ABSTRACT: The aim of this study was to identify a neurophysiological marker of upper motoneuron involvement in patients with sporadic amyotrophic lateral sclerosis (ALS). For this purpose we evaluated the after-effects of transcranial direct-current stimulation (tDCS) on excitability of the motor cortex of eight ALS patients and eight healthy controls. Healthy controls showed a transient polarity-specific change in corticospinal excitability of about ±45%, with anodal tDCS inducing facilitation and cathodal tDCS leading to inhibition, whereas no change could be induced in ALS patients after either type of tDCS. It is likely that the lack of tDCS after-effects in ALS is the result of alterations of the motoneuronal membrane or, alternatively, may represent an electrophysiological correlate of disordered glutamate neurotransmission. Further studies are warranted to confirm these results. The present findings may lead to a new, reliable electrophysiological marker of upper motoneuronal involvement in ALS.

MOTOR CORTEX ABNORMALITIES IN AMYOTROPHIC LATERAL SCLEROSIS WITH TRANSCRANIAL DIRECT-CURRENT STIMULATION

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Although the exact etiology of amyotrophic lateral sclerosis (ALS) is unclear, many studies have demonstrated that in the early stages of the disease the motor cortex is hyperexcitable, probably due to potentiated glutamate transmission causing excitotoxicity and neuronal death. The ability of the anti-glutamatergic agent riluzole to slow disease progression has been cited in support of this theory. However, motor cortex hyperexcitability might be related to changes in the membrane electrical properties of motoneurons.

Cortical excitability can be tested non-invasively in humans with transcranial magnetic stimulation (TMS). TMS allows transsynaptic activation of large pyramidal cells, which seem to be affected predominantly in ALS, and is therefore capable not only of testing the integrity of the corticospinal pyramidal tract neurons but also the efficiency of intracortical inhibitory and excitatory circuits.

Transcranial direct-current stimulation (tDCS) of the human motor cortex can elicit intracortical excitability changes whose direction depends on stimulation polarity: anodal stimulation increases excitability, and cathodal stimulation leads to inhibition. If tDCS is applied for several minutes, the changes outlast the stimulation by up to 1 hour. The after-effects of tDCS depend upon several mechanisms that may alter neuronal membrane function, and long-lasting after-effects may involve a synaptic mechanism at the level of N-methyl D-aspartate (NMDA) receptors. Given the potential of tDCS to modify cortical excitability through membrane polarization changes, we combined TMS and tDCS in an attempt to reveal new...
neurophysiological markers of upper motoneuron involvement in ALS patients.

**METHODS**

Eight patients (four men and four women, mean age 55 ± 8 years, range 43–68 years) with sporadic ALS were enrolled consecutively in the study. All patients were categorized as having clinically definite ALS according to the revised El Escorial criteria. Patients were excluded if motor evoked potentials (MEPs) with an amplitude <0.5 mV were elicited from the right first dorsal interosseous (FDI) muscle after TMS of the left primary motor cortex at the maximum stimulator output. None of the patients had any additional neurological or psychiatric diseases. The mean disease duration by history was 10.7 ± 9 months. The mean central conduction time recorded from the FDI was 6.9 ± 0.8 ms on the right side and 7.4 ± 1.1 ms on the left side. Only one patient was being treated with riluzole when the experiments were done. No patient had taken any other medications for at least 2 weeks prior to the experiments. All patients except one had a normal central motor conduction time as measured with the F-wave method to the right FDI. For comparison, eight healthy age- and gender-matched controls (four men and four women, mean age 56 ± 10 years, range 37–67 years, all without medication) were tested. All patients and control subjects gave written informed consent and the study was approved by the local ethics committee.

