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RESEARCH ARTICLE

Repeated cathodal transspinal pulse and direct current stimulation modulate cortical and corticospinal excitability differently in healthy humans

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Abstract
Noninvasive transspinal stimulation of the thoracolumbar region, where leg motor circuits reside, produces prominent plasticity of brain and spinal cord circuits. However, reorganization of cortical and corticospinal excitability after multiple sessions (i.e. repeated) remains elusive. In this study, we investigated changes in intracortical inhibition, intracortical facilitation, and corticospinal excitability after 10 sessions of cathodal transcutaneous delivery of pulse or direct current stimulation, termed here transspinal (tsPCS, tsDCS), in resting healthy humans. tsPCS was delivered at sub- and supra-threshold intensities, while intensity for tsDCS ranged from 2.24 to 2.34 mA within a session. Intracortical inhibition and facilitation were assessed based on the tibialis anterior (TA) motor evoked potential (MEP) amplitude following subthreshold transcranial magnetic stimulation (TMS) at the conditioning-test (C-T) intervals of 1, 2, 3, 10, 15, 20, 25, and 30 ms. The TA MEP recruitment input–output curves were also assembled to establish changes in corticospinal excitability. For both transspinal stimulation protocols, the active cathodal electrode was placed over the T10 spinal process. Results indicated that repeated tsPCS did not alter intracortical inhibition or intracortical facilitation but decreased corticospinal excitability for the right M1 and increased corticospinal excitability for the left M1. tsDCS decreased intracortical inhibition, increased intracortical facilitation, did not affect the maximal MEP amplitude but increased the slope of the right TA MEP input–output curve. Neurophysiological changes may be attributed to neural mechanisms involved in learning and memory. These results support that noninvasive transspinal stimulation alters both cortical and corticospinal neural excitability in resting healthy humans.

Keywords Pulse current · Direct current · Transspinal stimulation · Corticospinal excitability · Cortical mechanisms · Modulation · Plasticity

Introduction

Electrical stimulation-induced plasticity of the central nervous system is mediated largely by similar mechanisms to those of motor learning and training (Eccles 1987; Bennett 2000). Repeated stimulation potentiates excitatory transmission in dorsal horn neurons (Jeftinija and Urban 1994), and activity of inhibitory synapses stabilizing neuronal networks dynamics (Vogels et al. 2013), promoting release of endogenous neurotrophic factors as occurs with motor training (Lamy and Boakye 2013; Houle and Côté 2013).

Evidence suggests that transcutaneous delivery of pulse or direct current over the thoracolumbar region where leg motor circuits reside, termed here transspinal (tsPCS, tsDCS), can regulate brain neuronal activity. The tsPCS induced muscle responses (transspinal evoked potentials; TEPs) use the neuronal pathways that convey descending drive and sensory inputs from the periphery onto spinal neuronal circuits (Knikou 2014; Knikou and Murray 2018). Specifically, cortically-induced motor evoked potentials (MEPs) and spinally-induced TEPs summate at the muscle surface electromyogram (EMG) when the descending motor volleys are timed to arrive at the spinal cord before transspinal stimulation produces depolarization of motoneurons (Knikou 2014), supporting the ability of tsPCS to affect

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corticospinal tract excitability. Within this context, a single session of tsPCS in healthy humans results in depression of indirect waves (Murray et al. 2019) and facilitation of corticospinal tract excitability (Knikou et al. 2015; Dixon et al. 2016). Both cathodal and anodal tsPCS decreased the afferent-mediated MEP facilitation in a polarity-dependent manner (Knikou et al. 2015), further supporting the upstream effects of transspinal stimulation.

Neuroplasticity of brain activity is also evident when stimulation does not evoke direct depolarization of alpha motoneurons, as is the case for direct current. tsDCS modulates the brain spontaneous activity and its responsiveness to external somatosensory stimuli in a polarity-dependent manner in anaesthetized rats (Aguilar et al. 2011). Further, cathodal tsDCS altered the rhythmicity and anodal tsDCS increased the spike frequency and amplitude of cortically induced muscle contractions in mice, while the modulatory effects were different when assessed during or after tsDCS (Ahmed 2011). tsDCS-mediated potentiation of cortically induced muscle contractions was related to increases of extracellular glutamate concentration (Ahmed and Wieraszko 2012), while brain-derived neurotrophic factor (BDNF) genes were linked to tsDCS-induced neuroplasticity (Lamy and Boakye 2013). A single session of tsDCS in humans produces immediate changes in intracortical inhibition (Bocci et al. 2015a), and upregulation of corticospinal tract excitability as evident by changes in MEP amplitude or input–output curve (Lim and Shin 2011; Nierat et al. 2014; Bocci et al. 2015b; Murray et al. 2018), cortical silent period (Bocci et al. 2015c), and amplitude of somatosensory evoked potentials (Cogiamanian et al. 2008).

Collectively, these results support for complex neuromodulation of brain activity by tsPCS and tsDCS. However, evidence on neurophysiological changes after multiple sessions (e.g. repeated administration) of tsPCS and/or tsDCS in humans is lacking. The objectives of this study were to establish changes in intracortical inhibition, intracortical facilitation and corticospinal tract excitability after 10 sessions of cathodal tsPCS and tsDCS in resting healthy humans. We hypothesized that cortical and corticospinal tract excitability will change in a similar pattern regardless of the type of current (i.e. tsPCS, tsDCS). Such results are particularly attractive because tsPCS and tsDCS could provide a novel therapeutic tool for enhancing synaptic connections between spinal and supraspinal levels and improving motor performance through stimulation-induced motor learning and motor memory consolidation (Galea and Celnik 2009). To address our hypothesis, we delivered low-frequency cathodal tsPCS or tsDCS up to 45 min/session. Cortical neuroplasticity was assessed by changes in the amount of short-latency intracortical inhibition and medium-latency intracortical facilitation. Corticospinal neuroplasticity was assessed by amplitude changes in MEP recruitment input–output curves. Lastly, we characterized reorganization of left and right motor cortices after tsPCS because at suprathreshold intensities tsPCS produces synchronous muscle contractions of both legs.

Methods

Subjects

The Institutional Review Board (IRB) of the City University of New York approved all experimental procedures (IRB no. 515055). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent from each individual was obtained before study enrollment and participation. Individuals were excluded if they had a history of epilepsy, head injury, unexplained loss of consciousness, or were taking any medications or agents known to affect central nervous system performance. Subjects were instructed not to consume caffeinated drinks 24 h before each experiment, and a post-study TMS and tsDCS questionnaire were given to establish the presence of any adverse events. No significant changes were noted in the blood pressure of all participants during the experiments and stimulation sessions. Some participants reported a mild-moderate transient headache following the TMS experiments, with minor reports of sleepiness, neck pain, scalp discomfort and/or dental pain. Following tsDCS, the major complaint was skin redness or irritation which subsided within a few hours, followed by reports of tingling, burning or itchy sensations mainly during the ramp-up and down phase of stimulation. No discomfort or irritations were reported following tsPCS.

