Transspinal constant-current long-lasting stimulation: a new method to induce cortical and corticospinal plasticity

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Knikou M, Dixon L, Santora D, Ibrahim MM. Transspinal constant-current long-lasting stimulation: a new method to induce cortical and corticospinal plasticity. J Neurophysiol 114: 1486–1499, 2015. First published June 24, 2015; doi:10.1152/jn.00449.2015.—Functional neuroplasticity in response to stimulation and motor training is a well-established phenomenon. Transcutaneous stimulation of the spine is used mostly to alleviate pain, but it may also induce functional neuroplasticity, because the spinal cord serves as an integration center for descending and ascending neuronal signals. In this work, we examined whether long-lasting noninvasive cathodal (c-tsCCS) and anodal (a-tsCCS) transspinal constant-current stimulation over the thoracolumbar enlargement can induce cortical, corticospinal, and spinal neuroplasticity. Twelve healthy human subjects, blind to the stimulation protocol, were randomly assigned to 40 min of c-tsCCS or a-tsCCS. Before and after transspinal stimulation, we established the afferent-mediated motor evoked potential (MEP) facilitation and the subthreshold transcranial magnetic stimulation (TMS)-mediated flexor reflex facilitation. Recruitment input-output curves of MEPs and transspinal evoked potentials (TEPs) and postactivation depression of the soleus H reflex and TEPs was also established. We demonstrate that both c-tsCCS and a-tsCCS decrease the afferent-mediated MEP facilitation and alter the subthreshold TMS-mediated flexor reflex facilitation in a polarity-dependent manner. Both c-tsCCS and a-tsCCS increased the tibialis anterior MEPs recorded at 1.2 MEP resting threshold, intermediate, and maximal intensities and altered the recruitment input-output curve of TEPs in a muscle- and polarity-dependent manner. Soleus H-reflex postactivation depression was reduced after a-tsCCS and remained unaltered after c-tsCCS. No changes were found in the postactivation depression of TEPs after c-tsCCS or a-tsCCS. Our findings reveal that c-tsCCS and a-tsCCS have distinct effects on cortical and corticospinal excitability. This method can be utilized to induce targeted neuroplasticity in humans.

brain stimulation; spinal cord stimulation; neuroplasticity; transcortical magnetic stimulation; transspinal evoked potential; motor evoked potential

The brain and spinal cord are prone to functional and structural changes that can occur in distributed neural networks underlying motor behavior (Joseph 2013). Neuronal plasticity can be induced via repetitive, paired, or continuous electrical stimulation (Rossini et al. 2015). For example, high-frequency repetitive transcranial magnetic stimulation (TMS), repetitive TMS paired with peripheral nerve stimulation at long interstimulus intervals, and repetitive paired TMS pulses at I-wave periodicity increase motor evoked potential (MEP) sizes and thus potentiate corticospinal excitability (Pascual-Leone et al. 1994; Stefan et al. 2000; Thickbroom et al. 2006). Similarly, low-frequency repetitive TMS and TMS paired with peripheral nerve stimulation at shorter intervals have depressant effects on corticospinal excitability (Chen et al. 1997; Wolters et al. 2003). Possible mechanisms accounting for these neurophysiological changes are those underlying learning and memory, known as long-term potentiation (LTP) and long-term depression (LTD) (Dan and Poo 2006; Rioult-Pedotti et al. 2000; Stefan et al. 2002).

Corticomotoneuronal connections have a systematic synaptic pattern with alpha motoneurons (Bawa and Lemon 1993; Edgley et al. 1997), and corticospinal systems corresponding to each leg interact at the spinal level (Brus-Ramer et al. 2009). These potent characteristics of corticomotoneuronal connections along with their distinct ability to change after electrical stimulation (Carmel et al. 2010) support the notion that long-lasting stimulation of the spinal cord can influence cortical and corticospinal excitability. In anesthetized spinal cats, polarizing currents passed across the spinal cord in a dorsal-ventral direction alter the amplitude of membrane potentials and the excitatory postsynaptic potentials (EPSPs) of spinal motoneurons (Eccles et al. 1962). Furthermore, repetitive stimulation produces hyperpolarization of nerve terminals, thereby enhancing synaptic transmission via postactivation potentiation (Hubbard and Willis 1962).

Electrical stimulation delivered epidurally or transcutaneously to the spinal cord generates compound action potentials or transspinal evoked potentials (TEPs) in arm and leg muscles with distinct neurophysiological characteristics (Eimhoin et al. 2013; Knikou 2013a, 2013b, 2014; Maruyama et al. 1982). TEPs are mediated mostly by direct nonsynaptic activation of motoneurons and indirect transsynaptic excitation of descending projections and local spinal interneuron circuits (Maruyama et al. 1982; Sharpe and Jackson 2014). In people with spinal cord injury, current delivered transcutaneously or via epidural stimulation increases motor activity of paralyzed muscles (Harkema et al. 2011; Jilge et al. 2004), induces rhythmic locomotor-like motor activity (Minassian et al. 2007), and entrains output of previously silent muscles during robotic-driven assisted stepping (Minassian et al. 2015). These modulatory effects on motor output can be mediated by plastic changes in a network(s) of cortical or spinal circuits, but it is not known whether long-lasting noninvasive transspinal stimulation can induce neuroplasticity.

