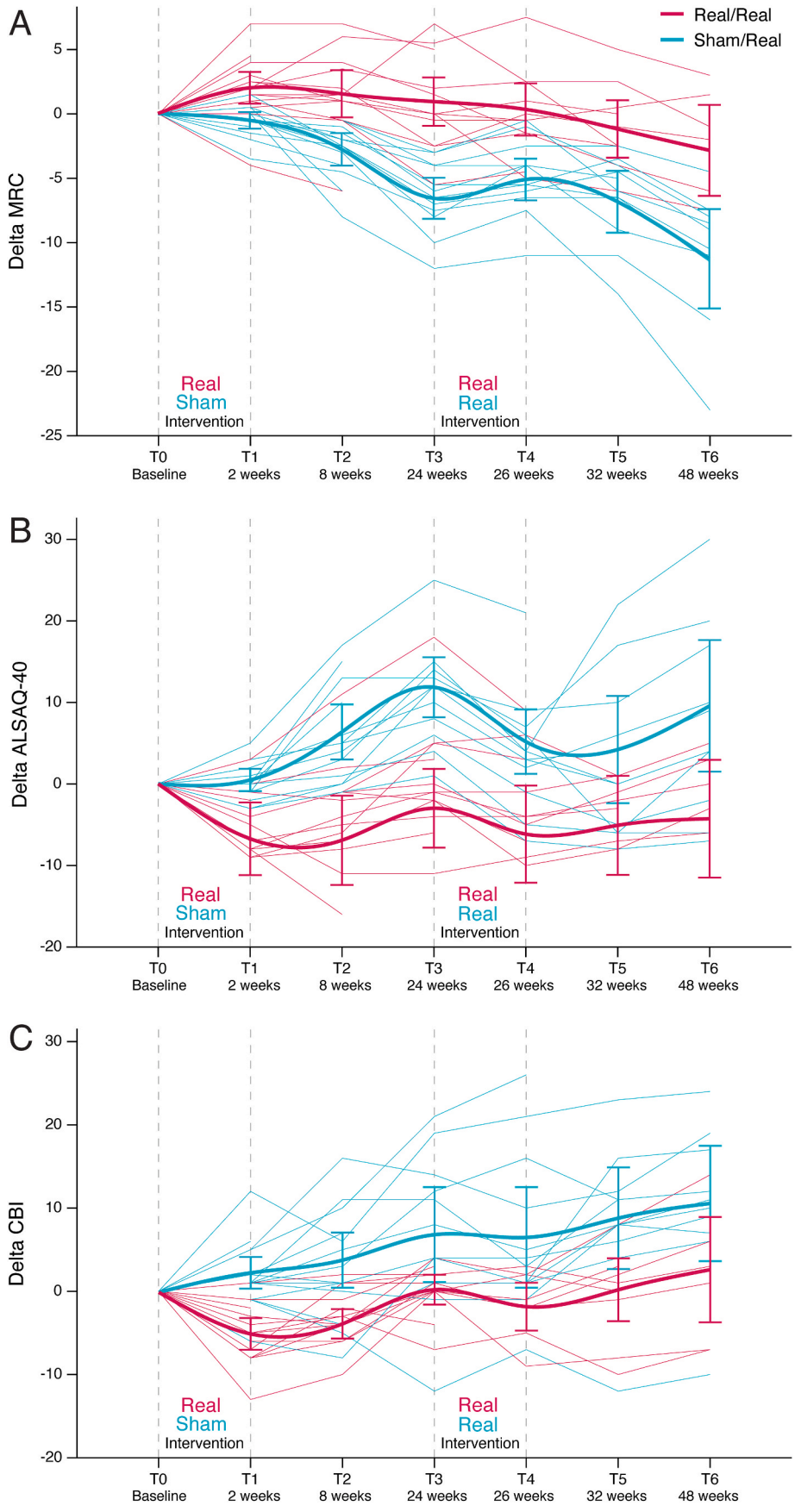

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Fig. 3. Clinical measures of included participants at different time points for A) global MRC scores, B) ALSAQ-40 and C) CBI.
Legend. Average Δ compared to T0 (baseline) of clinical assessments at T1 (after 2-week treatment of randomized sham (Group 1) or real (Group 2) tDCS, at T2 (8-week follow-up), T3 (24-week follow-up), T4 (after 2-week open-label real tDCS treatment),T5 (32-week follow-up), T6 (48-week follow-up). tDCS = transcranial direct current stimulation; MRC = strength evaluated with the Medical Research Council megascore; ALSAQ-40 = amyotrophic lateral sclerosis assessment questionnaire-40-item scale; CBI = caregiver burden inventory. Thick lines represent median and 95 % CI while thin lines represent single participants.