Noninvasive transspinal stimulation

tsPCS protocol

In this stimulation intervention protocol, 5 females and 5 males with an average age of 30.9 ± 14 (mean $\pm$ SD) participated. With subjects seated, the T10 spinous process was identified via palpation and in consolidation with anatomical landmarks (T1 spinal process, end of sternum, and end of rib cage). A single cathode electrode (Uni-Patch™ EP84169, 10.2×5.1 cm^2, Minnesota, USA) was placed at T10 equally spaced along the vertebrae ending at L1-2 (Fig. 1a). These vertebral levels correspond to spinal segments and segmental innervation of the muscles from which action potentials were recorded in this study. Subjects were then transferred to a supine position, with hip and knee joints flexed at 30°. Two inter-connected electrodes (anode, same type as the cathode), were placed bilaterally on abdominal muscles or iliac crests depending on each subject's reported comfort (Knikou 2013; Knikou et al. 2015). These electrodes were connected

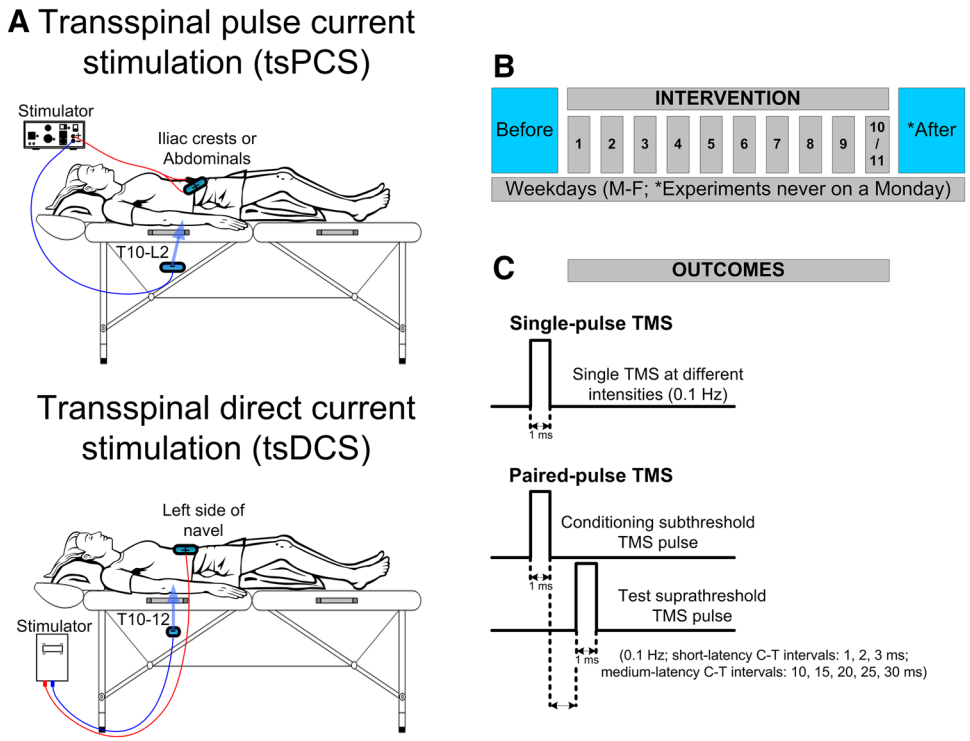

Fig. 1 Transspinal Stimulation for Neuromodulation. **a** Position of subject and placement of electrodes for transspinal pulse and direct current stimulation (tsPCS, tsDCS). **b**, **c** Intervention and outcomes before and after tsPCS and tsDCS. Illustration of single TMS pulses delivered for assembling the MEP recruitment curves. Paired TMS pulses at different conditioning-test (C-T) intervals were used to study intracortical inhibition and intracortical facilitation

to a constant current stimulator (DS7A, Digitimer, UK) that was triggered by Spike 2 scripts (Cambridge Electronics Design Ltd., Cambridge, UK).

tsPCS was delivered daily, excluding weekends and holidays, as a single 1-ms pulse at 0.2 Hz based on the well-described neural interactions between TEPs and MEPs, and between TEPs and muscle spindle primary afferent volleys (Knikou 2014; Knikou and Murray 2018). All subjects received 10 stimulation sessions (39.8 ± 0.1 min per session) except one subject who received 12 sessions because post-stimulation assessments were arranged to be performed during a weekday but not after 2-days of no stimulation (e.g. weekend). At each stimulation session, intensities ranged from 0.7 ± 0.1 to 10.3 ± 2.9 (6.4 ± 1.7) of the right tibialis anterior (TA) TEP resting threshold established at baseline, increasing from an average of 105.1 ± 21.5 mA in the first five sessions to an average of 130.3 ± 24.3 mA in the last five sessions. Within a session, stimulation was delivered in 10-min and 5-min alternating blocks of suprathreshold and subthreshold TEP intensity. The rationale for this approach was based on the fact that TEP maximal amplitudes decrease after 10–15 min of continuous transspinal stimulation (Murray and Knikou 2017; Murray et al. 2019).

tsDCS protocol

In this stimulation intervention protocol, 7 females and 3 males with an average age of 27.2 ± 4.98 (mean ± SD) participated. Four individuals took part in both tsPCS and tsDCS protocols more than 1 year apart. Cathodal tsDCS, which has more persistent neuromodulatory effects than anodal tsDCS (Bindman et al. 1964; Bocci et al. 2014; Murray et al. 2018), was delivered through a rubber encased saline-soaked sponge with a stainless steel mesh backing (3.2 cm × 3.2 cm; Amrex Electrotherapy Equipment, California, USA) connected to a battery-driven direct current stimulator (neuroConn DC stimulator plus, Ilmenau, Germany). Identification of the cathode electrode placement at T10 was identical to the tsPCS protocol but due to size, this electrode covered from T10 to T12 vertebral levels. The subject was then transferred to a supine position and a reference electrode (rubber pad saline-soaked sponge electrode 10.16 cm × 10.16 cm; Amrex Electrode, USA) was positioned adjacent to the navel on the left side (Fig. 1a) to avoid placement over the appendix, and because maximal electric field potentials are directed longitudinal along the spinal cord and cauda equina with this montage (Parazzini et al. 2014). The reference electrode was not positioned exactly on the navel (Parazzini et al. 2014) because of safety concerns. All subjects received 10 stimulation sessions (45 ± 0.27 min per session) except one subject whom received 11 sessions in order for post-assessment experiments not to occur after the weekend (Fig. 1b). Stimulation intensity increased from 2.24 ± 0.023 mA in the first five sessions to 2.34 ± 0.031 mA in the last five sessions. To avoid significant skin irritation by continuous daily tsDCS on the active electrode skin site, stimulation was delivered in blocks of 10-min with intensity ranging between 2.0 to 2.7 mA. The overall average current