We have demonstrated that the transspinal stimulation-induced TEPs in arm and leg muscles are increased when the excitability of peripheral nerves is altered or when descending and ascending inputs are synchronized to meet at the spinal...
cord (Einhorn et al. 2013; Knikou 2013a, 2013b, 2014). On the basis of these findings, we hypothesized that transspinal stimulation induces cortical, corticospinal, and spinal neuroplasticity. To test our hypotheses, we delivered cathodal (c-tsCCS) and anodal (a-tsCCS) transspinal constant-current stimulation for 40 min. Cortical neuroplasticity was assessed based on changes in the amount of afferent-mediated MEP facilitation and subthreshold TMS-mediated flexor reflex facilitation. Corticospinal neuroplasticity was assessed based on changes in the amplitude of resting MEPs and MEP recruitment input-output curves. Spinal plasticity was assessed based on changes in the amplitude of resting TEPs, recruitment curves of TEPs, and amount of soleus H-reflex and TEP postactivation depression. Part of this study has been published in abstract form (Knikou et al. 2015).

METHODS

Subjects

Twelve healthy subjects (5 men, 7 women; 24 ± 1.67 yr, mean age ± SD) with right leg dominance participated in this study. All subjects gave their written consent to the experimental procedures, which were conducted in compliance with the Declaration of Helsinki after full Institutional Review Board (IRB) approval by the City University of New York IRB committee. Subjects with tooth implants, metal implants in the body, assistive hearing devices, pacemaker, history of seizures, or history of depression and those who were taking medications known to alter central nervous system (CNS) excitability were excluded from the study. The blood pressure of all participants was monitored periodically during the experiments, and no significant changes were noted. MEPs and spinal reflexes were recorded with subjects seated (hip angle, 120°; knee angle, 160°; ankle angle, 110°) with both feet supported by foot rests. Transspinal stimulation was delivered with subjects supine. Both knee joints were flexed at 30°, and ankle joints were supported by foot rests positioned in neutral. The supine position was selected because TEP amplitude depends on the position of the body (Danner SM, Krenn M, personal communication).

Recordings. In all subjects, electromyography (EMG) was recorded bilaterally from the medial gastrocnemius (MG), soleus (SOL), tibialis anterior (TA), rectus femoris (RF), vastus medialis (VM), medial hamstrings (MH), and hip adductor gracilis (GRC) muscles via single bipolar differential electrodes (MA300-28, Motion Lab Systems, Baton Rouge, LA). EMG signals were amplified, filtered (10-1,000 Hz), sampled at 2,000 Hz (1401 plus running Spike 2, Cambridge Electronics Design), and stored as coded data files on a password-protected personal computer for off-line analysis.

Stimulation

Transcranial. Single TMS pulses over the left primary motor cortex were delivered with a Magstim 200² stimulator (Magstim) with a double-cone coil (diameter 110 mm) placed so the current of the coil flowed from a posterior to an anterior direction. Procedures were similar to those we have previously utilized (Hanna-Boutrous et al. 2015; Knikou 2014; Knikou et al. 2013). The point where the lines between the inion and glabellum and the left and right ear tragus met was marked on an EEG cap. The center of the double-cone coil was placed 1 cm posterior to and 1 cm to the left of this intersection point. With the double-cone coil held at this position, the stimulation intensity was gradually increased from zero and MEPs recorded from the right TA, SOL, and MG muscles were observed on a digital oscilloscope. When TA MEPs could not be evoked at low stimulation intensities with the subject at rest in three of five consecutive TMS pulses, the magnetic coil was moved by a few millimeters to the left and the procedure was repeated. When the optimal position was determined, the TA MEP resting threshold was established and corresponded to the lowest stimulation intensity that induced repeatable MEPs with peak-to-peak amplitude of at least 100 μV in three of five consecutive single TMS pulses (Rossini et al. 2015). All subjects wore a mouth guard and earplugs to minimize discomfort due to TMS. Subjects answered a post-TMS questionnaire the day after the experiment. A few subjects reported mild but short-lived headaches.

Transspinal. TEPs in leg muscles were elicited via a single cathode electrode (Uni-Patch EP84169, 10.2 cm × 5.1 cm, Wabasha, MA) placed along the vertebrae equally between the left and right paravertebral sides. The T₁₀ spinous process was identified via palpation. Because of its size, the electrode covered from T₁₀ to L₂ vertebral levels. These vertebral levels correspond to L₃ to S₂ spinal segments and thus to the segmental innervation of the muscles from which TEPs were recorded in this study (Kendall et al. 1993). Two reusable self-adhered electrodes (anode; same type as the cathode), connected to function as a single electrode, were placed on the left and right iliac crests. The cathode and anode electrodes were connected to a constant-current stimulator (DST7A, Digitimer, Welwyn Garden City, UK) that was triggered by Spike 2 scripts. The cathode electrode was held under constant pressure throughout the experiment and maintained in place via Pre-Wrap.

Posterior tibial nerve. A stainless steel plate electrode (anode) 4 cm in diameter was placed and secured proximal to the right patella. Rectangular single-pulse stimuli of 1 ms were delivered to the tibial nerve at the popliteal fossa. The most optimal stimulation site was established via a handheld monopolar stainless steel head electrode used as a probe (Knikou 2008). An optimal stimulation site corresponded to the site at which the M wave had a shape similar to that of the H reflex at low and high stimulation intensities, and at low stimulus intensities an H reflex could be evoked without an M wave (Knikou 2008). When the optimal site was identified, the monopolar electrode was replaced by a pregelled disposable electrode (cathode; Suretrace, Conmed, Utica, NY) that was maintained under constant pressure throughout the experiment.