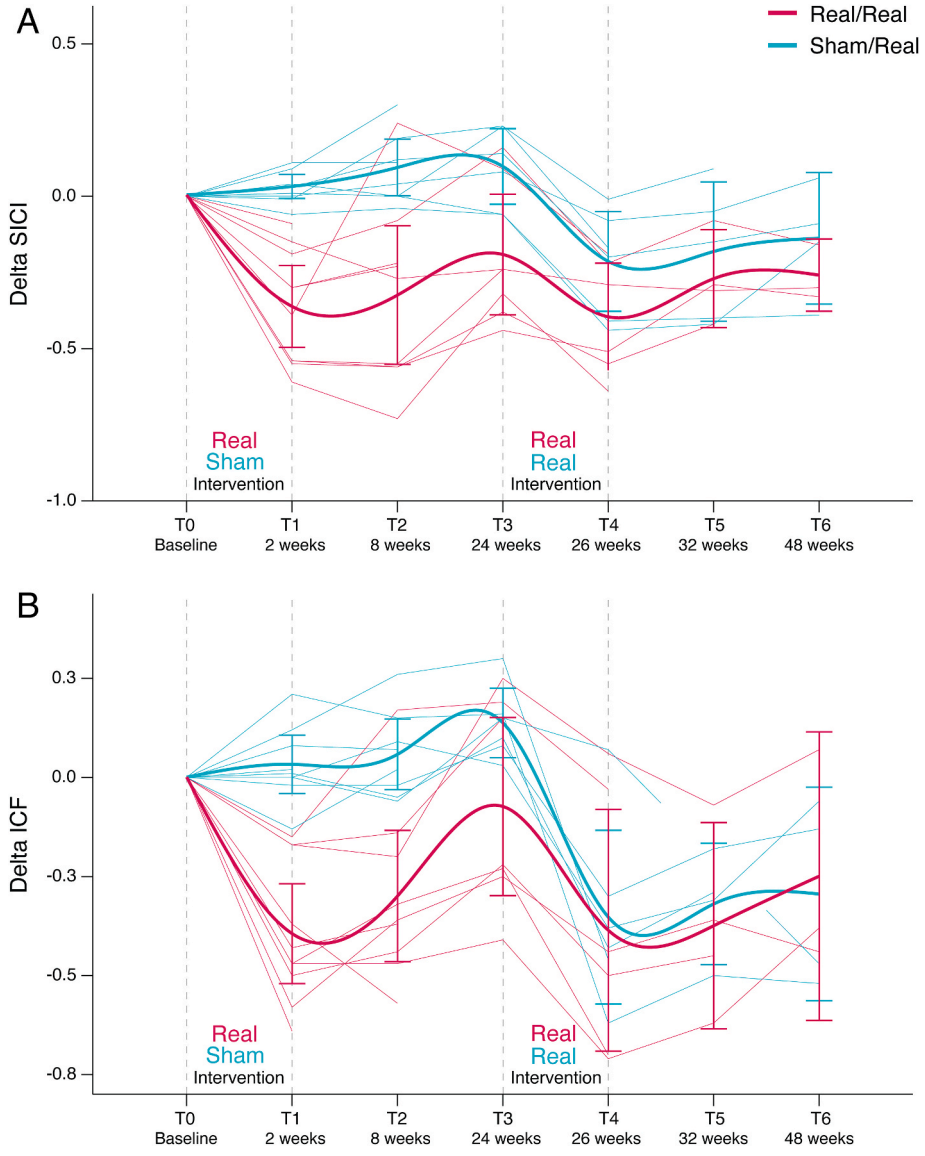

Fig. 4. Neurophysiological measures of included participants at different time points for A) SICI and B) ICF.
Legend. Average Δ compared to T0 (baseline) of clinical assessments and neurophysiological parameters at T1 (after 2-week treatment of randomized sham (Group 1) or real (Group 2) tDCS, at T2 (8-week follow-up), T3 (24-week follow-up), T4 (after 2-week open-label real tDCS treatment), T5 (32-week follow-up), T6 (48-week follow-up). tDCS = transcranial direct current stimulation; SICI = mean short interval intracortical inhibition (1, 2, 3 ms); ICF = mean intracortical facilitation (7, 10, 15 ms). Thick lines represent median and 95 % CI while thin lines represent single participants.

aspect of ALS pathophysiology by providing valuable insights into the functioning of motor circuits in the human brain [70]. A decrease in SICI, a $GABA_A$-mediated phenomenon, and an increase in ICF, a glutamatergic-mediated circuit, have been reported, both indicative of cortical hyperexcitability [63,66,71]. These findings suggest that abnormalities in cortical GABA-ergic and glutamatergic signaling are implicated in the pathophysiology of ALS, providing potential targets for therapeutic intervention. Indeed, recent research has demonstrated that anodal tDCS can lead to increased SICI and reduced ICF, potentially due to its modulatory effects on superficial interneuronal populations [49]. These results imply that cortico-spinal tDCS might help modulate the dysregulated glutamatergic and GABAergic neurotransmission that is characteristic of ALS pathology.

Additionally, our study highlights the potential impact of cortico-spinal tDCS on exploratory survival outcomes in ALS patients. We observed that the number of completed 2-week tDCS treatments significantly influenced survival rates, suggesting a possible dose-dependent effect of tDCS on disease progression. This finding is particularly noteworthy, as it highlights the potential of cortico-spinal tDCS as a disease-modifying therapy in addition to its symptomatic benefits. Supporting this proposition is the significant interaction observed for serum NfL levels over the total observation period (T0-T6), despite the