density was 0.22 mA/cm^2 (ranging from 0.20–0.23 mA/cm^2) and the overall average total charge was 602.66 C/cm^2 (ranging from 573.54–620 mC/cm^2). These values are ten times lower than the current density threshold for invasive spinal stimulation (2.3 mA/cm^2), and pulse electrical stimulation causing tissue damage (25 mA/cm^2) reported in the literature (McCreery et al. 1990; Wesselink et al. 1998; Cogiamanian et al. 2012).

Neurophysiological assessments before and after repeated transspinal stimulation

In the tsPCS intervention protocol, experiments were performed randomly for the left and right legs 1 or 2 days after cessation of stimulation. In the tsDCS intervention protocol, experiments were performed 1-day after cessation of stimulation (Fig. 1b).

EMG recordings

Surface EMG activity was recorded from the left and right soleus (SOL) and TA muscles by single bipolar differential electrodes (MA300-28, Motion Lab Systems Inc., Louisiana, USA). EMG signals were amplified, filtered (10–1000 Hz), sampled at 2000 Hz via a 1401 plus (Cambridge Electronics Design Ltd., England), and stored for offline analysis.

Transcranial magnetic stimulation

TMS was delivered with a 110 mm diameter double-cone coil via two Magstim 200 stimulators connected with a BiStim module (The Magstim Company Ltd., Whitland, UK) positioned such that the current flowed from a posterior to an anterior direction. With the double-cone coil held 1 cm lateral, posterior, and diagonal from the vertex, the stimulation intensity was gradually increased from zero maximum stimulator output (MSO). When MEPs in the TA muscle could not be evoked without concomitant SOL MEPs at low MSOs, the magnetic coil was moved, and the procedure was repeated. When the optimal position was determined (hot spot), the TA MEP resting threshold was established and corresponded to the lowest MSO that induced reproducible MEPs of at least ~ 50 µV in 4 out of 5 consecutive single TMS pulses.

In all subjects, control MEPs were recorded at 1.3 TA MEP resting threshold following single TMS pulses at 0.1 Hz (Fig. 1c). Control MEPs were recorded in two blocks of 12 consecutive single TMS pulses randomly with the conditioned MEPs. Conditioned MEPs were recorded following subthreshold TMS at the conditioning-test (C-T) intervals of 1, 2, 3, 10, 15, 20, 25, and 30 ms (Fig. 1c). Twelve conditioned TA MEPs were recorded at 0.1 Hz for each C-T interval. For each subject, control and conditioned MEPs were recorded at exactly the same stimulation intensities before and after repeated transspinal stimulation. Across subjects, the subthreshold and suprathreshold TMS pulses before repeated tsPCS were delivered at 0.77 ± 0.01 (36.4 ± 2.3 MSO) and at 1.23 ± 0.01 (58.33 ± 3.04 MSO) times the TA MEP resting threshold, respectively. Similarly, the subthreshold and suprathreshold TMS pulses after repeated tsPCS were delivered at 0.76 ± 0.02 (36 ± 2.02 MSO) and at 1.24 ± 0.02 (58.78 ± 3.24 MSO) times the TA MEP resting threshold, respectively. Similar intensities were utilized for sub- and suprathreshold TMS pulses to study intracortical inhibition and facilitation before and after repeated tsDCS.

Over the same hot spot, MEP recruitment input–output curves were assembled with single pulse TMS at 0.1 Hz in ascending order from subthreshold intensities with absent TA MEP responses until maximum amplitudes were obtained. Stimulation intensities increased in steps of at least 4 MSO, with 4 MEPs recorded at each intensity. For each subject, the TA MEP recruitment curves were assembled at the exact same intensities before and after tsPCS and tsDCS. Conditioned MEPs at short-latency and medium-latency C-T intervals, and MEP recruitment curves before and after tsPCS were recorded from the left and right TA muscle following stimulation of the right and left M1, respectively. Recordings before and after tsDCS were taken from the right TA muscle following stimulation of the left M1 and not bilateral due to grant time constraints.

Data analysis and statistics

TA MEPs were measured as the area of the full-wave rectified EMG signal (Spike 2, Cambridge Electronics Design Ltd., UK). For each subject, the conditioned TA MEP evoked upon paired TMS pulses at different C-T intervals before and after tsPCS and/or tsDCS were normalized to the mean homonymous control MEP size. The mean conditioned TA MEP amplitude from each subject was grouped based on the time of testing, C-T interval, and stimulation intervention protocol. The Shapiro–Wilk test was performed to test data for normal distribution. A repeated-measures analysis of variance (rmANOVA) was performed separately for conditioned MEPs recorded at short- and medium-latency C-T intervals to determine the main effect of time (before vs. after) and C-T interval. When a statistically significant main effect of time was found, Bonferroni *t*-tests for multiple comparisons were used to establish significant interactions between time and C-T intervals.

For each MEP recruitment curve recorded from each subject before and after transspinal stimulation, a Boltzmann sigmoid function (Eq. 1; SigmaPlot 11, Systat Software Inc., California, USA) was fitted to the non-normalized TA MEP sizes plotted against the actual stimulation intensities. Equation 1 estimated the predicted maximal MEP, *m* function

of the slope, and stimulation intensity corresponding to the 50% of the maximal MEP (S50-MEPmax). Equations 2–4 were used to estimate the slope of the MEP input–output curve, and stimuli corresponding to the MEP threshold (MEPth), and maximal MEP. Each predicted parameter from the sigmoid fit for each subject was grouped based on the time of testing, and a two-way rmANOVA was applied to the data. When a statistically significant main effect of time was found, Bonferroni *t*-tests for multiple comparisons were used to establish significant interactions between time and stimulation intensities:

