Transcutaneous Spinal Direct Current Stimulation (tsDCS) Modulates Human Corticospinal System Excitability

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Abstract

This study aimed to assess the effects of thoracic anodal and cathodal transcutaneous spinal direct current stimulation (tsDCS) on upper- and lower-limb corticospinal excitability. Yet, despite studies assessing thoracic tsDCS influences the spinal ascending tract and reflexes, none assessed the effects of this technique over upper- and lower-limb corticomotorneuronal connections.

In 14 healthy subjects we recorded motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) from abductor hallucis (AH) and hand abductor digiti minimi (ADM) muscles before (baseline, B), and at a different time-points (0 and 30 minutes) after anodal or cathodal tsDCS (2.5 mA, 20 minutes, T9-T11 level). In 8 of the 14 subjects we also tested the soleus H-reflex, the F-waves from AH and ADM before and after tsDCS.

Both anodal and cathodal tsDCS left the upper-limb MEPs and F-wave unchanged. Conversely, while leaving lower-limb H-reflex unchanged, they oppositely affected lower-limb MEPs: whereas anodal tsDCS increased resting motor threshold ({mean±SEM} 107.33 ± 3.3%, increase immediately after tsDCS, and 108.37 ± 3.2% increase 30 min after tsDCS compared to baseline), and had no effects on MEP area and latency, cathodal tsDCS increased MEP area (139.71 ± 12.9% increase immediately after tsDCS and 132.74 ±22.0% increase 30 min after tsDCS compared to baseline) without affecting resting motor threshold and MEP latency.

Our results show that tsDCS induces polarity specific changes in corticospinal excitability that last for more than 30 min after tsDCS offset and selectively affect responses in lower-limb muscles innervated by lumbar and sacral motorneurons.

Key words: direct current stimulation; spinal cord stimulation; spinal cord; corticospinal system; tsDCS; tDCS; TMS; motor potentials.

Introduction

Transcutaneous spinal direct current stimulation (tsDCS) is a simple, painless and noninvasive technique for modulating spinal cord function in humans (Cogiamanian et al. 2012; Cogiamanian et al. 2011; Lamy and Boakye 2013b; Priori et al. 2014). tsDCS consists in delivering a constant direct current (DC) at 1.5-2.5mA over the spinal cord through a pair of sponge electrodes. The technique induces effects lasting from minutes to hours (Cogiamanian et al. 2008; Lamy et al. 2012; Lim and Shin 2011; Winkler et al. 2010) and is well tolerated by subjects. After the first reports (Cogiamanian et al., 2008; Winkler et al., 2010), this non-invasive method for spinal neuromodulation has come into increasingly widespread use (Cogiamanian et al. 2011; Hubli et al. 2013; Lamy and Boakye 2013b). Although different settings and stimulation parameters have been used, the "monopolar" montage (active electrode over the lower thoracic spinal cord, return electrode over the right arm) reduces the spread of the current towards the higher spinal cord levels or to the brainstem (Parazzini et al. 2014). Equally important, longitudinal electrical fields, as those induced by this montage, may have important implications for rehabilitation as they promote axonal regrowth and prevent fiber degeneration (Hernandez-Labrado et al. 2011).

In earlier research in our laboratory (Cogiamanian et al., 2008), we found that thoracic anodal tsDCS depresses the cervico-medullary SEP component (P30). tsDCS also modulates post-activation H-reflex dynamics (Winkler et al., 2010; Lamy et al., 2012) and the flexion reflex in the human lower limb (Cogiamanian et al., 2011). Further experiments reported that tsDCS impairs conduction in the ascending nociceptive spinal pathways thus increasing pain tolerance in healthy subjects (Truini et al. 2011). Cervical spinal DC stimulation increased upper-limb muscle motor evoked potential (MEP) amplitudes (Lim and Shin, 2011). The effects induced by tsDCS could arise from the influence of electric field on impulse

conduction, membrane excitability, GABAergic and glutamatergic transmission (Priori et al. 2014). Whatever the mechanisms, by modulating spinal cord function, tsDCS could provide a novel therapeutic tool complementary to drugs and invasive spinal cord stimulation in managing various pathological conditions, including pain, spasticity and movement disorders.

Having more information on the underlying mechanisms is an essential prerequisite for designing future clinical applications and, more specifically, to understand whether and how tsDCS influences the corticospinal system would be relevant. Though interesting, the work of Lim & Shin (2011) on upper limb motor responses cannot rule out possible non specific effects. Hence, to expand the knowledge about the tsDCS effects on the human corticospinal pathways, we tested upper and lower limb MEPs elicited by transcranial magnetic stimulation (TMS) in a group of healthy subjects before and after tsDCS. To evaluate possible motorneuronal or reflex excitability changes induced by tsDCS we also tested the F-wave from the lower limb abductor hallucis (AH) and the upper limb abductor digiti minimi (ADM) muscles and the H-reflex from soleus muscle.