The study protocol included two experimental sessions separated by approximately 1 week. In each session, participants received either anodal or cathodal tDCS for 7 minutes. Patients and healthy controls were blinded to the type of tDCS. The order of tDCS conditions was pseudo-randomized and balanced within both groups (patients, controls). Constant direct current (DC) of 1 mA was delivered by a purpose-built DC stimulator (Schneider Electronic, Gülen, Germany) and applied via two large-sized (7 × 5 cm), saline-soaked, sponge-covered electrodes positioned over the optimal motor cortical representation (as revealed by TMS) of the right FDI muscle and on the contralateral frontal pole. The tDCS polarity refers to the electrode over the motor cortex. The tDCS was applied for 7 minutes. Before (pre) and twice after tDCS (post 1 = after 10 minutes, post 2 = after 20 minutes), the following parameters were obtained by TMS of the left motor cortex: active and resting motor thresholds (AMT, RMT), single-pulse MEP, as well as short-latency intracortical inhibition (SICI) and intracortical facilitation (ICF), using paired-pulse TMS with interstimulus intervals of 2 ms and 12 ms, respectively. The intensity of the test pulse was adjusted to evoke MEP amplitudes of approximately 0.6–1 mV in the relaxed FDI muscle and kept identical throughout the experiment. The intensity of the conditioning pulse for SICI and ICF was set to 90% of the individual AMT. At each time-point (pre, post 1, post 2) motor thresholds were determined first. RMT was defined as the minimum stimulator intensity eliciting MEPs of 50 μV in 5 of 10 consecutive trials in the relaxed FDI muscle. AMT was defined as the minimum stimulator intensity eliciting MEPs of 200 μV in the tonically contracting FDI muscle in at least 5 of 10 consecutive trials with a force level of approximately 10%–15% of maximum voluntary contraction. Then a randomized protocol was run to measure single-pulse MEP, SICI, and ICF. This consisted of a total of 120 stimuli given at 0.25 Hz; 60 stimuli were unconditioned in order to measure corticospinal excitability, and 60 were conditioned pulses for SICI and ICF determination. Each block of measurements lasted approximately 10 min, that is, a period of approximately 20 min after tDCS was covered by the two latter measurements.

All TMS measurements were done using two monophasic Magstim 200 HP magnetic stimulators (Magstim, Whitland, UK), which were connected through a bistim module to a standard double 90-mm figure-eight coil (Magstim). The coil was placed flat on the skull with the handle pointing backward and rotated about 45° away from the midline. Thus, the current induced in the brain was approximately perpendicular to the central sulcus. This orientation of the induced electrical field is thought to be optimal for a predominantly transynaptic mode of activation of the corticospinal system. Surface electromyographic (EMG) activity of the FDI muscle was recorded with Ag–AgCl surface electrodes using a belly–tendon montage. Both auditory (speakers) and visual (oscilloscope) feedback of EMG activity was given to the subjects to ensure complete relaxation. EMG signals were amplified and filtered using a time constant of 3 ms and a high-pass filter set at 3 kHz (Neurolog System; Digitimer, Ltd., Welwyn Garden City, Hert, UK). Signals were acquired at a rate of 5 kHz (CED 1401 Laboratory Interface; Cambridge Electronic Design, Cambridge, UK) on a personal computer for off-line analysis.

For each block of measurements, the peak-to-peak amplitude of each MEP (in millivolts) was measured off-line, and the mean MEP amplitudes were calculated for each stimulation condition (single-pulse MEP, SICI, and ICF). For SICI and ICF, the conditioned MEP to unconditioned MEP ratio was calculated from individual data. Motor thresholds
(as a percentage of maximum stimulator output) and MEP amplitudes from the different stimulation conditions and time-points were entered separately in three-way repeated-measures analyses of variance (ANOVAs) with time (pre, post 1, post 2) and tDCS (anodal, cathodal) as within-subject factors and group (patients, controls) as between-subjects factor. The Greenhouse–Geisser method was used to correct for nonphericity. Conditional on a significant F-value, paired-sample two-tailed t-tests were used for post hoc comparisons. P < 0.05 was considered significant. Data are given as mean ± standard error.

**RESULTS**

None of the patients or controls reported any adverse effects during or after the experiments.