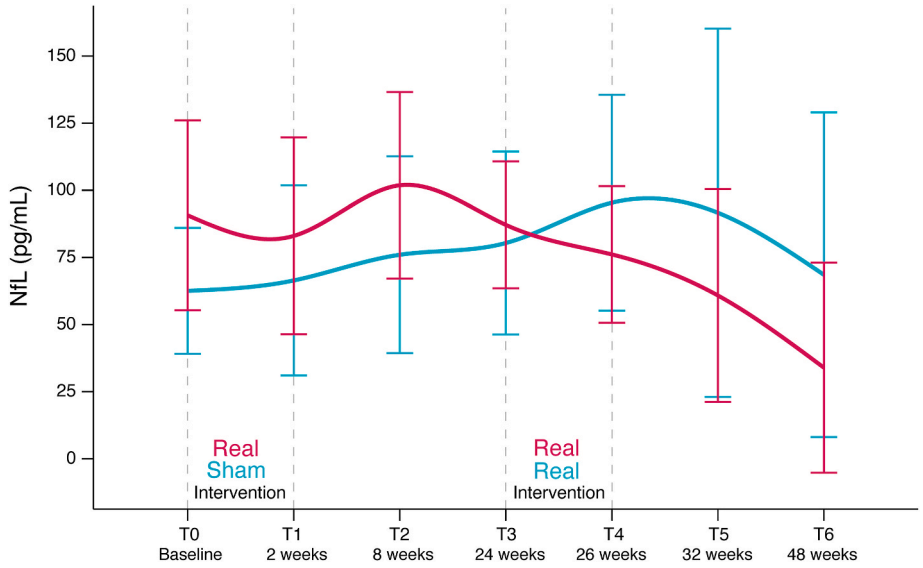

Fig. 5. Serum neurofilament light measurements of included participants at different time points.
Legend. Average serum neurofilament light concentration at T0 (baseline), at T1 (after 2-week treatment of randomized sham (Group 1) or real (Group 2) tDCS, at T2 (8-week follow-up), T3 (24-week follow-up), T4 (after 2-week open-label real tDCS treatment),T5 (32-week follow-up), T6 (48-week follow-up). tDCS = transcranial direct current stimulation; NfL = serum neurofilament light. Thick lines represent median and 95 % CI.

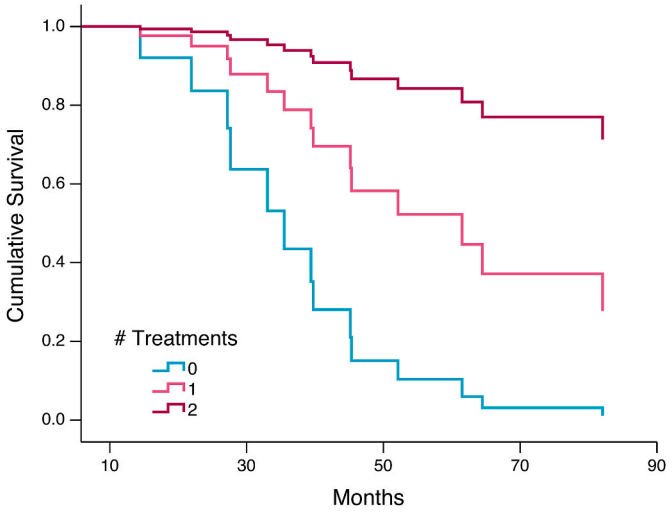

Fig. 6. Cumulative survival curves.
Legend. # Treatments represent the number of completed 2-week treatments. 0: sham transcranial Direct Current Stimulation (tDCS) stimulation; 1: one round real tDCS; 2: two rounds real tDCS.

absence of a significant main effect of treatment during the initial phase (T0-T3). An elevation in serum NfL levels has been previously correlated with neuronal damage in a spectrum of neurodegenerative conditions, including ALS. Notably, in ALS, increased NfL concentrations have been shown to not only correlate with disease severity, but also to predict disease progression and prognosis [72,73]. Therefore, a decrease in these levels, as observed in our study, could potentially suggest a neuroprotective effect of tDCS.

Indeed, we made substantial changes in methodological approach compared to previous studies on tDCS in ALS. In the present work, we stimulated motor cortex bilaterally (vs. left motor cortex) [74], we increased the constant current to 2 mA per each electrode (vs. 1 mA) [75], and we considered the stimulation of spinal motor neurons.

Building upon the foundation of prior research, our study introduces several distinguishing features that set it apart from our previous study [25]. Firstly, we incorporated a new patient population, which not only broadened the scope of our research but also reinforced the findings from the earlier study. Secondly, our study design uniquely included an open-label phase, allowing us to assess whether two rounds of tDCS treatment offer cumulative benefits compared to just one. This approach provided insights into the potential long-term and repeated effects of the intervention. Thirdly, we extended our follow-up duration to 1 year, offering a more in-depth look into the sustained impacts of tDCS over time. Fourthly, our research took a step further by evaluating survival through a comprehensive survival analysis, shedding light on the potential life-prolonging effects of the treatment. Lastly, we delved into the evaluation of serum neurofilament light chain (NfL) levels, providing a more holistic understanding of the intervention's impact on prognostic ALS markers.