Materials and Methods

Transcutaneous spinal DC stimulation (tsDCS)

With participants lying supine on a comfortable couch, tsDCS (2.5 mA, 20 min) was delivered by a constant current programmable electrical stimulator (HDCStim^{TM}, Newronika, Italy) connected to a pair of electrodes, one centred over the spinous process of the 10th thoracic vertebra with the major axis longitudinally placed so that it spanned from 9th to 11th thoracic vertebrae, and the other above the right shoulder on the deltoid muscle (Cogiamanian et al., 2008, 2011). Because the tibial nerve arises from L4 to S3 spinal levels that correspond to 9th to 12th vertebral level, the active tsDCS electrode was placed over lumbar and sacral motorneurons. tsDCS electrodes were thick (6 mm) rectangular pieces of saline-soaked synthetic sponge (7 x 5 cm, 35 cm^2). We applied current at a density of 0.071 mA/cm^2 and

delivered a total charge density of 85.7 mC/cm^2 which is below the threshold values for tissue damage (Liebetanz et al. 2009; McCreery et al. 1990). The wide electrode surface avoided the possible harmful effects of high current density. Apart from occasional, transient and short-lasting tingling and burning sensations below the electrodes, tsDCS remained below the conscious sensory threshold throughout the experimental session. tsDCS polarity (cathodal or anodal) refers to the electrode over the spinal cord.

Motor evoked potentials (MEPs)

Transcranial magnetic stimulation (TMS) was delivered by a Novametrix Magstim 200 stimulator (Magstim®, Whitland, Carmarthenshire, UK) through a flat coil (outer diameter 13.5 cm) in which current flows clockwise (viewed from above). The coil was kept in a constant position centered over the vertex for both upper and lower limb; for the upper limb one edge of the coil was slightly tilted towards the hemisphere to be stimulated (Groppa et al., 2012). Motor evoked potentials (MEPs) were recorded at rest by two standard non-polarizable Ag/AgCl surface electrodes (diameter 10 mm; Technomed Europe®), one placed over the belly of the *abductor digiti minimi* (ADM) muscle, and the other on the skin overlying the first metacarpophalangeal joint of the fifth finger of the left hand; for lower limbs, MEPs were recorded through one electrode placed over the belly of the *abductor hallucis* (AH) muscle and the other on the first metatarsophalangeal joint of the left toe. Because both ADM and AH have been used in many TMS studies in normal subjects and in patients (Chen et al. 1998; Nakanishi et al. 2006; Osei-Lah and Mills 2004), and our laboratory uses both muscles in routine TMS studies, we selected these two muscles for our experiments.

Stimulation intensity was set at 120% of resting motor threshold (RMT) defined as the minimum stimulator output that evoked MEPs higher than 50 μV in at least five out of 10

trials when muscle is completely relaxed (Di Lazzaro et al. 1999; Ni et al. 2007). The threshold was set differently for each muscle and was analyzed at each time point (B, T0, T30). However, to compare MEP modifications across time we used the same stimulation intensity (baseline). A total of ten MEPs were collected at approximately 10 s intervals and averaged for each time point. MEPs were amplified and filtered (bandwidth 3Hz–3kHz, Nicolet Viking IV P). Three different variables were measured: RMT (% of stimulator output), onset latency (ms) and area-under-the curve (mVms) of motor response. RMT was measured before and after tsDCS, MEP area and latency were measured off-line on the MEPs averaged from ten sweeps.

H-reflex

H-reflexes were elicited in eight subjects by delivering 1 ms rectangular pulses through Ag/AgCl electrodes (10-mm diameter), placed over the left tibial nerve at the popliteal fossa (inter-electrode distance 20 mm), and recorded from the soleus muscle, through Ag/AgCl electrodes (10-mm diameter) placed 2 cm apart over the muscle belly. The leg was fixed, with the hip semi-flexed (~110°), the knee slightly flexed (~150°), and the ankle in approximately 10° plantar flexion. The current intensity was progressively increased to obtain H-reflex threshold (defined as the minimum stimulation intensity that evoked reproducible response higher than 50 uV), maximal H-reflex, and maximal compound muscle action potential (CMAPmax). To avoid post-activation effects, the tibial nerve was stimulated at intervals randomly varying between 10 and 20 s.