Repeated-measures ANOVAs on motor thresholds revealed a significant main effect on active motor threshold for group \( F_{1,14} = 7.4, P = 0.01 \) and a trend toward a significant main effect on resting motor threshold for group \( F_{1,14} = 3.9, P = 0.06 \) (see Table 1 for raw data). Comparison between groups showed that mean active motor threshold was about 8% higher in ALS subjects than healthy controls \((t\text{-test}; P = 0.02)\) and that resting motor threshold tended to be about 6% higher in ALS than healthy subjects \((t\text{-test}; P = 0.09)\). The stimulation intensity for the test response was not statistically different between patients \((77 ± 14% \text{ of maximum stimulator output})\) and controls \((70 ± 7%\) \((t\text{-test}; P = 0.2)\).

tDCS caused a polarity-specific shift in corticospinal excitability in the control group but not in the ALS group. Repeated-measures ANOVA on MEPs revealed significant interactions for group × DC × time \( F_{1,8,12.7} = 16.966, P < 0.001 \). Post hoc t-tests demonstrated that MEPs after anodal tDCS compared to baseline were facilitated in the control group \((P < 0.001)\) but not in the ALS group \((P = 0.89)\). Equally, MEPs after cathodal tDCS, compared to baseline, were inhibited in the control group \((P < 0.001)\) but not in the ALS group \((P = 0.75)\). Post 2 MEP amplitudes were not significantly different compared to baseline level, irrespective of group and DC. Percentage changes in MEP size induced by anodal and cathodal tDCS are shown in Figure 1.

**Table 1.** Cortical excitability measures before and after tDCS.

<table>
<thead>
<tr>
<th></th>
<th>RMT (%)</th>
<th>AMT (%)</th>
<th>MEP (mV)</th>
<th>SICI (%)</th>
<th>ICF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anodal tDCS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TMS: pre</td>
<td>ALS 59 ± 10</td>
<td>ALS 54 ± 10</td>
<td>ALS 0.8 ± 0.1</td>
<td>ALS 70 ± 4</td>
<td>ALS 140 ± 19</td>
</tr>
<tr>
<td></td>
<td>Controls 51 ± 5</td>
<td>Controls 44 ± 5</td>
<td>Controls 0.8 ± 0.1</td>
<td>Controls 38 ± 4</td>
<td>Controls 147 ± 9</td>
</tr>
<tr>
<td>TMS: post 1</td>
<td>ALS 58 ± 10</td>
<td>ALS 54 ± 9</td>
<td>ALS 0.8 ± 0.1</td>
<td>ALS 70 ± 10</td>
<td>ALS 150 ± 23</td>
</tr>
<tr>
<td></td>
<td>Controls 50 ± 5</td>
<td>Controls 44 ± 4</td>
<td>Controls 1.2 ± 0.1</td>
<td>Controls 40 ± 6</td>
<td>Controls 150 ± 7</td>
</tr>
<tr>
<td>TMS: post 2</td>
<td>ALS 58 ± 10</td>
<td>ALS 53 ± 9</td>
<td>ALS 0.8 ± 0.1</td>
<td>ALS 61 ± 6</td>
<td>ALS 140 ± 16</td>
</tr>
<tr>
<td></td>
<td>Controls 50 ± 6</td>
<td>Controls 44 ± 4</td>
<td>Controls 0.8 ± 0.1</td>
<td>Controls 34 ± 6</td>
<td>Controls 159 ± 13</td>
</tr>
<tr>
<td><strong>Cathodal tDCS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMS: pre</td>
<td>ALS 57 ± 11</td>
<td>ALS 51 ± 7</td>
<td>ALS 0.7 ± 0.1</td>
<td>ALS 60 ± 9</td>
<td>ALS 144 ± 12</td>
</tr>
<tr>
<td></td>
<td>Controls 52 ± 6</td>
<td>Controls 43 ± 4</td>
<td>Controls 0.8 ± 0.1</td>
<td>Controls 35 ± 4</td>
<td>Controls 140 ± 9</td>
</tr>
<tr>
<td>TMS: post 1</td>
<td>ALS 57 ± 10</td>
<td>ALS 50 ± 8</td>
<td>ALS 0.7 ± 0.1</td>
<td>ALS 52 ± 10</td>
<td>ALS 154 ± 12</td>
</tr>
<tr>
<td></td>
<td>Controls 50 ± 6</td>
<td>Controls 44 ± 4</td>
<td>Controls 0.5 ± 0.1</td>
<td>Controls 37 ± 6</td>
<td>Controls 157 ± 15</td>
</tr>
<tr>
<td>TMS: post 2</td>
<td>ALS 58 ± 10</td>
<td>ALS 50 ± 7</td>
<td>ALS 0.7 ± 0.1</td>
<td>ALS 60 ± 11</td>
<td>ALS 134 ± 6</td>
</tr>
<tr>
<td></td>
<td>Controls 51 ± 5</td>
<td>Controls 44 ± 5</td>
<td>Controls 0.7 ± 0.1</td>
<td>Controls 39 ± 6</td>
<td>Controls 120 ± 8</td>
</tr>
</tbody>
</table>