Medial arch of the foot. The medial arch of the right foot was stimulated with a 30-ms pulse train with a constant-current stimulator (DST7A, Digitimer) at a site at which the lowest stimulation intensities TA responses in the right leg were evoked, with absent responses in the ipsilateral SOL and/or MG muscles. The bipolar electrode was replaced by two disposable pregelled Ag-AgCl electrodes (Suretrace adhesive gel electrodes; Conmed) that were maintained in place via Pre-Wrap. The TA flexor reflex behavior in response to low and high stimulation intensities was reestablished, and the lowest stimulus intensity that induced an initial EMG response in the right TA muscle was identified as reflex threshold.

**Transspinal Stimulation for Neuroplasticity**

Eligible participants received randomly c-tsCCS (n = 11) or a-tsCCS (n = 9) on two different days at least 4 wk apart. c-tsCCS and a-tsCCS were delivered for 40 min (480 single 1-ms pulses at 0.2 Hz) at similar intensities (c-tsCCS: 61.2 ± 4.73 mA; a-tsCCS: 64.4 ± 4.86 mA). The stimulation intensity was selected based on the amplitude of the TA and SOL TEPs. Specifically, the TA TEPs were matched to be equivalent to the TA MEPs recorded at 1.2 resting MEP threshold, and the SOL TEPs were matched to be equivalent to the soleus H reflex that was ∼20–30% of the maximal M wave. Electrodes were positioned in a manner similar to that utilized to evoke TEPs in both leg muscles. However, the polarity of the electrodes was switched to deliver either c-tsCCS or a-tsCCS through a single electrode to the thoracolumbar enlargement.
Neurophysiological Tests Conducted Before and After c-tsCCS and a-tsCCS

The neurophysiological tests described below were conducted before and after transspinal stimulation randomly within and across subjects. The time at which each neurophysiological test was conducted after 40 min of c-tsCCS or a-tsCCS varied significantly and ranged from 15 to 60 min after transspinal stimulation.

Cortical neuroplasticity. Neuroplasticity at the cortical level was assessed by establishing changes in the amounts of 1) afferent-mediated TA MEP facilitation and 2) subthreshold TMS-mediated TA flexor reflex facilitation (Fig. 1A). Stimulation of mixed peripheral nerves delivered 20–50 ms before TMS induces a significant facilitation of MEPs (Kasai et al. 1992; Khaslavskai et al. 2002; Mariorenzi et al. 1991; Nielsen et al. 1997) that coincides with absent changes in soleus H reflex and cervicomedullary evoked potentials (Mariorenzi et al. 1991; Roy and Gorassini 2008). Thus afferent-mediated facilitation of MEPs is mostly cortical in origin. The medial arch of the foot was stimulated at a two-fold sensory threshold, preceded the TMS pulses at the conditioning-test (C-T) intervals of 0, 40, 50, 60, 70, and 80 ms. The C-T intervals correspond to the interval between the onset of the conditioning pulse train to the medial arch of the foot and the onset of the TMS test stimulus. Across subjects, control and conditioned MEPs were recorded randomly at 1.2 ± 0.009 (47.45 ± 2.16% maximum stimulator output) resting MEP threshold for c-tsCCS and at 1.28 ± 0.03 (46.78 ± 2.53% maximum stimulator output) resting MEP threshold for a-tsCCS. Under control conditions and at each C-T interval, 15 TA MEPs were recorded at a stimulation frequency of 0.1 Hz.

TMS delivered at an intensity that does not evoke direct depolarization of spinal α-motoneurons modulates segmental spinal reflexes, ongoing EMG activity, and MEPs mostly through cortical internuncial circuits because descending spinal cord volleys are suppressed upon paired TMS (Di Lazzaro et al. 1998). Subthreshold TMS increases the polysynaptic spinal TA flexor reflex size when TMS pulses are delivered 40–100 ms after the end of the test pulse train stimulation (Mackey et al. 2015). By convention these intervals are negative. Furthermore, at the negative C-T intervals of 40–80 ms, the delay is sufficient for the interaction between conditioning TMS and test afferent volleys to occur at the somatosensory cortex.

In this study, subthreshold conditioning TMS was delivered at 0.7 ± 0.005 TA MEP resting threshold for c-tsCCS and at 0.71 ± 0.009 TA MEP resting threshold for a-tsCCS. The effects of subthreshold TMS on the innocuous TA flexor reflex evoked by medial arch foot stimulation were established at C-T intervals that ranged from −80 ms (TMS delivered after foot stimulation) to 20 ms (TMS delivered before foot stimulation). Flexor reflexes for c-tsCCS were elicited at 1.36 ± 0.07 and for a-tsCCS at 1.50 ± 0.12 TA flexor reflex threshold. Under control conditions and for each C-T interval, 10 TA flexor reflexes at a stimulation frequency of 0.1 Hz were recorded.

Corticospinal neuroplasticity. Corticospinal neuroplasticity after transspinal stimulation was assessed by establishing changes in the TA MEP recorded at 1.2 TA MEP resting threshold and from changes in the TA MEP recruitment curve (Fig. 1B). For each subject, the TA MEP recruitment curve was assembled starting from stimulation intensities corresponding to 0.6 TA MEP resting threshold until maximal amplitudes were obtained. TA MEPs for both cases were recorded at similar stimulation intensities for each subject before and after a-tsCCS and c-tsCCS.