To define threshold and maximum size of H-reflex, stimulation began at 0 mA intensity and increased in 1 mA steps up to the intensity eliciting the maximal H-reflex. Signals were amplified and band-pass filtered (3Hz–3kHz). We measured the H-reflex size (peak-to-peak amplitude, mV) and we calculated the Hmax/CMAPmax ratio.

158

159 *F-wave*

160 F-waves were elicited in eight subjects with a 25% supra maximal stimulation applied
161 to the tibial nerve and recorded from the abductor hallucis (AH) muscle, or to the ulnar nerve
162 and recorded from the abductor digiti minimi (ADM) muscle through a pair of 10 mm surface
163 Ag/AgCl electrodes in a belly-to-tendon configuration. Tibial-nerve evoked F-waves from the
164 AH muscle were obtained by 20 stimuli delivered to the left ankle and at an interstimulus
165 interval of 1 s (1 Hz). Similarly, ulnar F-waves from ADM muscles were elicited by 20
166 stimuli delivered to the ulnar nerve at the left wrist with an interstimulus interval of 1 s (1
167 Hz). Subjects were asked to fully relax. To ensure the absence of muscular activity we
168 recorded the audio EMG feedback from the same muscles used for MEP recording at all time
169 points. F-wave mean latency (ms), minimal latency (ms), mean amplitude (mV), mean
170 temporal dispersion (ms) were collected and analysed. The filter setting was 15-1500Hz and
171 skin temperature at the ankle and wrist was kept above 32°C.

172

173 *Subjects and experimental procedure*

174 A group of 14 healthy right-handed volunteers (nine women and five men, mean
175 [±SD] age 25.6 ± 4.3 years) participated in the study, which was approved by the institutional
176 review board. Before enrollment, the study protocol was explained to each subject and
177 informed written consent was obtained. The experimental procedures were conducted in
178 accordance with the declaration of Helsinki.

179 Subjects were studied before and after anodal and cathodal tsDCS. Cathodal and
180 anodal tsDCS in each subject were tested in random order and at least 1 week elapsed
181 between sessions. The subjects were blinded about tsDCS polarity. Because preliminary
182 experiments showed that anodal and cathodal tsDCS elicited MEP changes in the opposite

directions and subjects were unable to discriminate stimulation polarity (as for brain tDCS), as in previous works (Cogiamanian et al. 2008; Truini et al. 2011), we avoided using sham stimulation.

Soleus H-reflex, lower (AH) and upper (ADM) limb MEPs and F-wave were recorded before tsDCS (baseline), immediately after tsDCS offset (T0), and at 30 min after tsDCS offset (T30); all tests were performed in the same order as we have listed them above. For all the electrophysiological recordings we chose the left side to avoid any possible modification on motor response on the right side due to the current flowing through the reference tsDCS electrode possibly acting on peripheral nerves. During each tDCS session, subjects were interviewed to assess the general tolerability of the procedure, and were asked to report any adverse effect, particularly itching, tingling, burning, and pain sensations.

Data analysis

We analyzed statistical significant changes for the following variables: MEP threshold (percentage of the maximum stimulator output), MEP area (mVms), MEP latency (ms); H-reflex threshold, H-reflex latency (ms), H-reflex amplitude (μV), and Hmax/CMAPMax amplitude ratio; F-wave mean latency (ms), minimal latency (ms), amplitude (μV) and temporal dispersion (ms). All the neurophysiological measures were considered as independent variables and were analyzed separately. Each variable is expressed throughout the text as a percentage of baseline values (= 100%), after anodal and cathodal tsDCS, at T0 and T30.

First, to verify the absence of biases due to intra-subject changes across tsDCS sessions, a t-test was run to compare baseline data for anodal and cathodal tsDCS. Values of $p<0.05$ were considered to indicate statistical significance.

Then, tsDCS-induced changes in each variable were tested with a two-way repeated measure analysis of variance (ANOVA) (STATISTICA 5.5, StatSoft Inc.) with main factors ‘‘stimulation”, two levels (anodal and cathodal), and “time”, three levels (B, T0 and T30). Bonferroni corrected t-tests were used for *post hoc* comparison ($p<0.025$). Values in the text and figures are expressed as mean±SEM.

Results

All participants tolerated the procedure well and none of them reported adverse effects. Participants occasionally referred a slight tingling or itching sensation below the stimulating electrodes (not distinguishable between the two polarities) that disappeared within few seconds or after wetting the electrode sponges.

tsDCS effects in the lower limb muscles

No differences were found between anodal and cathodal tsDCS in baseline values for any of the measured variables in the lower-limb muscles. Neither anodal nor cathodal tsDCS induced changes in H-reflex and F-wave variables (Table 1).