\*Percent of maximum stimulator output.
\*Percent of unconditioned MEP.

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**FIGURE 1.** Lack of tDCS after-effects in ALS. The upper panel shows MEP data (given as percentage of pre-tDCS control) for healthy controls (open bars) and ALS patients (filled bars) at two time-points (post 1, post 2) after anodal tDCS, the lower panel after cathodal tDCS. In healthy controls, tDCS induced polarity-specific MEP modulation in the range of approximately ±45%, which wore off by the second time-point (-10–20 min after DC stimulation). In the group of ALS patients, no significant change in MEP size could be observed in response to tDCS.
Repeated-measures ANOVA on SICI revealed a significant main effect for group \( [F_{1,7} = 13.291, P < 0.001] \) but no main effect for time, DC, or any interaction. Mean SICI at a time-point before tDCS, given as percentage of the unconditioned MEP and averaged from both tDCS conditions, was 36.5 \( \pm \) 2\% in the control group and 65 \( \pm \) 7\% in the ALS group (Table 1). Repeated-measures ANOVA on ICF did not show any significant main effect for the factors group and time, because patients showed a similar amount of ICF at baseline, with respect to controls, which was not affected by tDCS (see Table 1).

**DISCUSSION**

We confirmed that weak tDCS for 7 minutes can induce sustained excitability changes in healthy subjects of about \( \pm \) 45\%, with anodal tDCS inducing facilitation and cathodal tDCS leading to inhibition.\(^{25}\) In contrast, the excitability of the primary motor cortex of ALS patients was not affected by anodal or cathodal tDCS.

There are different possible explanations for this observation. First, the lack of tDCS after-effects on corticospinal excitability in ALS patients may have been due to a ceiling effect of MEP size related simply to the numerical loss of cortical neurons. This is theoretically possible but extremely unlikely because central motor conduction times were predominantly normal in our patient group and motor thresholds were only slightly elevated, indicating an early–middle stage of the disease.

The lack of a cathodal effect could be due to the fact that cathodal tDCS for 7 minutes was not sufficient to decrease cortical excitability due to the pre-existing hyperexcitability within the motor cortex of ALS patients.\(^{26,31}\) We have recently shown that in patients with writer’s cramp, where the excitability of inhibitory circuits is also reduced, the normal inhibitory effect of cathodal tDCS cannot be induced.\(^{27}\) However, this assumption is not consistent with our data as the patients had higher active motor thresholds, suggesting reduced motor cortex excitability rather than increased cortical excitability.