Spinal neuroplasticity. Spinal neuroplasticity was assessed by establishing changes in the 1) TEP recruitment curves and 2) amount of soleus H-reflex and TEP postactivation depression (Fig. 1C). TEPs in response to cathodal transspinal stimulation were recorded at interstimulus intervals of 5 s and 1 s and at different intensities to assemble the TEP input-output recruitment curve. At the intensity at which most TEPs of ankle muscles were 30% of their maximal amplitude, 10 TEPs elicited every 5 s (or 0.2 Hz) or every 1 s (1 Hz) were recorded. For each recruitment curve assembled, at least 80 TEPs at different stimulation intensities were recorded. To establish the soleus H-reflex postactivation depression, the maximal M wave following posterior tibial nerve stimulation was evoked, measured as peak-to-peak amplitude, and saved for off-line analysis. The stimulus intensity was then adjusted to evoke an H reflex on the ascending part of the recruitment curve that ranged from 20% to 40% of the maximal M wave across subjects (Knikou 2008). Twenty soleus H reflexes were recorded randomly at interstimulus intervals of 5 s and 1 s, as well as upon paired pulses at an interstimulus interval of 50 ms and a stimulation frequency of 0.2 Hz.

Off-Line Data Analysis

MEPs, TEPs, M waves, H reflexes, and flexor reflexes were measured as the area of the full-wave-rectified EMG signal (Spike 2, Cambridge Electronics Design). The latency of the TA MEP and TEP of each muscle was measured based on the cumulative sum technique on the rectified waveform average (Brinkworth and Türker 2003; Ellaway 1978; Knikou 2014). For each subject, the TA MEP recorded at different stimulation intensities (recruitment curve) was normalized to the maximal associated MEP size recorded before 40 min of transspinal stimulation. A Boltzmann sigmoid function (Eq. 1) was fitted to all recorded nor-
nalized MEPs plotted against the stimulation intensity. The MEP slope and stimuli corresponding to MEP threshold and maximal MEP were estimated based on Eqs. 2, 3, and 4, respectively. The predicted MEP threshold intensity was used to normalize the TMS intensities. This was done for each subject separately so that MEP amplitudes at different stimulation intensities could be grouped across subjects based on multiples of MEP resting threshold. The average normalized MEP size was calculated in steps of 0.05 MEP resting threshold for each subject and then across subjects. This was done separately for MEPs recorded before and after c-tsCCS and a-tsCCS and for TEPs. Recruitment curves recorded bilaterally from distal and proximal leg muscles.

\[
\text{MEP}(s) = \frac{\text{MEP}_{\text{max}}}{(1 + \exp(m(s50 - s)))}
\]  
\[
\text{MEP}_{\text{slope}} = \frac{m \times \text{MEP}_{\text{max}}}{4}
\]  
\[
\text{MEP}_{\text{th stim}} = \frac{s - 2}{m}
\]  
\[
\text{MEP}_{\text{max stim}} = \frac{s + 2}{m}
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The soleus H reflex recorded at 0.2 Hz and at 1 Hz and upon paired pulses at 0.2 Hz was normalized to the associated maximal M wave. TA MEPs conditioned by sensory stimulation and TA flexor reflexes conditioned by subthreshold TMS were normalized to the unconditioned associated control value. The average amplitude of the conditioned MEPs or flexor reflexes from each subject was grouped based on time, C-T interval, and transspinal stimulation polarity.

Statistics

Two-way repeated-measures analysis of variance (RM ANOVA) was performed to determine the effect of time and C-T interval on the overall amplitude of TA MEPs conditioned by cutaneous stimulation. Two-way RM ANOVA was performed to determine the effect of time and C-T interval on the overall amplitude of TA flexor reflexes conditioned by subthreshold TMS. Three-way RM ANOVA was performed to establish the effect of polarity, time, and C-T interval on the MEPs conditioned by cutaneous stimuli and on the flexor reflexes conditioned by subthreshold TMS. The same analysis was performed to assess the effects of polarity, time, and muscle on the percent change of TEPs and soleus H reflexes recorded at different stimulation frequencies. Post hoc Bonferroni, Holm-Sidak, or Student-Newman-Keuls t-tests for multiple comparisons were used to test for significant differences between polarity, time, muscle, and C-T interval. Additional ANOVA and paired t-tests or rank sum tests were performed for each group and between groups as needed. Significance was set at \( P < 0.05 \). Group data are presented as means ± SE.

RESULTS

Cortical Plasticity After Transspinal Stimulation: Afferent-Mediated MEP Facilitation

Figure 2, A and C, illustrate waveform averages (n = 10) of conditioned TA flexor reflexes from two representative subjects (subjects 7 and 10) at an interval that subthreshold TMS was delivered 60 ms after medial arch foot stimulation before and after c-tsCCS and a-tsCCS. Note that the amplitude of the conditioned TA flexor reflexes for both participants was increased after c-tsCCS but decreased after a-tsCCS.

In the c-tsCCS group, the conditioned TA flexor reflex amplitude from all subjects was significantly different based on time of testing \( [F_{(1,4)} = 8.76, P = 0.004] \) and C-T interval \( [F_{(1,4)} = 10.02, P < 0.001] \). The conditioned TA MEPs from all subjects were significantly reduced after a-tsCCS at the C-T interval of 60 ms (Fig. 2B). Specifically, the conditioned TA MEPs were significantly reduced after c-tsCCS compared with those observed before c-tsCCS at C-T intervals of 40 and 60 ms (Fig. 2B). In the a-tsCCS group, the conditioned MEP amplitude from all subjects was significantly different based on time of testing \( [F_{(1,4)} = 8.76, P = 0.004] \) and C-T interval \( [F_{(1,4)} = 10.02, P < 0.001] \). The conditioned TA MEPs from all subjects were significantly reduced after a-tsCCS at the C-T interval of 60 ms (Fig. 2D). For c-tsCCS and a-tsCCS groups, three-way ANOVA showed a significant effect of time \( [F_{(1,4,1)} = 8, P < 0.001] \) and C-T interval \( [F_{(1,4,1)} = 10.02, P < 0.001] \) but not of stimulation polarity \( [F_{(1,4,1)} = 0.134, P = 0.71] \). However, post hoc Holm-Sidak multiple comparisons revealed that at C-T intervals of 40 ms (\( t = 3.17, P = 0.002 \)), 50 ms (\( t = 3.84, P < 0.001 \)), and 60 ms (\( t = 3.18, P = 0.002 \)) the effect of a-tsCCS was stronger compared with that of c-tsCCS.