Though MEP latency showed an un-specific effect of time (two-way ANOVA, factor “time”: $p = 0.009$) (baseline latency values were in general shorter than latency values at T30 (*post-hoc* analysis: baseline vs T30 103.53 ± 0.95%, $p=0.008$), MEP latency was not affected by tsDCS (two-way ANOVA, factor “stimulation”: $p = 0.83$), nor by the interaction between tsDCS and time (two-way ANOVA, interaction “stimulation x time”: $p = 0.85$).

RMT was affected by tsDCS (two-way ANOVA, factor “stimulation”: $p = 0.012$; factor “time” $p=0.11$; interaction “stimulation x time”, $p=0.011$). More specifically, after anodal tsDCS (Figure 1A) RMT increased (*post hoc* analysis anodal tsDCS: $T0_A$ vs. B_A: 107.33 ± 3.3%, $p = 0.006$; $T30_A$ vs. B_A: 108.37 ± 3.2%, $p = 0.002$), whereas after cathodal

tsDCS (Figure 1B and Figure 2A) it remained unchanged (*post hoc* analysis, cathodal tsDCS: $T0_C$ vs. B_C: 96.83 ± 2.3%, p = 0.15; $T30_C$ vs. B_C: 100.60 ±2.1%, p = 0.8).

MEP area was also modulated after tsDCS (two-way ANOVA, factor "stimulation": p = 0.96; factor "time" p=0.78 interaction "stimulation x time", p=0.008) (Figure 2B): whereas after anodal tsDCS MEP area failed to change (*post hoc* analysis anodal tsDCS: $T0_A$ vs. B_A: 66.09 ±9.1%, p = 0.24; $T30_A$ vs. B_A: 58.12 ±10.48%, p = 0.13), after cathodal tsDCS MEP area increased (*post hoc* analysis cathodal tsDCS: $T0_C$ vs. B_C: 139.71 ± 12.9%, p =0.018; $T30_C$ vs. B_C: 132.74 ±22.0%, p = 0.02).

In conclusion, anodal tsDCS increases RMT whereas cathodal tsDCS increases the MEP area in lower limb muscles.

tsDCS effects in the upper limb muscles

No differences were found between anodal and cathodal tsDCS in baseline values for any of the measured variables in the upper-limb muscles. Neither anodal nor cathodal tsDCS induced changes in F-wave variables (Table 1).

The two-way ANOVA disclosed no anodal or cathodal tsDCS-induced effect on MEP variables (Figure 1C and 1D, Figure 2C and 2D). RMT did not change over time and across sections (Figure 2C: two-way ANOVA, factor "stimulation": p = 0.54; factor "time" p=0.14; interaction "stimulation x time", p=0.48), nor it did MEP area (Figure 2D: two-way ANOVA, factor "stimulation": p = 0.58; factor "time" p=0.63; interaction "stimulation x time", p=0.23). MEP latency was not affected by stimulation (two-way ANOVA, factor "stimulation": p = 0.26; interaction "stimulation x time", p=0.29) but showed the unspecific time-related increase at T30 compared to baseline (two-way ANOVA, "time" p=0.013; *post-hoc* analysis: baseline vs T30 103.53 ± 0.95%, p=0.011).

Discussion

Whereas tsDCS leaves H-reflex, F-wave and upper-limb RMT and MEPs size unchanged, it modulates the excitability in corticospinal projections to lower limb muscles for at least 30′ after stimulation offset, inducing polarity-dependent excitability changes: anodal tsDCS significantly increases RMT, whereas cathodal stimulation increases MEP area.

The absence of F-wave changes in our experiments therefore argues against the occurrence of changes in postsynaptic motorneuronal excitability after tsDCS. The absence of H/M ratio changes in this and a previous study from our group (Cogiamanian et al. 2011) agrees with previous observations (Lamy et al. 2012; Winkler et al. 2010).

The tsDCS-induced corticospinal excitability changes at lower limb level are in line with our previous observation that tsDCS modulates conduction along human spinal ascending pathways (Cogiamanian et al., 2008; Truini et al., 2011) and are consistent with the effects of anodal spinal DC on motor potentials elicited by cortical stimulation in the mouse *triceps surae* (Ahmed, 2011).