Foci of gliosis in layers II and III may parallel the loss of pyramidal neurons in the motor cortex in ALS patients. Thus, another explanation for the lack of effect of tDCS on cortical excitability in ALS may pertain to changes in the electrical field as a result of the anatomical alterations in the motor cortex. In this case, the electrical current might not even be reaching the motor cortex at an adequate level because gliosis could have a shield effect on the electrical current induced by tDCS.\(^{25}\) However, it is likely that gliosis is relevant only in the later stages of the disease, whereas most of our patients were in an earlier stage.

Alternatively, the lack of tDCS after-effects in ALS could reflect an abnormality of the properties of the upper motoneuron membrane. Although the mechanisms of tDCS-induced changes of cortical excitability are not fully understood, it has been proposed that tDCS may change excitability by altering the membrane potential of corticospinal cells.\(^{1}\) tDCS could induce changes in the properties and number of ion channels from alterations in transmembrane proteins and from electrolysis-related changes in \([H^{+}]\) induced by exposure to a constant electric field, thus affecting the overall level of neuronal activity (non-synaptic plasticity).\(^{1,7}\) Changes in the excitability properties of peripheral motor axons have been reported in patients with ALS due to the involvement of potassium and sodium channels.\(^{13}\) In addition, intracellular recordings in spinal motoneurons of mutant superoxide dismutase 1 (SOD1) mice have demonstrated very early abnormalities in the electrophysiological properties of spinal motor circuitry due to changes in sodium and potassium currents.\(^{16}\)

A variety of TMS studies have detected early electrophysiological abnormalities in the motor cortex of ALS patients.\(^{8}\) At the beginning of the illness, MEPs with a low threshold and large amplitude can be recorded.\(^{11}\) As the disease progresses the threshold rises and MEP amplitude decreases. As an extension of these findings, the lack of tDCS effects on cortical excitability may be a correlate of early changes in the electrical properties of the cortical motoneuron membrane.\(^{2,6,20}\)

Moreover, the lack of tDCS after-effects on cortical excitability in ALS patients could be related to altered glutamate transmission.\(^{4}\) In healthy subjects the absence of both anodal and cathodal tDCS-induced excitability shifts could only be achieved by administration of the NMDA-receptor antagonist dextromethorphan, indicating that glutamatergic neurotransmission is implicated in the after-effects of tDCS.\(^{19,21}\)

Finally, it is possible that tDCS effects can be produced in ALS patients but that the threshold for their induction is higher than in healthy controls. This could have been excluded by applying longer-duration tDCS protocols (11–13 minutes), because tDCS after-effects correlate with current strength and duration.\(^{22,24}\)

As previously reported, SICI is reduced in ALS,\(^{31}\) but there was no effect of tDCS on intracortical inhibitory or facilitatory mechanisms in our patients or controls. Because tDCS in control subjects led to changes in the amplitude of the test response, it is possible that these could have affected the amount of SICI and ICF.\(^{30}\) However, as suggested by Ridding et al.,\(^{28}\) we
expected that the amount of SICI or ICF would remain approximately constant given the rather limited range in amplitude of the test MEPs that we observed. The lack of effect of strength of SICI and ICF after tDCS contrasts with recent findings of Nitsche et al., who showed that anodal tDCS of the primary motor cortex reduces intracortical inhibition and enhances facilitation in healthy controls. Methodological differences may account for this discrepancy; in fact, the intensity of the conditioning pulse was 70% of the AMT that produced a small amount of inhibition and facilitation at baseline. In our study we used an intensity of the conditioning pulse of 90% of the AMT, with a greater amount of inhibition and facilitation at baseline, possibly causing a floor or ceiling effect.

In conclusion, corticospinal neurons of ALS patients cannot be polarized by anodal and cathodal tDCS. At the present time, it is not clear whether the lack of tDCS after-effects in ALS is the result of an alteration of motoneuronal membrane or an electro-physiological correlate of disordered glutamate neurotransmission, or is simply related to a higher threshold for inducing tDCS after-effects. Regardless, the lack of effect after tDCS polarization may provide a new key for understanding the pathogenesis of ALS.

REFERENCES