Corticospinal Plasticity After Transspinal Stimulation

The latency of the TA MEP recorded at 1.2 TA MEP resting threshold did not change in the c-tsCCS (before: 31.24 ± 0.78 ms, after: 31.15 ± 0.71 ms; \( P = 0.46 \)) or a-tsCCS (before: 31.56 ± 0.76 ms, after: 31.43 ± 0.79 ms; \( P = 0.45 \)) group. Figure 4, A and D, illustrate nonrectified waveform averages of TA MEPs recorded at 1.2 TA MEP resting threshold from three representative participants (subjects 12, 4, and 3) before and after c-tsCCS and a-tsCCS.
and after c-tsCCS and a-tsCCS. Note that both c-tsCCS and a-tsCCS increased the MEP amplitude.

The TA MEP amplitude recorded at 1.2 MEP resting threshold from all subjects increased by 41.03 ± 12.38% and the maximal MEP amplitude increased by 74.31 ± 34.74% after c-tsCCS (Fig. 4B). The TA MEP recruitment curves support increased corticospinal excitability of the leg motor cortex area after c-tsCCS (Fig. 4C). RM ANOVA showed that the actual normalized MEP amplitudes recorded at different intensities varied significantly as a function of time \([F_{(1,11)} = 11.95, P < 0.001]\) for stimulation intensities of 55%, 60%, and 70%, which correspond largely to 1.2 MEP resting threshold intensities. The predicted maximal MEP amplitude from the sigmoid function fitted to each MEP recruitment curve separately changed from an overall mean amplitude of 113.21 ± 9.97% before a-tsCCS to 146.96 ± 24.9% after a-tsCCS (\(t = 77, P = 0.48\)). However, RM ANOVA showed that the actual normalized MEP amplitudes recorded at different intensities changed significantly as a function of time \([F_{(1,14)} = 41.09, P < 0.001]\) for stimulation intensities of 67 up to 85 (in increments of 3), which largely correspond to intensities of 1.3 MEP resting threshold and maximal MEPs.

For c-tsCCS and a-tsCCS groups, two-way RM ANOVAs on the MEP recruitment curve sigmoid function parameters showed a significant effect of polarity \([F_{(1,1)} = 7.02, P = 0.012]\) but not of time \([F_{(1,1)} = 1.37, P = 0.24]\) for m function, a significant effect of polarity \([F_{(1,1)} = 8.19, P = 0.007]\) but not of time \([F_{(1,1)} = 0.8, P = 0.37]\) for MEP slope, a nonsignificant effect of polarity \([F_{(1,1)} = 0.27, P = 0.6]\) and time \([F_{(1,1)} = 2.53, P = 0.24]\) for stimuli at MEP threshold, a significant effect of polarity \([F_{(1,1)} = 7.59, P = 0.009]\) but not of time \([F_{(1,1)} = 0.083, P = 0.77]\) for stimuli at MEP maximal, and a nonsignificant effect of polarity \([F_{(1,1)} = 0.001, P = 0.941]\).
Spinal Plasticity After Transspinal Stimulation: Recruitment Curves of TEPs

TEPs were present at similar latencies in left and right leg muscles, with shorter latencies observed for the RF and MH muscles compared with the more distal ankle flexors/extensors (Table 1), consistent with the TEP latencies we have previously reported (Knikou 2013a, 2013b). The latency of TEPs tested in all leg muscles at an interstimulus interval of 5 s did not change before and after c-tsCCS and a-tsCCS (for all TEP latencies before and after, Table 1).

Figure 5 illustrates the mean TEP amplitude from all subjects and muscles at different intensities (recruitment curves) before and after c-tsCCS. For TEPs recorded from right leg muscles, c-tsCCS changed the recruitment order and increased the predicted maximal TEP amplitude based on the sigmoid function fit for the MG (P = 0.04; Fig. 5D) and decreased for the GRC (P = 0.02; Fig. 5H).

Figure 6 illustrates the mean TEP amplitude from all subjects and muscles at different intensities (recruitment curves) before and after a-tsCCS. No significant changes in the recruitment order or maximal TEP amplitudes were found for TEPs recorded from the right leg muscles (P > 0.05). A tendency for a-tsCCS to negatively affect the recruitment of the left MG and SOL TEPs was noted (Fig. 6, D and F). However, a statistically significant difference between the predicted maximal TEP amplitudes before and after stimulation was not found (P > 0.05). A nonsignificant effect of time was found for the sigmoid function m (P = 0.35), slope (P = 0.73), and stimuli corresponding to TEP threshold (P = 0.7) and maximal TEP amplitude (P = 0.73). These results are for the left SOL TEP, but similar findings were found for all remaining TEPs.