How tsDCS influences the corticospinal system remains hypothetical. First, because tDCS influences neurotransmitters in the brain (Rango et al. 2008), tsDCS could do the same in the human spinal cord, ultimately modulating the corticospinal output as in animals (Ahmed and Wieraszko 2012). As recently shown in animals (Ahmed, 2013), cathodal tsDCS can amplify segmental responses to supraspinal drive by increasing glutamate release at the spinal level, although mice typically lack the monosynaptic corticomotoneuronal synapse of higher primates. Another possibility is that tsDCS could influence neural activity in ascending spinal pathways, ultimately modulating the excitability in their cortical targets including the motor areas, as changes in RMT suggest. Possible support for a cortical mechanism comes

from a report that invasive spinal stimulation seems to modulate intracortical facilitation (Schlaier et al. 2007). Thus, our data expand, rather than be simply contradictory, previous knowledge on putative tsDCS targets, including supra-spinal, and possibly polarity-specific, effects of spinal current polarization; this possibility also agree with recent evidence in rats that non-invasive spinal stimulation modulates the activity of gracile nucleus and primary somatosensory cortex (Aguilar et al. 2011).

Finally, tsDCS could influence the conductive properties of the corticospinal tract, for example by decreasing/increasing the number of axons conducting an action potential. For instance, anodal tsDCS can induce a hyperpolarizing conduction block thus blocking action potentials along the pyramidal tract (Bhadra and Kilgore 2004). However, given that we used low current intensities, anodal block could not be the sole explanation for the effects of anodal tsDCS; moreover, it's known that, also in routinary electrodiagnostic testings, geometry and tissue distribution of electrical fields are additional critical parameters for inducing a hyperpolarizing block (Dreyer et al. 1993; Kirshblum et al. 1998).

Although the mechanisms underlying the tsDCS-induced changes in the corticospinal system remain speculative, our finding that anodal tsDCS seems mainly to affect the RMT whereas cathodal tsDCS predominantly influences MEP area is intriguing. A possible explanation is that the mechanisms underlying cathodal and anodal tsDCS differ and could have a different putative circuit(s)-pathway(s)-neurotransmitters, i.e. anodal current might preferentially act on one target system, whereas cathodal current acts on another. This possibility agrees with evidence that anodal and cathodal brain tDCS act through different brain neurotransmitters: for instance, whereas anodal tDCS reduces gamma aminobutyric acid (GABA), cathodal tDCS decreases glutamate (Stagg et al. 2009). Increase in MEP area following cathodal tsDCS is in line with previous reports (Aguilar et al. 2011; Ahmed 2013; 2011; Alanis 1953; Eccles et al. 1962) and agree with data in animals showing an increased

recruitment of larger motor units following cathodal polarization (Ahmed and Wieraszko 2012); as suggested in a recent work by our group, cathodal, but not anodal, stimulation could have a trans-synaptic effect mediated by spinal interneurons likely involving Renshaw cells network (Bocci et al. 2014).

Among methodological issues related to our study the first is that to avoid subjecting participants to another experimental session, we decided not to test them under sham conditions. Sham testing was also unnecessary given that even though changes induced by cathodal tsDCS and anodal tsDCS go in opposite directions, subjects cannot distinguish between the two polarities. We therefore considered each polarity as the best possible control for the other as previously reported (Cogiamanian et al. 2008; Lamy and Boakye 2013a; Truini et al. 2011). Besides, as recently showed by Kessler and colleagues (Kessler et al. 2012), sham stimulation may be an inappropriate control condition for some studies, because sensory side effects seem to be more frequent and severe in active than in sham tDCS.

A second methodological issue is about possible pitfalls concerning the use of H-reflex and F-wave to assess spinal motorneuron excitability. The absence of H-reflex changes suggests no modification to small motorneurons and the lack of F-wave effects rules out changes in large motorneurons (McNeil et al. 2013). However, the classical view that H-reflex and F-wave represent separate and complementary events at spinal level, i.e. presynaptic inhibition versus changes in intrinsic motorneuron excitability (Fisher 1992; Leis et al. 1995), is questionable. In fact, while H-reflex could be also affected by post-activation depression and changes in axonal excitability itself (McNeil et al. 2013; Pierrot-Deseilligny et al. 1981), many reports have suggested that F-waves offers only a flawed measure of motorneuron excitability (Hultborn and Nielsen 1995).

A further important point is to compare our data with those by Lim and Shim (Lim and Shin, 2011) who found that cervical spinal DC stimulation influences upper-limb MEPs. But

they did not report polarity-specific effects. Their results cannot be compared with ours because they studied a different anatomical region, with a different recording montage and stimulation intensity, and used smaller electrodes. Finally, there are known features in how corticospinal excitability differs between the upper and lower body, which may account, at least in part, for differences between our results and data obtained by Lim and Shin. Particularly, monosynaptic corticomotorneuronal projections, with fast conducting motor units, are more prominent for hand than for proximal arm or lower limb muscles (Brouwer and Ashby 1992; Dalpozzo et al. 2002; Palmer and Ashby 1992); in this view, lack of changes in MEPs amplitude following anodal tsDCS may be caused by the greater desynchronization of corticomotorneuronal input compared with upper limb muscles, related to the involvement of fibers with different sizes or activation of polysynaptic descending pathways.