Despite the positive findings, our study has several limitations that warrant consideration. Firstly, the relatively small sample size and the high attrition rate may have impacted the generalizability of our findings. Secondly, the single-center design of the trial may have introduced selection bias. Future studies should address these limitations by utilizing larger, multicenter samples and employing rigorous methodological designs to minimize biases. Thirdly, while we observed significant improvements in several clinical outcomes, there was no significant effect on the ALSFRS-R. Future studies should explore the reasons for this discrepancy and examine the relationship between various clinical outcomes and tDCS treatment more comprehensively. Fourthly, the sham-controlled phase of the study was limited to the initial part of the trial. The subsequent open-label phase involved administering real tDCS to all participants, which could introduce biases.

The promising results from our study highlight the potential of cortico-spinal tDCS as a therapeutic intervention for ALS patients, and they open up several avenues for future research. One possible direction involves exploring the synergistic effects of combining tDCS with other therapeutic interventions, such as pharmacological treatments or physical therapy, which could potentially amplify their effects by modulating neural networks and promoting neuroplasticity, leading to better clinical outcomes. In addition, incorporating at-home tDCS treatment options into personalized care plans could further enhance treatment accessibility and patient adherence, resulting in more consistent and sustained therapeutic benefits. The use of advanced

neuroimaging or neurophysiological techniques could provide deeper insights into the mechanisms underlying the observed effects of tDCS in ALS patients.

In summary, our study provides compelling evidence for the potential benefits of cortico-spinal tDCS as a therapeutic and rehabilitative approach for patients with ALS. Larger and more comprehensive clinical trials are necessary to confirm these results and further investigate the mechanisms underlying these effects.

CRediT authorship contribution statement

Alberto Benussi: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization. **Valentina Cantoni:** Investigation, Writing – review & editing. **Mario Grassi:** Methodology, Formal analysis, Writing – review & editing. **Ilenia Libri:** Investigation, Writing – review & editing. **Maria Sofia Cotelli:** Investigation, Writing – review & editing. **Barbara Tarantino:** Formal analysis, Writing – review & editing. **Abhishek Datta:** Formal analysis, Writing – review & editing. **Chris Thomas:** Writing – review & editing. **Nadine Huber:** Investigation, Writing – review & editing. **Sari Kärkkäinen:** Investigation, Writing – review & editing, Investigation, Writing – review & editing. **Sanna-Kaisa Herukka:** Investigation, Writing – review & editing. **Annakaisa Haapasalo:** Investigation, Writing – review & editing. **Massimiliano Filosto:** Investigation, Writing – review & editing. **Alessandro Padovani:** Investigation, Writing – review & editing. **Barbara Borroni:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2023.11.008.