For c-tsCCS and a-tsCCS groups, three-way RM ANOVA on the predicted maximal TEP amplitudes recorded from ankle muscles showed a significant effect of polarity [F(5,1,1) = 10.98, P = 0.001], and an interaction between time and polarity [F(5,1,1) = 7.98, P = 0.005], but not for muscle [F(5,1,1) = 0.73, P = 0.59]. In contrast, three-way RM ANOVA on the predicted maximal TEP amplitudes recorded from knee/thigh muscles showed a significant effect of muscle [F(7,1,1) = 2.45, P = 0.02] but not a significant effect of stimulus polarity [F(7,1,1) = 0.08, P = 0.77] with an exception for the right GRC (t = 2.17, P < 0.05), right VM...
subjects of each group before and after c-tsCCS and a-tsCCS.

transspinal stimulation.

Spinal Plasticity After Transspinal Stimulation: to elucidate this, we recorded TEPs and may be due to changes in spinal inhibition induced by transspinal stimulation. 

Fig. 4. Corticospinal plasticity after transspinal stimulation. A and D: nonrectified waveform averages of TA MEPs recorded at 1.2 MEP resting threshold before and after 40 min of transspinal stimulation. B and E: % of change of TA MEPs at 1.2 MEP resting threshold and % of change of maximal MEP sizes from all subjects of each group before and after c-tsCCS and a-tsCCS. C and F: TA MEP recruitment curves from all subjects of each group before and after transspinal stimulation. x-Axis shows multiples of TA MEP resting threshold. y-Axis shows TA MEP sizes as % of the associated maximal MEP size obtained before transspinal stimulation.

(t = 3.66, P < 0.05), and right RF (t = 0.077, P < 0.05) muscles. An interaction between muscle and time was also found [F(7,1,1) = 1.87, P = 0.033].

Spinal Plasticity After Transspinal Stimulation: Postactivation Depression

The altered multisegmental spinal output described above may be due to changes in spinal inhibition induced by transspinal stimulation. To elucidate this, we recorded TEPs and soleus H reflexes at an interstimulus interval of 5 s and 1 s before and after transspinal stimulation.

Figure 7A illustrates TEPs from one participant (subject 12) before and after 40 min of c-tsCCS and a-tsCCS elicited at an interstimulus interval of 5 s. It should be noted that the shape of TEPs induced by cathodal stimulation is similar to those evoked by anodal stimulation (see Fig. 2 in Kniik 2013b), but similarities and/or differences in the neurophysiological properties of TEPs evoked by anodal and cathodal stimulation require further investigation. Figure 7, B and C, illustrate the overall percent change in TEP sizes recorded at an interstimulus interval of 5 s after c-tsCCS and a-tsCCS from all subjects. TEP sizes from thigh muscles (right MH, right/left GRC) were decreased (P = 0.003) after c-tsCCS and a-tsCCS, while no differences were found for the remaining TEPs.

TEP postactivation depression is easily recognized by the overall percent change observed in the TEP amplitude when

Table 1. Latency of transspinal evoked potentials before and after 40 min of transspinal stimulation

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<th>R MG</th>
<th>R SOL</th>
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<td>Before</td>
<td>15.58 ± 0.5</td>
<td>16.97 ± 0.79</td>
<td>19.51 ± 0.58</td>
<td>11.74 ± 1.36</td>
<td>12.54 ± 0.37</td>
<td>12.63 ± 0.67</td>
<td>9.60 ± 0.58</td>
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<td>After</td>
<td>15.46 ± 0.59</td>
<td>16.48 ± 0.64</td>
<td>20.04 ± 0.65</td>
<td>10.46 ± 0.65</td>
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<td>Before</td>
<td>16.97 ± 0.89</td>
<td>18.08 ± 1.02</td>
<td>19.54 ± 0.87</td>
<td>12.88 ± 1.12</td>
<td>13.22 ± 0.33</td>
<td>14.04 ± 0.64</td>
<td>10.24 ± 0.78</td>
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<tr>
<td>After</td>
<td>16.35 ± 1.03</td>
<td>17.64 ± 0.91</td>
<td>19.06 ± 0.76</td>
<td>11.13 ± 0.96</td>
<td>13.25 ± 0.59</td>
<td>15.13 ± 0.17</td>
<td>9.66 ± 0.73</td>
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<th>L TA</th>
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<td>Before</td>
<td>15.36 ± 0.45</td>
<td>17.16 ± 0.50</td>
<td>19.14 ± 0.65</td>
<td>10.89 ± 0.81</td>
<td>12.64 ± 0.33</td>
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<td>9.28 ± 0.38</td>
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<td>After</td>
<td>15.57 ± 0.66</td>
<td>17.65 ± 0.76</td>
<td>18.98 ± 0.72</td>
<td>10.97 ± 0.93</td>
<td>12.71 ± 0.21</td>
<td>12.07 ± 0.86</td>
<td>9.16 ± 0.40</td>
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<tr>
<td>Before</td>
<td>16.81 ± 0.86</td>
<td>17.52 ± 0.61</td>
<td>19.98 ± 0.71</td>
<td>9.13 ± 0.71</td>
<td>12.53 ± 0.91</td>
<td>11.15 ± 0.68</td>
<td>9.95 ± 0.70</td>
</tr>
<tr>
<td>After</td>
<td>16.18 ± 0.79</td>
<td>17.68 ± 0.78</td>
<td>20.05 ± 0.53</td>
<td>9.73 ± 0.97</td>
<td>12.90 ± 0.57</td>
<td>12.68 ± 0.81</td>
<td>9.84 ± 0.53</td>
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Values (in ms) are means ± SE. c-tsCCS, a-tsCCS, cathodal and anodal transspinal constant-current stimulation; R, right; L, left; TA, tibialis anterior; MG, medial gastrocnemius; SOL, soleus; RF, rectus femoris; MH, medial hamstrings; GRC, hip adductor gracilis; VM, vastus medialis. Latencies before and after transspinal stimulation for all transspinal evoked potentials P > 0.05.
evoked at 1 s from 5 s before transspinal stimulation (Fig. 8, A and B). The percentage of change in TEP amplitude from all subjects was statistically significant different across muscles \([F_{(12,1)} = 8.52, P < 0.001]\) but not based on time of testing \([F_{(12,1)} = 0.52, P = 0.46]\) or on their interaction \([F_{(12,1)} = 0.6, P = 0.82]\) after c-tsCCS. Similarly, no difference was found for TEP sizes evoked at 1 s before and after a-tsCCS. c-tsCCS did not affect the soleus H-reflex size evoked at 5 s (\(P = 0.63\)) or 1 s (\(P = 0.16\)) after paired pulses at an interstimulus interval of 50 ms at a constant stimulation frequency of 0.2 Hz (\(P = 0.16\); Fig. 8C). a-tsCCS reduced the amount of soleus H-reflex depression at low frequencies, i.e., at an interstimulus interval of 1 s (\(P = 0.039\); Fig. 8D), but did not affect the H-reflex size at 5 s (\(P = 0.11\)). Repeated-measures ANOVA on the normalized H reflexes from all subjects showed a significant effect of interstimulus interval \([F_{(1,1,1)} = 15.1, P < 0.001]\) but not of polarity \([F_{(1,1,1)} = 1.4, P = 0.23]\) or time \([F_{(1,1,1)} = 2.7, P = 0.1]\). A significant interaction between polarity and time \([F_{(1,1)} = 4.88, P = 0.031]\) was found.