Overall, our data support the conclusion that tsDCS induces changes in corticospinal tract excitability. The first clinical observations with tsDCS are encouraging: Hubli (Hubli et al., 2013) found that anodal tsDCS can improve gait in patients with spinal cord injury. Together with a previous report describing how tsDCS influences ascending spinal pathways (Aguilar et al., 2011), the present experiments suggest that the spinal cord could act as a "highway" for conveying tsDCS-induced changes to the brain thereby inducing suprasegmental effects in the brain and brainstem. Because tsDCS is simple, safe and non-invasive, our observation opens the way to new approaches using this technique in widely ranging neurological conditions characterized by corticospinal and spinal cord dysfunction and possibly even in brain disorders.

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360 of interest.

361

362 **Figure Legends**

363 *Figure 1*. **A, B -** Lower-limb motor evoked potentials (MEPs) before (baseline, B),
364 immediately after (T0) and at 30 min (T30) after anodal (**A**) or cathodal (**B**) tsDCS in a
365 representative subject. Horizontal calibration 10 ms; vertical calibration 1 mV; each trace is
366 the superimposition of five sweeps. The table on the right side reports the average values of
367 RMT, MEP area, and MEP latency for the represented subject. Note that whereas anodal
368 tsDCS decreased the MEP area at T0 and T30, cathodal tsDCS increased the MEP area.
369 Vertical arrows represent the stimuli. **C, D -** Upper-limb motor evoked potentials (MEPs)
370 before (baseline, B), immediately after (T0) and at 30 min (T30) after anodal (**C**) or cathodal
371 (**D**) tsDCS in a representative subject. Horizontal calibration 10 ms; vertical calibration 0.5
372 mV; each trace is the superimposition of five sweeps. The table on the right side reports the
373 average values of RMT, MEP area, and MEP latency for the represented subject. Note that
374 neither anodal nor cathodal tsDCS affected MEPs. Vertical arrows represent the stimuli.
375
376 *Figure 2*. **A, B -** Effects of transcutaneous spinal direct current stimulation (tsDCS) on resting
377 motor threshold (**A**, RMT) and motor evoked potentials (**B**, MEP) area when responses were
378 recorded from abductor hallucis muscle (AH; data are expressed as % of baseline). Group
379 data are presented as mean ± SEM changes induced by anodal (gray) or cathodal (dark gray)
380 tsDCS immediately after current offset (T0) and 30 minutes later (T30; error lines are
381 standard error of the mean, SEM). Note that anodal and cathodal tsDCS induced significantly
382 different, opposite changes in RMT and MEP area. **C, D -** Effects induced by tsDCS on RMT
383 (**C**) and MEP size (**D**) when responses were recorded from abductor digiti minimi (ADM)
384 muscles. Group data are presented as mean ± SEM changes induced by anodal (gray) or
385 cathodal (dark gray) tsDCS immediately after current offset (T0) and 30 minutes later (T30;