References

[1] Edmond EC, Stagg CJ, Turner MR. Therapeutic non-invasive brain stimulation in amyotrophic lateral sclerosis: rationale, methods and experience. J Neurol Neurosurg Psychiatry 2019;90:1131–8. https://doi.org/10.1136/jnnp-2018-320213. Preprint at.
[2] van den Bos MAJ, Geevasinga N, Higashihara M, Menon P, Vucic S. Pathophysiology and diagnosis of ALS: insights from advances in neurophysiological techniques. Int J Mol Sci 2019;20. https://doi.org/10.3390/ijms20112818. Preprint at.
[3] Riviere M, Meininger V, Zeisser P, Munsat T. An analysis of extended survival in patients with amyotrophic lateral sclerosis treated with riluzole. Arch Neurol 1998;55:526–8.
[4] Abe K, et al. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2017;16:505–12.
[5] Fang T, et al. Stage at which riluzole treatment prolongs survival in patients with amyotrophic lateral sclerosis: a retrospective analysis of data from a dose-ranging study. Lancet Neurol 2018;17:416–22.
[6] Quartarone A, et al. Motor cortex abnormalities in amyotrophic lateral sclerosis with transcranial direct-current stimulation. Muscle Nerve 2007;35:620–4.
[7] Di Lazzaro V, et al. Long-term motor cortex stimulation for amyotrophic lateral sclerosis. Brain Stimul 2010;3:22–7.
[8] Munneke MAM, et al. Cumulative effect of 5 daily sessions of theta burst stimulation on corticospinal excitability in amyotrophic lateral sclerosis. Muscle Nerve 2013;48:733–8.
[9] Di Lazzaro V, et al. Effects of repetitive TMS of the motor cortex on disease progression and on glutamate and GABA levels in ALS: a proof of principle study. Brain Stimul 2017;10:1003–5.
[10] Di Lazzaro V, et al. Motor cortex stimulation for ALS: a double blind placebo-controlled study. Neurosci Lett 2009;464:18–21.
[11] Di Lazzaro V, et al. Motor cortex stimulation for ALS: open label extension study of a previous small trial. Brain Stimul 2014;7:143–4.
[12] Dileone M, et al. Repetitive transcranial magnetic stimulation for ALS. CNS Neurol Disord: Drug Targets 2010;9:331–4.
[13] Di Lazzaro V, et al. Repetitive transcranial magnetic stimulation for ALS. A preliminary controlled study. Neurosci Lett 2006;408:135–40.
[14] Di Lazzaro V, et al. Motor cortex stimulation for amyotrophic lateral sclerosis. Time for a therapeutic trial? Clin Neurophysiol 2004;115:1479–85.
[15] Ceccanti M, et al. Modulation of human corticospinal excitability by paired associative stimulation in patients with amyotrophic lateral sclerosis and effects of Riluzole. Brain Stimul 2018;11:775–81.
[16] Di Lazzaro V, et al. Transcranial static magnetic field stimulation can modify disease progression in amyotrophic lateral sclerosis. Brain Stimul 2021;14:51–4. https://doi.org/10.1016/j.brs.2020.11.003. Preprint at.
[17] Gibbons C, Pagnini F, Friede T, Young CA. Treatment of fatigue in amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev 2018;2018. https://doi.org/10.1002/14651858.CD011005.pub2. Preprint at.
[18] Fang J, Zhou M, Yang M, Zhu C, He L. Repetitive transcranial magnetic stimulation for the treatment of amyotrophic lateral sclerosis or motor neuron disease. Cochrane Database Syst Rev 2013;2013. https://doi.org/10.1002/14651858.CD008554.pub3. Preprint at.
[19] Ng L, Khan F, Young CA, Galea M. Symptomatic treatments for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev 2017;2017. https://doi.org/10.1002/14651858.CD011776.pub2. Preprint at.