$$\text{MEP (s)} = \frac{\text{MEPmax}}{(1 + \exp(m(s50 - s)))} \quad (1)$$

$$\text{MEPslope} = \frac{m \times \text{MEPmax}}{4} \quad (2)$$

$$\text{MEPth stim} = \frac{s - 2}{m} \quad (3)$$

$$\text{MEPmax stim} = \frac{s + 2}{m}. \quad (4)$$

For each MEP recruitment curve recorded from each subject, the MEPs recorded before and after transspinal stimulation were normalized to the maximal MEP recorded before transspinal stimulation. The TMS MSO intensities used before transspinal stimulation were expressed in multiples of the homonymous S50-MEPmax, while the TMS MSO intensities used after transspinal stimulation were expressed in multiples of the predicted S50-MEPmax obtained both before and after transspinal stimulation. The normalized MEPs were then grouped within and across subjects based on multiples of stimulation intensities. The average normalized MEP size was calculated in increments of 0.05 multiples of S50-MEPmax for each subject and across subjects, and rmANOVA was performed to establish the main effects of time. When a statistically significant main effect of time was found, post hoc Bonferroni *t* tests for multiple comparisons were used to test for significant interactions between time and stimulation intensities. Results are presented as mean ± standard error of the mean. Significance was considered with a $p < 0.05$.

Results

Intracortical excitability changes after repeated cathodal tsPCS

Non-rectified waveform averages of conditioned MEPs from two representative subjects recorded from the left and right TA muscles at short- and medium-latency C-T intervals reflecting the amount of intracortical inhibition and intracortical facilitation before and after tsPCS are shown in Fig. 2a, b, respectively. rmANOVA for the left TA MEPs recorded at short-latency C-T intervals showed a non-significant main effect of time ($F_{(1,48)} = 0.35$, $p = 0.55$), among C-T intervals ($F_{(2,48)} = 1.45$, $p = 0.24$), or in their interaction ($F_{(2,48)} = 0.14$, $p = 0.86$; Fig. 2c). Similar results were found for the right TA MEPs (Fig. 2d) recorded at short-latency C-T intervals (time: $F_{(1,54)} = 0.38$, $p = 0.53$; C-T intervals: $F_{(2,54)} = 1.02$, $p = 0.36$). These results suggest for unchanged intracortical inhibition in the right and left M1 after repeated tsPCS.

rmANOVA for the left TA MEPs recorded at medium-latency C-T intervals (Fig. 2c) showed a significant main effect of time ($F_{(1,71)} = 5.66$, $p = 0.02$) but not between C-T intervals ($F_{(4,71)} = 1.55$, $p = 0.19$) or in their interaction ($F_{(4,71)} = 0.16$, $p = 0.95$). While a significant effect of time was found, post hoc Bonferroni *t*-tests showed that the left conditioned TA MEPs at any of the medium-latency C-T interval recorded before and after tsPCS were not significantly different ($p = 0.08$ for all) suggesting that more subjects may be required to reach statistical significance. Finally, rmANOVA for the right TA MEPs recorded at medium-latency C-T intervals (Fig. 2d) showed a non-significant main effect of time ($F_{(1,87)} = 0.15$, $p = 0.70$), suggesting for unaltered intracortical facilitation in the left M1 after repeated cathodal tsPCS.

Intracortical excitability changes after repeated cathodal tsDCS

Non-rectified waveform averages of the conditioned MEPs recorded from the right TA muscle are shown in Fig. 3a from a representative subject (same subject as the conditioned MEPs shown before and after tsPCS in Fig. 2a), and depict clear changes in intracortical modulatory mechanisms after tsDCS. rmANOVA for the right TA MEPs recorded at short-latency C-T intervals showed a non-significant effect of time ($F_{(1,54)} = 1.06$, $p = 0.31$), but a significant difference across short-latency C-T intervals ($F_{(2,54)} = 4.63$, $p = 0.014$). In contrast, rmANOVA for the right TA MEPs recorded at medium-latency C-T intervals showed a significant main effect of time ($F_{(1,90)} = 4.04$, $p = 0.04$) but not between C-T intervals ($F_{(4,90)} = 0.66$, $p = 0.63$) or in their interaction ($F_{(4,90)} = 0.07$, $p = 0.99$). Post hoc Bonferroni *t*-tests showed that the TA MEPs at 2 ($t = -2.03$, $p = 0.04$), 25 ($t = -5.0$, $p = 0.02$) and 30 ($t = -8.0$, $p = 0.04$) ms were significantly different before and after tsDCS (Fig. 3b). These results suggest that intracortical inhibition at 2 ms was decreased, and intracortical facilitation at 25 and 30 ms was increased after repeated tsDCS.