**DISCUSSION**

Transspinal constant-current-long-lasting stimulation induced cortical, corticospinal, and spinal plasticity in healthy human subjects. Our results support our hypothesis that prolonged transcutaneous stimulation over the thoracolumbar enlargement can induce functional neuroplasticity. The neurophysiological changes and mechanisms of neuroplasticity after transspinal stimulation along with limitations of this study are discussed below.

**Neurophysiological Changes After Transspinal Stimulation**

c-tsCCS and a-tsCCS decreased the afferent-mediated MEP facilitation (Fig. 2, B and D). Furthermore, c-tsCCS increased the subthreshold TMS-mediated TA flexor reflex facilitation but a-tsCCS decreased the subthreshold TMS-mediated TA flexor reflex facilitation (Fig. 3, B and D). These neurophysiological changes most likely reflect plasticity of cortical neuronal circuits. This is supported by the finding that the increased MEP sizes and firing rate of single TA motor units following stimulation of mixed peripheral or sensory nerves at similar time delays (Aimoñetti and Nielsen 2001; Deletis et al. 1992; Deuschl et al. 1991; Devanne et al. 2009; Kasai et al. 1992; Khaslavskaia et al. 2002; Mackey et al. 2015; Nielsen et al. 1997; Roy and Gorassini 2008; Tamburin et al. 2001) coincide with absent changes in the cervicomedullary MEPs (Roy and Gorassini 2008), increases in intracortical excitation (Devanne et al. 2009; Di Lazzaro et al. 1999; Roy and Gorassini 2008), and absent changes of soleus H-reflex excitability (Mariorenzi et al. 1991). Furthermore, at the C-T intervals ranging from −80 to −50 ms, cutaneous afferents have ample time to reach the somatosensory cortex and via thalamocortical...
circuits (Fig. 1A) affect the conditioned MEPs’ amplitude at the site of their origin. In addition, the parallel facilitation of flexor reflexes by subthreshold TMS before transspinal stimulation supports further involvement of cortical neuronal circuits, since subthreshold TMS suppresses the descending spinal cord volleys and the muscle responses evoked by a subsequent suprathereshold TMS pulse through corticocortical inhibitory circuits (Chen et al. 1998; Di Lazzaro et al. 1998; Kujirai et al. 1993).

Our findings suggest that transspinal stimulation can change activity in cortical neuronal circuits. Indeed, during spinal cord stimulation the cortical silent period is prolonged and the intracortical inhibition is increased (Schlaier et al. 2007). Moreover, cathodal transspinal direct-current stimulation (ts-DCS) decreases intracortical inhibition, while anodal tsDCS decreases the amplitude of somatosensory evoked potentials (Bucci et al. 2015; Cogiamanian et al. 2008). In light of the similar effects of c-tsCCS and a-tsCCS on the afferent-mediated MEP facilitation and their different influences on the subthreshold TMS-mediated effects on the TA flexor reflex (compare Figs. 2 and 3), it is possible that the observed changes are the result of different cortical neuronal circuits on which transspinal stimulation has distinct influences.

While the changes observed on the afferent-mediated TA MEP facilitation and subthreshold TMS-mediated TA flexor reflex facilitation point toward cortical plasticity induced by transspinal stimulation, both c-tsCCS and a-tsCCS potentiated corticospinal excitability. The TA MEPs recorded at 1.2 MEP resting threshold, intermediate, and maximal intensities were substantially increased after stimulation (Fig. 4, B, C, E, and F). The latencies and thresholds of MEPs recorded at 1.2 MEP resting threshold intensities remained unaltered, suggesting that the same neuronal populations were recruited by TMS before and after tsCCS, and that membrane excitability changes are not a major mechanism contributing to MEP facilitation.