[20] Pateraki G, et al. Therapeutic application of rTMS in neurodegenerative and movement disorders: a review. J Electromyogr Kinesiol 2022;62.
[21] Farnad L, Ghasemian-Shirvan E, Mosayebi-Samani M, Kuo MF, Nitsche MA. Exploring and optimizing the neuroplastic effects of anodal transcranial direct current stimulation over the primary motor cortex of older humans. Brain Stimul 2021;14:622–34.
[22] Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527 Pt 3:633–9.
[23] Fritsch B, et al. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 2010;66:198–204.
[24] Vucic S, Ziemann U, Eisen A, Hallett M, Kiernan MC. Transcranial magnetic stimulation and amyotrophic lateral sclerosis: pathophysiological insights. J Neurol Neurosurg Psychiatry 2013;84:1161–70.
[25] Benussi A, et al. Cortico-spinal tDCS in ALS: a randomized, double-blind, sham-controlled trial. Brain Stimul 2019;12:1332–4.
[26] Menon P, et al. Sensitivity and specificity of threshold tracking transcranial magnetic stimulation for diagnosis of amyotrophic lateral sclerosis: a prospective study. Lancet Neurol 2015;14:478–84.
[27] Verde F, et al. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2019;90:157–64.
[28] Lu C-H, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology 2015;84:2247–57.
[29] Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2009;1:293–9.
[30] Trojsi F, et al. Comorbidity of dementia with amyotrophic lateral sclerosis (ALS): insights from a large multicenter Italian cohort. J Neurol 2017;264:2224–31.
[31] Chiò A, et al. Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. J Neurol Neurosurg Psychiatry 2011;82:740–6.
[32] Bikson M, et al. Safety of transcranial direct current stimulation: evidence based update 2016. Brain Stimul 2016;9:641–61.
[33] Monte-Silva K, et al. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul 2013;6:424–32.
[34] Lefaucheur JP. A comprehensive database of published tDCS clinical trials (2005–2016). Neurophysiologie Clinique/Clinical Neurophysiology 2016;46:319–98.
[35] Lefaucheur JP, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol 2017;128:56–92.
[36] Mehta P, et al. Recruitment of patients with amyotrophic lateral sclerosis for clinical trials and epidemiological studies: descriptive study of the national ALS registry's research notification mechanism. J Med Internet Res 2021;23.
[37] Ho KA, et al. The effect of transcranial direct current stimulation (tDCS) electrode size and current intensity on motor cortical excitability: evidence from single and repeated sessions. Brain Stimul 2016;9:1–7.
[38] Sorenson EJ. A comparison of muscle strength testing techniques in amyotrophic lateral sclerosis. Neurology 2003;61:1503–7.
[39] Cedarbaum JM, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. J Neurol Sci 1999;169:13–21.
[40] Palmieri A, et al. Quality of life and motor impairment in ALS: Italian validation of ALSAQ. Neurol Res 2010;32:32–40.
[41] Pinto S, de Carvalho M. Health status perspectives in amyotrophic lateral sclerosis. Neurodegener Dis 2017;17:323–9.
[42] Novak M, Guest C. Application of a multidimensional caregiver. Gerontol 1989;29:798–803.
[43] Benussi A, et al. Classification accuracy of TMS for the diagnosis of mild cognitive impairment. Brain Stimul 2021;14:241–9.