386 error lines are standard error of the mean, SEM). Neither anodal nor cathodal tsDCS
387 significantly changed RMT or MEP area. ** = $p<0.05$.
388
389 *Table 1* -Changes in F-wave and H-reflex variables over time (B, T0, T30), in both
390 experimental conditions (anodal and cathodal stimulation). We found no significant changes
391 in F-wave and H-reflex variables across the different time points. p values refer to two-way
392 repeated measures ANOVA with "stimulation" and "time" as factor (interaction effects). Data
393 are shown as mean values ± SEM. Minimal, mean latencies and temporal dispersion are
394 expressed in ms, H-reflex area as mVms.
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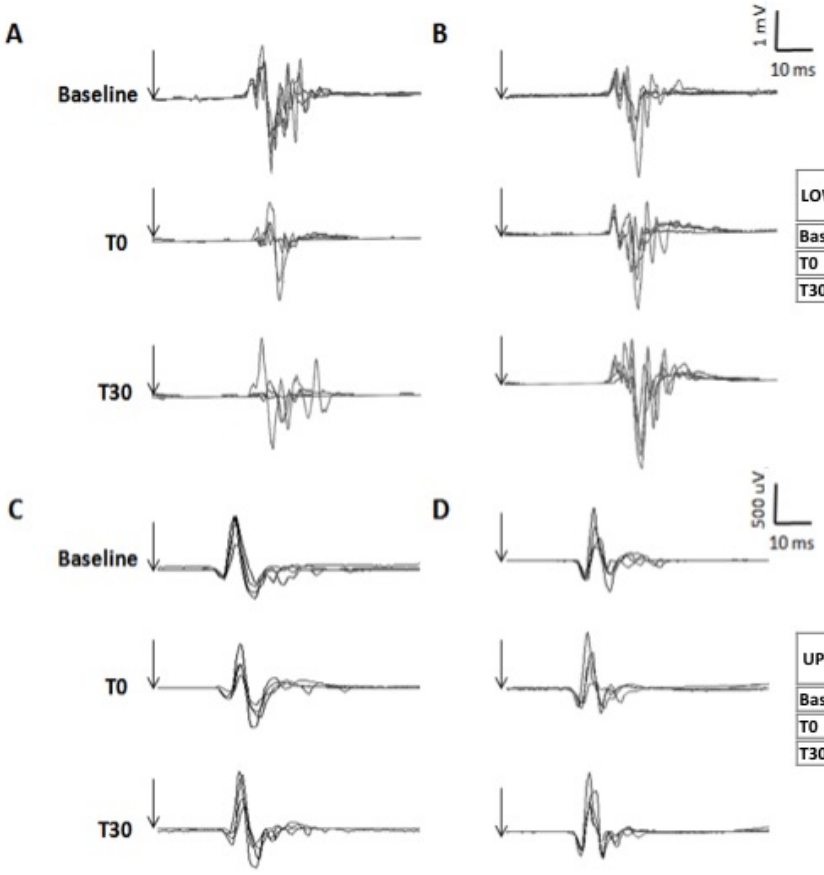
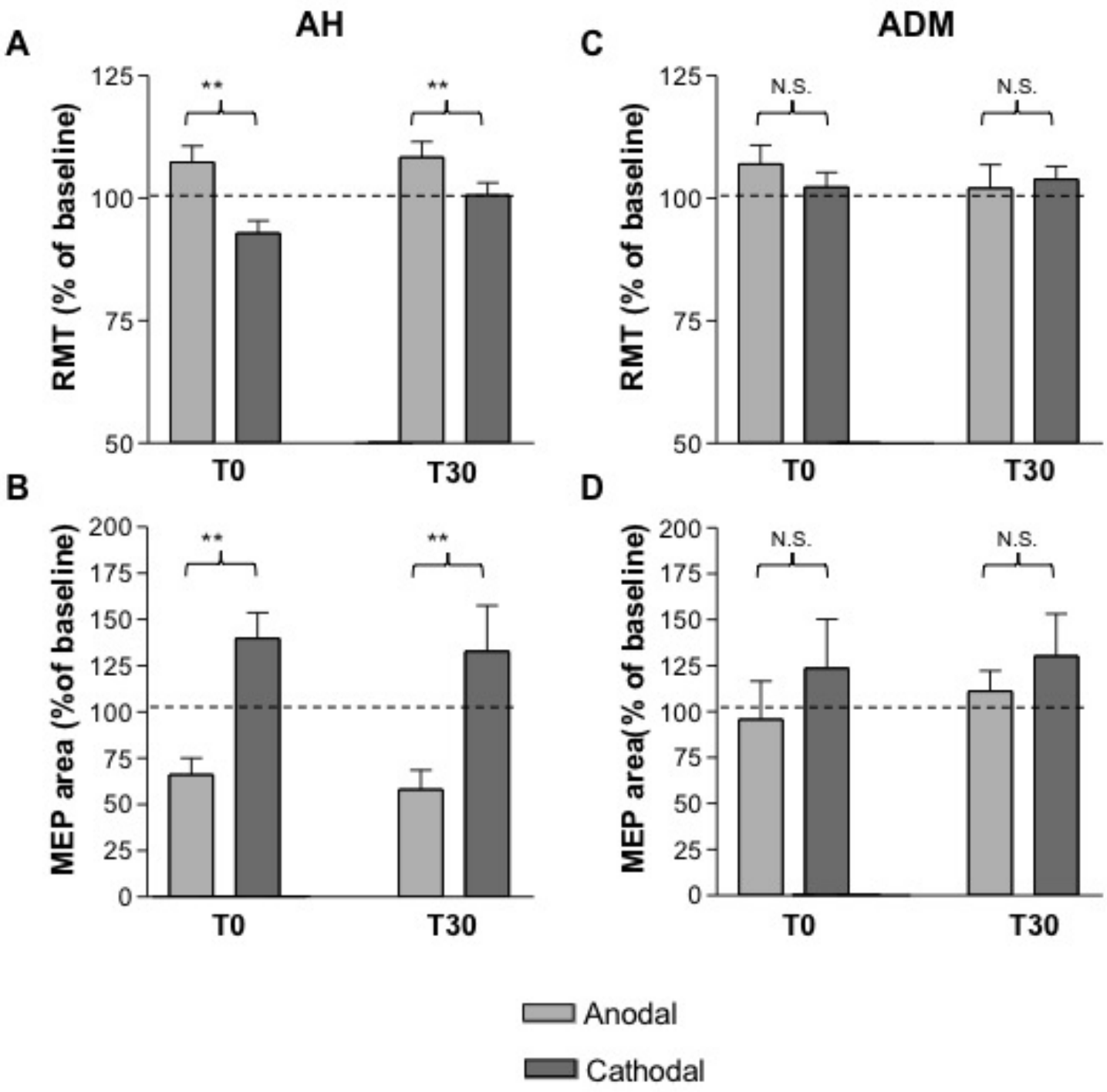