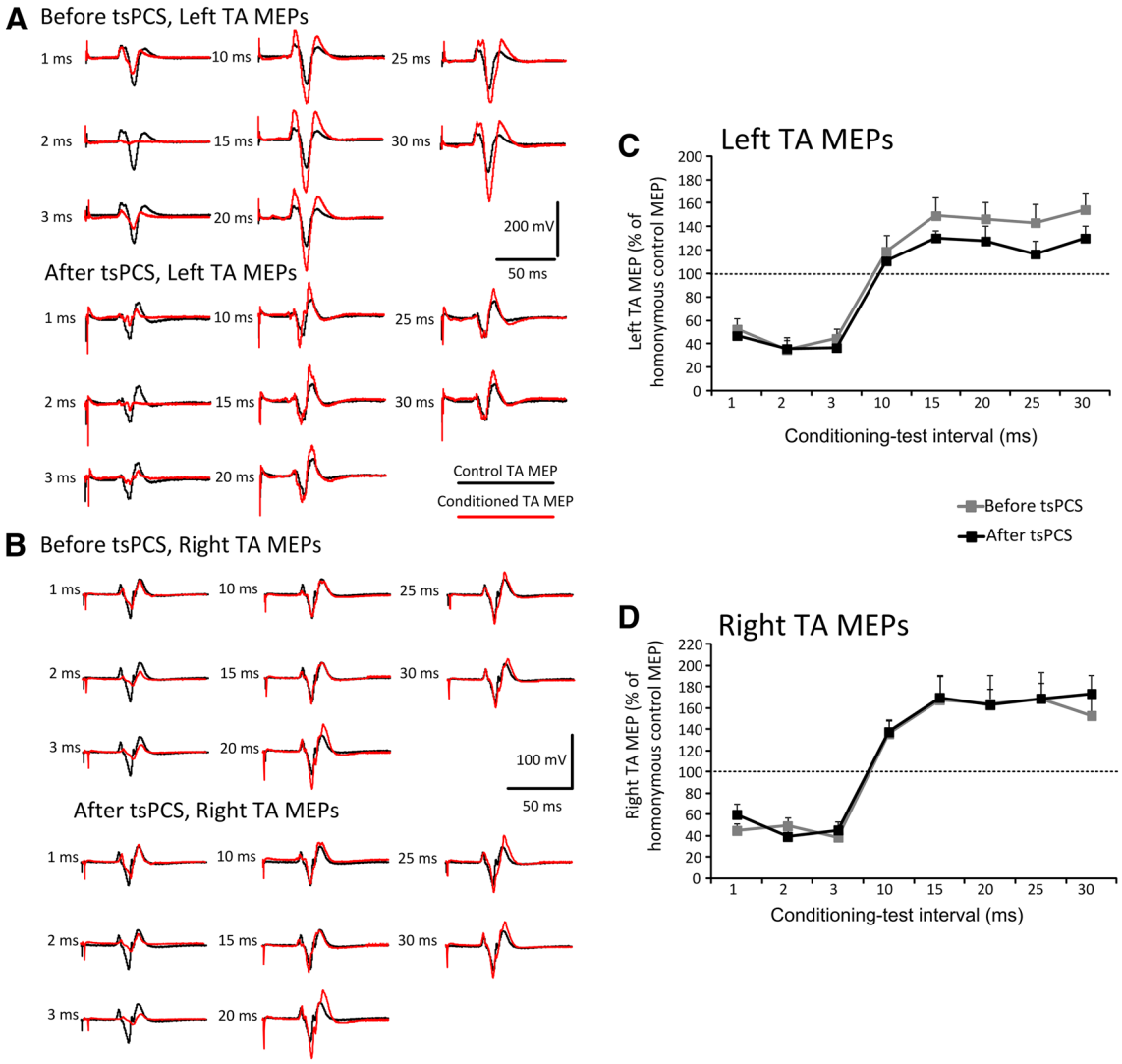

Fig. 2 Intracortical inhibition before and after repeated tsPCS. **a**, **b** Non-rectified waveform averages of conditioned tibialis anterior motor evoked potentials (TA MEPs) by subthreshold transcranial magnetic stimulation (TMS) from two subjects before and after tsPCS recorded from the left and right TA muscle. **c**, **d** Group amplitude of MEPs recorded from the left and right TA muscles conditioned by subthreshold TMS. Analysis showed a tendency for decreased intracortical facilitation after tsPCS in the left leg, and no significant changes in intracortical inhibition and/or intracortical facilitation in the right leg. Error bars indicate SE

Corticospinal excitability changes after repeated cathodal tsPCS and tsDCS

The mean normalized MEPs recorded from the left and right TA muscle before and after tsPCS, and from the right TA muscle before and after tsDCS from all subjects are shown in Fig. 4. For all cases, the MEPs are expressed as a percentage of the maximal MEP observed before transspinal stimulation, and are plotted against the stimulation intensities expressed in multiples of the predicted S50-MEPmax observed before and after transspinal stimulation, and are shown along with the sigmoid fit curve.

The MEP input–output, recorded from the left TA muscle after tsPCS exhibited a shift to the right when MEPs were grouped based on stimulation intensities normalized to the predicted S50-MEPmax observed before and after tsPCS (Fig. 4a). This shift, however, coincided without significant changes in the slope of MEP input–output curve (Table 1). No significant difference was found for any of the predicted sigmoid parameters (m function, slope, and stimuli corresponding to MEP threshold and MEPmax) from the sigmoid analysis conducted for each subject and MEP input–output curve separately (Table 1). However, both the predicted maximal MEPs and the normalized MEPs were significantly decreased after tsPCS ($p = 0.005$) when stimulation intensities were normalized to the homonymous S50-MEPmax. Furthermore, an overall significant effect of time ($F_{(2,277)} = 12.9$, $p < 0.001$) and stimulation intensity

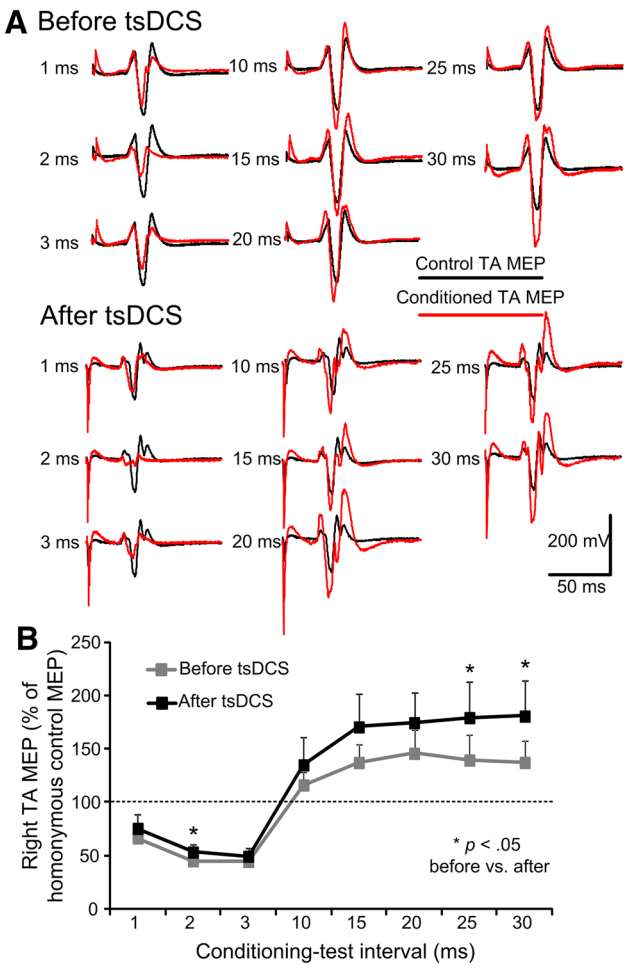

Fig. 3 Intracortical inhibition and facilitation before and after repeated tsDCS. **a** Non-rectified waveform averages of conditioned TA MEPs (black lines) by subthreshold transcranial magnetic stimulation (TMS) from one subject before and after tsDCS. **b** Group amplitude of TA MEPs conditioned by subthreshold TMS are plotted against short and medium latency interstimulus intervals tested. Analysis showed a significant effect of time with intracortical inhibition to decrease and intracortical facilitation to increase after tsDCS. Error bars indicate SE; $*p < 0.05$