[44] Benussi A, et al. TMS for staging and predicting functional decline in frontotemporal dementia. Brain Stimul 2020;13:386–92.
[45] Gazzina S, et al. Neuroanatomical correlates of transcranial magnetic stimulation in presymptomatic granulin mutation carriers. Brain Topogr 2018;31:488–97.
[46] Padovani A, et al. Transcranial magnetic stimulation and amyloid markers in mild cognitive impairment: impact on diagnostic confidence and diagnostic accuracy. Alzheimer's Res Ther 2019;11:95.
[47] Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 1996;496:873–81.
[48] Kujirai T, et al. Corticocortical inhibition in human motor cortex. J Physiol 1993;471:501–19.
[49] Vignaud P, Mondino M, Poulet E, Palm U, Brunelin J. Duration but not intensity influences transcranial direct current stimulation (tDCS) after-effects on cortical excitability. Neurophysiol Clin 2018;48:89–92.
[50] Powell ES, et al. Transvertebral direct current stimulation paired with locomotor training in chronic spinal cord injury: a case study. NeuroRehabilitation 2016;38:27–35.
[51] 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.
[52] Bocci T, et al. Transcutaneous spinal direct current stimulation modulates human corticospinal system excitability. J Neurophysiol 2015;114:440–6.
[53] Hubli M, Dietz V, Schrafl-Altermatt M, Bolliger M. Modulation of spinal neuronal excitability by spinal direct currents and locomotion after spinal cord injury. Clin Neurophysiol 2013;124:1187–95.
[54] Datta A, Truong D, Minhas P, Parra LC, Bikson M. Inter-individual variation during transcranial direct current stimulation and normalization of dose using MRI-derived computational models. Front Psychiatr 2012;3:91.
[55] Christ A, et al. The Virtual Family—development of surface-based anatomical models of two adults and two children for dosimetric simulations. Phys Med Biol 2009;55:N23–38.
[56] Oberg AL, Mahoney DW. Linear mixed effects models. Topics Biostatistics. Methods Mol Biol 2007;404:213–34.
[57] DeSouza CM, Legedza ATR, Sankoh AJ. An overview of practical approaches for handling missing data in clinical trials. J Biopharm Stat 2009;19:1055–73. https://doi.org/10.1080/10543400903242795. Preprint at.
[58] Munsat TL, Andres PL, Finison L, Conlon T, Thibodeau L. The natural history of motoneuron loss in amyotrophic lateral sclerosis. Neurology 1988;38:409–13.
[59] Goldstein LH, Atkins L, Leigh PN. Correlates of quality of life in people with motor neuron disease (MND). Amyotroph Lateral Scler 2002;3:123–9.