LOWER LIMB	RMT (%)		MEP area (mV)		MEP Latency (ms)	
	Anodal tsDCS	Catodal tsDCS	Anodal tsDCS	Catodal tsDCS	Anodal tsDCS	Catodal tsDCS
Baseline	60	55	3.11	2.09	33.68	34.85
T0	75	60	1.42	2.95	38	37.8
T30	75	60	1.63	3.75	36.93	36.9

UPPER LIMB	RMT (%)		MEP area (mV)		MEP Latency (ms)	
	Anodal tsDCS	Catodal tsDCS	Anodal tsDCS	Catodal tsDCS	Anodal tsDCS	Catodal tsDCS
Baseline	55	50	1.15	1.08	18.8	20.6
T0	55	50	1.23	1.28	20.5	19.6
T30	55	50	1.2	1.36	19.3	20.5


A
AH
RMT (% of baseline)
125
100
75
50
**
**
T0
T30
C
ADM
N.S.
N.S.
B
MEP area (%of baseline)
200
175
150
125
100
75
50
25
0
D
MEP area(% of baseline)
Anodal
Cathodal

		F-wave						H-reflex			
		AH			ADM			Soleus			
		Minimal Latency (ms)	*Mean Latency (ms)*	*Dispersion (ms)*	*Minimal Latency (ms)*	*Mean Latency (ms)*	*Dispersion (ms)*	*Threshold (ms)*	*Latency (ms)*	*Area (mVms)*	*Ratio H/M*
Anodal (mean ± SEM)	*B*	46.4 ± 1.3	48.2 ± 1.5	4.3 ± 0.6	25.8 ± 0.6	27.9 ± 0.8	2.9 ± 0.5	14.7 ± 3.5	28.9 ± 1.0	7.4 ± 1.0	0.4 ± 0.1
	T0	47.0 ± 1.5	49.3 ± 1.4	4.3 ± 0.5	26.2 ± 1.0	27.8 ± 0.8	2.8 ± 0.7	13.9 ± 2.8	28.3 ± 1.3	7.1 ± 0.6	0.4 ± 0.05
	T30	47.2 ± 1.4	50.5 ± 2.5	4.4 ± 0.7	26.3 ± 0.9	28.3 ± 1.9	3.0 ± 0.4	13.9 ± 1.8	29.3 ± 1.4	7.7 ± 0.9	0.4 ± 0.13
Cathodal (mean ± SEM)	*B*	46.3 ± 1.2	48.6 ± 1.2	4.4 ± 0.5	25.3 ± 1.2	27.8 ± 1.5	3.0 ± 0.7	14.1 ± 3.0	28.0 ± 1.2	7.7 ± 0.6	0.4 ± 0.04
	T0	46.3 ± 1.4	48.8 ± 1.7	4.7 ± 0.3	25.5 ± 1.5	28.0 ± 1.0	3.1 ± 0.4	14.1 ± 2.5	27.8 ± 1.3	7.6 ± 1.1	0.4 ± 0.1
	T30	47.2 ± 1.6	49.1 ± 1.5	4.8 ± 0.7	26.0 ± 1.1	28.3 ± 1.0	2.8 ± 0.6	13.8 ± 2.7	28.9 ± 0.8	7.9 ± 1.4	0.3 ± 0.3
p-value		*0.6*	*0.2*	*0.8*	*0.3*	*0.9*	*0.3*	*0.8*	*0.7*	*0.8*	*0.3*