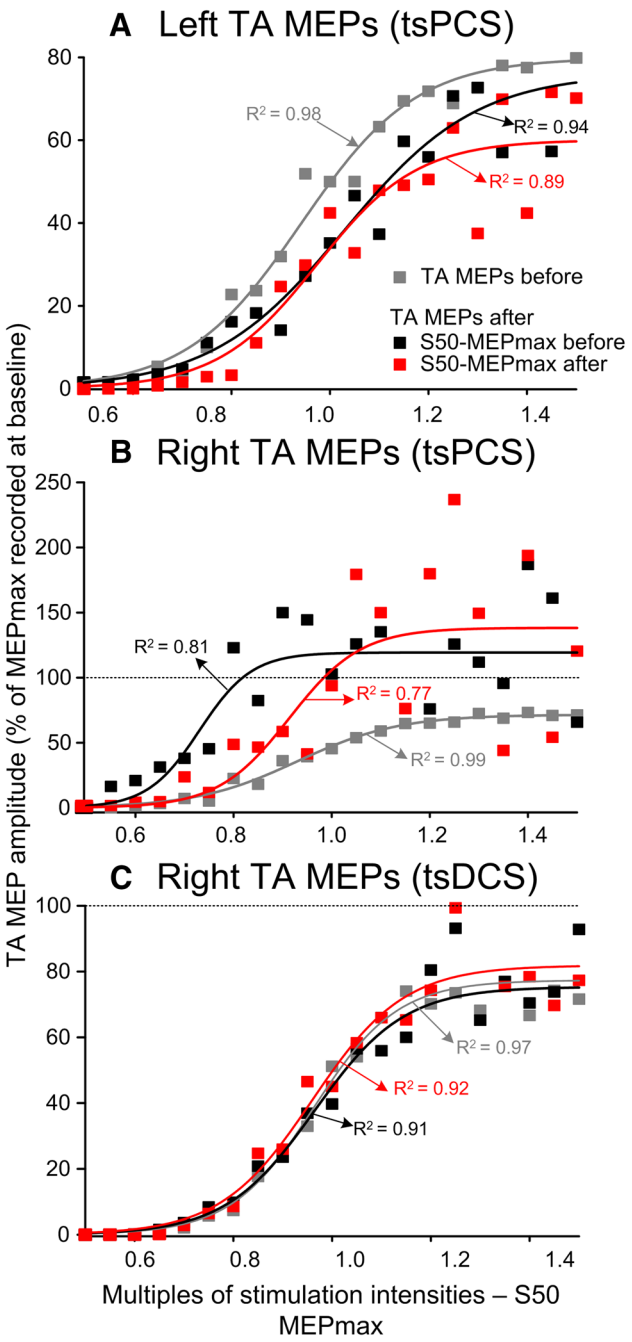

Fig. 4 Corticospinal excitability before and after repeated tsPCS and tsDCS. TA MEPs recruitment curves from all subjects before (grey lines) and after (red, black lines) tsPCS recorded from the left and right TA muscles (**a**, **b**), and repeated tsDCS recorded from the right TA muscle (**c**). For all cases, TA MEPs are normalized to the maximal MEP size obtained before transspinal stimulation. The TA MEPs recorded before transspinal stimulation are grouped based on multiples of stimulation intensities corresponding to 50% of the associated maximal MEP (S50-MEPmax) (grey lines). The TA MEPs recorded after transspinal stimulation are grouped based on stimulation intensities that were normalized to the S50-MEPmax obtained before (black lines) and after (red lines) stimulation. For all cases, a sigmoid fit to the data is also shown. Analysis showed a significant effect of time for the tsPCS in the right TA MEPs with increased MEP sizes at 1.05 and at 1.25 multiples of S50-MEPmax and decreased MEP sizes in the left leg

($F_{(20,277)} = 28.28$, $p < 0.001$) on the normalized MEPs but not in the interaction between time and stimulation intensities ($F_{(40,277)} = 0.93$, $p = 0.58$) was found. Bonferroni *t*-tests showed decreased MEP sizes at 1.3 and at 1.45 multiples of S50-MEPmax ($p < 0.05$) (Fig. 4a), supporting for decreased corticospinal excitability after tsPCS in the left TA MEPs.

The MEP input–output, recorded from the right TA muscle after tsPCS exhibited a shift to the left when MEPs were grouped based on stimulation intensities normalized to the predicted S50-MEPmax observed before tsPCS (Fig. 4b). This shift coincided with an overall increase in the *m* function and slope of the MEP input–output curve. However, the sigmoid analysis conducted for each subject and MEP input–output curve separately,

Table 1 TA MEP sigmoid function parameters

	MEPmax	*m*	slope	S50-MEPmax	Sth-MEP	Smax-MEP
tsPCS left TA MEP						
Before	102.54 ± 8.95	9.27 ± 2.31	0.28 ± 0.05	1.04 ± 0.04	0.76 ± 0.04	1.33 ± 0.08
After S50MEPmax.T1	62.52 ± 10.25	9.70 ± 1.77	0.29 ± 0.08	1.96 ± 0.99	1.67 ± 0.92	2.24 ± 1.07
p value	**0.005**	0.44	0.18	0.48	0.16	0.20
After S50MEPmax.T2	62.45 ± 10.27	9.33 ± 1.98	0.38 ± 0.16	2.87 ± 1.87	2.49 ± 1.71	3.26 ± 2.03
p-value	**0.005**	0.49	0.28	0.17	0.16	0.17
tsPCS right TA MEP						
Before	82.05 ± 4.37	9.65 ± 1.05	0.23 ± 0.03	1.00 ± 0.01	0.76 ± 0.03	1.23 ± 0.03
After S50MEPmax.T1	230.11 ± 66.25	19.26 ± 6.59	0.30 ± 0.10	1.16 ± 0.20	0.86 ± 0.10	1.46 ± 0.30
p-value	**0.01**	0.08	0.26	0.21	0.19	0.22
After S50MEPmax.T2	230.11 ± 66.25	15.67 ± 4.49	0.26 ± 0.08	1.13 ± 0.14	0.87 ± 0.08	1.39 ± 0.21
p-value	**0.01**	0.10	0.37	0.18	0.12	0.23
tsDCS right TA MEP						
Before	81.86 ± 6.32	14.35 ± 2.17	0.17 ± 0.02	1.00 ± 0.00	0.84 ± 0.02	1.17 ± 0.03
After S50MEPmax.T1	89.66 ± 19.3	9.09 ± 1.07	0.25 ± 0.04	1.31 ± 0.31	1.06 ± 0.31	1.56 ± 0.31
p-value	0.33	**0.02**	**0.03**	0.15	0.23	0.09
After S50MEPmax.T2	84.94 ± 17.35	9.08 ± 0.92	1.21 ± 0.2	1.21 ± 0.20	0.97 ± 0.21	1.46 ± 0.19
p-value	0.39	**0.04**	0.08	0.14	0.23	0.08