[60] de Wit J, et al. Caregiver burden in amyotrophic lateral sclerosis: a systematic review. Palliat Med 2018;32:231–45.
[61] Van Den Bos MAJ, et al. Imbalance of cortical facilitatory and inhibitory circuits underlies hyperexcitability in ALS. Neurology 2018;91:E1669–76.
[62] Vucic S, Pavey N, Haidar M, Turner BJ, Kiernan MC. Cortical hyperexcitability: diagnostic and pathogenic biomarker of ALS. Neurosci Lett 2021;759.
[63] Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. Brain 2006;129:2436–46.
[64] Vucic S, Kiernan MC. Transcranial magnetic stimulation for the assessment of neurodegenerative disease. Neurotherapeutics 2016;14:91–106.
[65] Menon P, Kiernan MC, Vucic S. Cortical hyperexcitability precedes lower motor neuron dysfunction in ALS. Clin Neurophysiol 2015;126:803–9.
[66] Vucic S, Nicholson GA, Kiernan MC. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. Brain 2008;131:1540–50.
[67] Vucic S, Howells J, Trevillion L, Kiernan MC. Assessment of cortical excitability using threshold tracking techniques. Muscle Nerve 2006;33:477–86.
[68] Vucic S, Cheah BC, Kiernan MC. Defining the mechanisms that underlie cortical hyperexcitability in amyotrophic lateral sclerosis. Exp Neurol 2009;220:177–82.
[69] Geevasinga N, Menon P, Yiannikas C, Kiernan MC, Vucic S. Diagnostic utility of cortical excitability studies in amyotrophic lateral sclerosis. Eur J Neurol 2014;21:1451–7.
[70] Vucic S, et al. Clinical diagnostic utility of transcranial magnetic stimulation in neurological disorders. Updated report of an IFCN committee. Clin Neurophysiol 2023;150:131–75.
[71] Vucic S, Cheah BC, Yiannikas C, Kiernan MC. Cortical excitability distinguishes ALS from mimic disorders. Clin Neurophysiol 2011;122:1860–6.
[72] Steinacker P, et al. Neurofilaments in the diagnosis of motoneuron diseases: a prospective study on 455 patients. J Neurol Neurosurg Psychiatry 2016;87:12–20.
[73] Zhou YN, et al. Role of blood neurofilaments in the prognosis of amyotrophic lateral sclerosis: a meta-analysis. Front Neurol 2021;12.
[74] Madhavan S, Sivaramakrishnan A, Bond S, Jiang QL. Safety and feasibility of transcranial direct current stimulation in amyotrophic lateral sclerosis - a pilot study with a single subject experimental design. Physiother Theory Pract 2019;35:458–63.
[75] Di Lazzaro V, Ranieri F, Capone F, Musumeci G, Dileone M. Direct current motor cortex stimulation for amyotrophic lateral sclerosis: a proof of principle study. Brain Stimul 2013:6 969–970. https://doi.org/10.1016/j.brs.2013.06.005. Preprint at.