Results of predicted parameters from the sigmoid input–output relation of tibialis anterior motor evoked potentials (TA MEPs) normalized to the maximal MEP recorded at baseline (before stimulation, T1), and stimulation intensities normalized to the predicted S50-MEPmax before (T1) and after (T2) transspinal stimulation. Mean averages are the result of sigmoid function fitted to each subject's MEP recruitment curve separately and grouped based on time and type of stimulation. Mean ± SE. *p*-values shown are for before and after transspinal pulse and/or direct current stimulation (tsPCS, tsDCS) comparisons

Significant differences for each parameter before and after transspinal stimulation is indicated in bold

showed that although the *m* function of the slope and the slope increased from 9.65 to 19.26 and from 0.23 to 0.3, respectively, no significant difference was found ($p = 0.083$; $p = 0.267$, respectively; Table 1). In contrast, the predicted maximal MEP was significantly increased after tsPCS ($p = 0.019$), regardless of the method used to group the MEPs per normalized stimulation intensities (S50-MEPmax before or after tsPCS). Furthermore, an overall significant effect of time ($F_{(2,362)} = 9.24$, $p < 0.001$) and stimulation intensity ($F_{(20,362)} = 4.61$, $p < 0.001$) on the normalized MEPs but not in the interaction between time and stimulation intensities ($F_{(40,362)} = 0.81$, $p = 0.78$) was found. Bonferroni *t*-tests showed increased MEP sizes at 1.05 and at 1.25 multiples of S50-MEPmax ($p < 0.05$) (Fig. 4b), supporting for increased corticospinal excitability in the right TA MEPs after repeated tsPCS.

The normalized MEPs recorded from the right TA muscle before and after tsDCS did not vary as a function of time ($F_{(2,295)} = 0.180$, $p = 0.83$; Fig. 4c). The sigmoid analysis for MEP input–output curves conducted for each subject separately showed that the *m* function was decreased when stimulation intensities were normalized to the predicted S50-MEPmax obtained before or after tsDCS ($p = 0.02$ and $p = 0.03$, respectively). The slope increased ($p = 0.034$) when stimulation intensities were normalized to the S50-MEPmax obtained at baseline (Table 1), suggesting for increased corticomotoneuron gain after tsDCS.

Discussion

The main findings of this study are that repeated transspinal stimulation with pulse or direct current produces complex bilateral corticospinal tract excitability changes in resting healthy humans. tsPCS decreased corticospinal excitability for the right motor cortex and increased corticospinal excitability in the left motor cortex that coincided with unaltered cortical neuronal mechanisms. tsDCS decreased intracortical inhibition, increased intracortical facilitation, did not alter maximal MEP amplitudes but increased the slope of the MEP input–output and thereby the corticomotoneuron gain.

We used a paired-pulse TMS paradigm to study changes in cortical neuronal mechanisms after repeated tsPCS and/or tsDCS over the thoracolumbar region. Results indicated that intracortical inhibition and intracortical facilitation upon paired TMS pulses (Figs. 2 and 3) occurred at C-T intervals consistent to those previously reported in the literature (Kujirai et al. 1993; Chen et al. 1998). Because intracortical inhibition and intracortical facilitation do not depend on changes in spinal excitability (Kujirai et al. 1993; Chen

et al. 1998), they can accurately measure changes in primary motor cortex (M1) activity. MEP depression at short C-T intervals has been ascribed to interneuronal circuits in M1, involving largely low-threshold γ-aminobutyric acid receptor-dependent inhibitory pathways (Ziemann et al. 1996; Ilic et al. 2002). MEP facilitation at medium C-T intervals occurs at the initial axon segment of cortical interneurons involving excitatory glutamatergic pathways (Di Lazzaro et al. 1998; Ilic et al. 2002).

Repeated cathodal tsPCS for the left MEPs showed a tendency for decreased intracortical facilitation (Fig. 2c) and significant decreased maximal MEP amplitudes (Fig. 4a). In contrast, for the right MEPs the unchanged intracortical neuronal mechanisms (Fig. 2d) coincided with increased MEP amplitudes (Fig. 4b). These results support for diametrically opposite changes in corticomotoneuron control for each limb, with cortical and corticospinal excitability changes after repeated tsPCS to occur in the same direction only within the same side of the body. Further, the increased MEP amplitudes from the input–output curve supports strengthened corticospinal projections after repeated tsPCS (Chen et al. 1998). We should note that definite conclusions on the effects of tsDCS and tsPCS cannot be made solely based on the right MEPs since left MEPs might have been affected but were not assessed in this study. Potential targets of neuromodulation by tsPCS include brain neural circuits via dorsal column axons and complex ascending neuronal pathways, local spinal circuits, and proprioceptive afferents (Fig. 5).

These sites of action are supported by the decreased afferent-mediated MEP facilitation after one session of tsPCS (Knikou et al. 2015), and depression of TA MEP facilitation peaks associated with late I-waves (Murray et al. 2019) that are considered to be under cortical control (Hanajima et al. 2002). Thus, there is great support for the involvement of neuronal pathways connecting thalamocortical axons and cortical interneurons. In addition to the sites of action, we should consider potential mechanisms of tsPCS-induced neuroplasticity. tsPCS at suprathreshold intensities evokes synchronous bilateral contractions of knee and ankle muscles, and thereby transsynaptic depolarization of motoneurons over multiple spinal segments. These synapses have shown to be glutamatergic because TEPs are blocked by cyanquixaline, an AMPA/kainate receptor antagonist (Hunanyan et al. 2012). The summation of TEPs and MEPs in the surface EMG (Knikou 2014), confirms that TEPs use the neuronal pathways that convey descending drive onto spinal neuronal circuits, and thus for a possibility of long-term depression (LTD) and long-term potentiation (LTP) of synaptic transmission accounting for the observed changes. LTD and LTP typically result from conventional paired associative stimulation protocols (Müller-Dahlhaus et al. 2010; Dan and Poo 2004). However, non-Hebbian LTD and LTP have been reported in cerebellar circuits (Piochon et al. 2013), hippocampus (Pandey and Sikdar 2014), thalamus (Sieber et al. 2013), and spinal lamina I neurons (Naka et al. 2013). Consequently, we can suggest that repeated single postsynaptic burst activity produced by tsPCS results in LTP and/or LTD, engaging mechanisms similar to those involved in spike-timing-dependent (STD) plasticity. The mechanisms involved in STD plasticity include activation of *N*-methyl-D-aspartate (NMDA) receptors (Fino et al. 2008), calcium and magnesium concentration changes (Malenka et al. 1988), as well as increases in the amount of transmitters released and size of dendritic spines, and new protein synthesis for maintenance of LTD and LTP (see review of Bennett 2000). Activation of NMDA receptors is required for the long-lasting facilitation of MEP amplitude after electromagnetic spinal cord stimulation in mid-thoracic lateral hemisection rats (Hunanyan et al. 2012), and is involved in induction of LTP-like action of neurotrophin 3 and BDNF in the spinal cord of neonatal rats (Arvanov et al. 2000; Arvanian and Mendell 2001; Arvanian et al. 2004). Additionally, the presence of BDNF secretion determines direct current stimulation induced LTP when delivered along with low-frequency stimulation in mouse M1 slices (Fritsch et al. 2010). Based on the cellular and molecular mechanisms underlying non-Hebbian LTD and LTP, these mechanisms may account

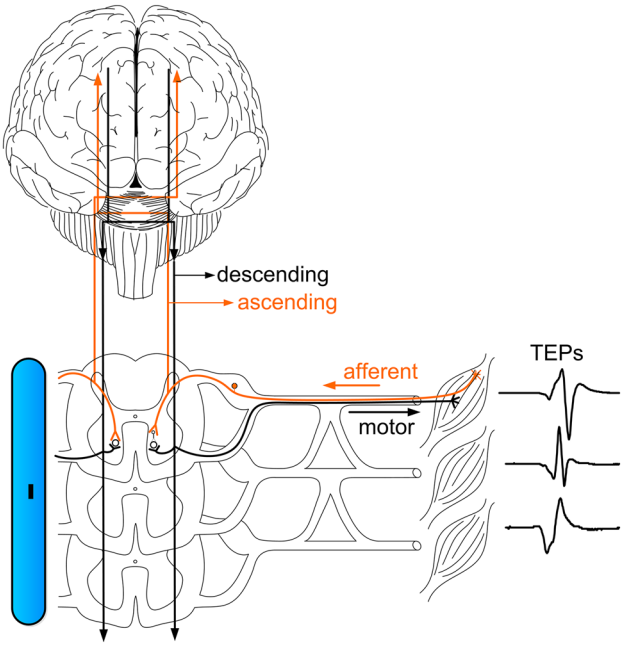

Fig. 5 Transspinal stimulation current flow and possible targets of neuromodulation include proprioceptive afferents, local neuronal circuits in both halves of the spinal cord, and brain circuits by dorsal column axons

for upregulation or downregulation of MEPs after tsPCS (Fig. 4a, b). LTD mechanisms after electrical stimulation can readily be recognized by the decreased MEP amplitude following repetitive transcortical low-frequency stimulation that increases the activity of inhibitory GABAergic interneurons (Kallioniemi et al. 2015).

Cathodal tsDCS decreased intracortical inhibition and increased intracortical facilitation (Fig. 3b), consistent to our recent findings after one session of tsDCS (Murray et al. 2018), and opposite to the unchanged intracortical facilitation of arm and leg MEPs assessed at a C-T interval of 10 ms (Bocci et al. 2015a). However, comparison of findings across studies is difficult given that the effects of tsDCS depend likely on the intensity, frequency-duration of administration, and montage of electrodes (Batsikadze et al. 2013).

tsDCS decreases spontaneous brain activity and somatosensory evoked potentials and increases local field potentials in the gracile nucleus in anaesthetized rats (Aguilar et al. 2011), increases excitability of myelinated axons within the dorsal columns that outlasts tsDCS application independent of activity (Jankowska et al. 2017), and produces long-lasting facilitation of cortical responses (Bindman et al. 1962). These studies support the notion that decreased intracortical inhibition and increased intracortical facilitation after repeated cathodal tsDCS in our study could have been mediated by persistent changes of spontaneous activity of cortical activity and membrane excitability changes (Priori et al. 2014) of complex ascending pathways. In contrast to tsPCS, tsDCS does not evoke any direct muscle responses and has been assigned to induce changes via non-synaptic mechanisms in humans (Ardolino et al. 2005). However, because direct current stimulation produces polarization of neuronal cells and sensory fibers (Eccles et al. 1962; Bindman et al. 1962, 1964; Bikson et al. 2004; Ahmed 2011; Bolzoni and Jankowska 2015; Jankowska et al. 2017), similar mechanisms to those described for the tsPCS may also account for tsDCS-induced neuroplasticity.

tsDCS induced no changes in the MEP amplitude (Fig. 4c) but increased the MEP slope (Table 1). An increased MEP slope reflects increased corticomotoneuron gain that is not associated with changes in the recruitment order of spinal motoneurons (Devanne et al. 1997). Increases in MEP slope have been attributed to changes in the subliminal fringe of cortical neurons and spinal motoneurons and interneurons (Capaday and Stein 1987). A large subliminal fringe after tsDCS is expected to lead to less rapidly increasing responses, explaining the non-significant MEP amplitude changes. This finding, however, is in contrast to those reported after one session of cathodal tsDCS on corticospinal excitability in humans (Lim and Shin 2011; Bocci et al. 2015b; Murray et al. 2018). Nonetheless, the effects of tsDCS have not been recorded after multiple sessions of stimulation, and thus a comparison on the results among different studies is difficult.

Limitations of the study

In this study, a sham stimulation group was not tested. This was largely based on the fact that the effects were assessed before and after the two interventions to show how different types of electrical fields led to dissociations in outcomes. Furthermore, it would be hard to disguise the difference between active and sham tsPCS, since at suprathreshold TA TEP intensities tsPCS produces synchronous extension of both legs with noticeable abdomen contractions, and tsDCS produces sensory side effects that are frequently reported following active stimulation. Although we delivered tsDCS within the suggested safety limits and based our choices on trying to maximize the current flow within the spinal cord at the thoracic level, the arrangement of stimulation parameters may not be optimal. A systematic investigation is warranted for both tsPCS and tsDCS applications, along with the identification of safety concerns specific to transcutaneous stimulation. We also did not systematically investigate the duration of after-effects and, therefore, cannot comment on the potential of either protocol to induce long-term neuroplasticity. However, the effects lasted more than 1 or 2 days after the end of stimulation supporting for strong neuromodulation effects.

Functional significance

This study provides evidence that repeated cathodal tsPCS and tsDCS produces strong but different neurophysiological changes in remote motor pathways involving both cortical and corticospinal neuronal circuits. Until recently, tsPCS or tsDCS mediated neuroplasticity has been reported immediately after stimulation. Our findings support that neural excitability changes persist 24 to 48 h after stimulation offset. Finally, tsDCS can be used to elevate intracortical facilitation, and tsPCS to strengthen corticomotoneuron excitability, while both stimulation protocols can be paired with other neuromodulatory methods to potentiate further neuroplasticity for improving motor performance.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest to report.

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