The increasing MEP amplitudes with increasing stimulation intensities (recruitment curve) have been related to the strength of corticospinal projections (Chen et al. 1998) and are affected by the excitability state of cortical neurons, spinal motoneurons, and spinal interneurons (Devanne et al. 1997). However, MEPs recorded at different stimulation intensities can reflect the cortical map reorganization after ischemia and limb amputation (Ridding and Rothwell 1997). Thus, while a distinction between organizational and excitability changes cannot readily be made based on the experimental protocol we utilized, the MEP recruitment curve can provide information on cortical reorganization.

MEP excitability is influenced by single-pulse transspinal conditioning stimuli, is decreased when EPSPs for MEPs and TEPs are summated (Knikou 2014), and is increased at intervals consistent with MEP facilitation by peripheral nerve
stimulation via cortical mechanisms (Roy and Gorassini 2008). Based on the summation of EPSPs induced by transspinal and transcranial stimulation, MEPs and TEPs likely share common neuronal pathways. This constitutes a possibility for LTP of synaptic transmission to account for the MEP facilitation we observed (Bennett 2000). While LTP typically results from repetitive stimulus pairs timed with presynaptic potentials to arrive before postsynaptic potentials, non-Hebbian LTP in the thalamus, cerebellar circuits, and spinal lamina I neurons has been reported (Naka et al. 2013; Piochon et al. 2013; Sieber et al. 2013). Increases in local field potentials of the gracile nucleus, changes in excitability of the primary motor cortex, and intracortical inhibition are reported after anodal and cathodal tDCS in humans (Bocci et al. 2015; Lim and Shin 2011) and animals (Aguilar et al. 2011; Ahmed 2011). These findings along with our current results support the notion that transspinal stimulation increases corticospinal excitability in a non-muscle-specific manner (Fig. 5). In contrast, a-tsCCS did not change the stimulus/response TEP recruitment curves recorded from extensors or flexors (Fig. 6). When TEPs were recorded at 0.2 Hz at similar stimulation intensities before and after 40 min of transspinal stimulation, both c-tsCCS and a-tsCCS decreased the TEPs tested in knee flexors of both legs (Fig. 7, B and C). It should be noted that pathological activity of knee flexors contributes to pathological synergistic movements in neurological disorders. These findings suggest that c-tsCCS can simultaneously affect different motoneuron pools, and this may be related to the anatomical topography of spinal motoneurons within the gray matter and local synaptic and nonsynaptic neuroplasticity.

The amplitude of TEPs evoked every 1 s was reduced by as much as 60% from that of TEPs evoked every 5 s, especially in those recorded from the distal ankle muscles. This phenomenon, known as homosynaptic (or postactivation or low frequency) depression (Fig. 1C), is apparent in the soleus H reflex and occurs at the Ia afferent-motoneuron synapse because of reduced transmitter release from the previously activated Ia afferents (Pierrot-Deseilligny and Burke 2012). The presence of postactivation TEP depression in this study is opposite to what we have previously observed on a-tsCCS and transcutaneous magnetic stimulation of the spine in semiprone seated subjects (Einhorn et al. 2013; Knikou 2013a, 2013b), suggesting that body position is key to some of the neurophysiological
properties of TEPs. However, postactivation TEP depression cannot readily be used to characterize TEPs as monosynaptic reflexes because α-motoneurons upon transspinal stimulation are depolarized mostly by transsynaptic actions (see *Excitation of Neuronal Elements upon tcsCCS*).

c-tscCCS or a-tscCCS did not affect the postactivation depression of TEPs (Fig. 8, A and B), but a-tscCCS decreased the soleus H-reflex postactivation depression (Fig. 8D). The latter finding is consistent with the reduced soleus H-reflex postactivation depression and the progressive increase of soleus H-reflex excitability after a-tscDCS (Lamy et al. 2012; Winkler et al. 2010). In our study, H-reflex excitability represented by the H reflexes recorded at 0.2 Hz did not show significant changes after transspinal stimulation, a finding also reported after a-tscDCS or c-tscDCS (Winkler et al. 2010). The decreased soleus H-reflex postactivation depression may be the result of membrane potential changes of primary afferent fibers (Eccles et al. 1962), facilitating transmitter release at the Ia fiber-motoneuron synapse.

To summarize, both c-tscCCS and a-tscCCS decreased afferent-mediated MEP facilitation, c-tscCCS increased TMS-mediated TA flexor reflex facilitation, and a-tscCCS decreased TMS-mediated TA flexor reflex facilitation. Furthermore, both c-tscCCS and a-tscCCS increased MEPs recorded at 1.2 MEP resting threshold, intermediate, and maximal intensities. While a-tscCCS did not alter the TEP recruitment curve, c-tscCCS changed their recruitment in a nonspecific pattern, but both a-tscCCS and c-tscCCS decreased the TEPs of knee flexors when recorded at a constant intensity. Finally, postactivation depression of TEPs remained unaltered but was decreased for the soleus H reflex after a-tscCCS and remained unaltered after c-tscCCS.

It is thus apparent that c-tscCCS and a-tscCCS delivered at identical levels of stimulation intensities can induce similar, opposite, or different effects. The differences in the effects of a-tscCCS and c-tscCCS on neuronal excitability suggest that these two types of stimulation act via different mechanisms. While c-tscCCS and a-tscCCS increased corticospinal excitability, subthreshold TMS-mediated flexor reflex facilitation, TEP amplitude, and postactivation depression of the soleus H reflex were affected differently based on the polarity of stimulation. While these differences may be related to interindividual variability of GABA<sub>A</sub>-GABA<sub>B</sub>-mediated inhibition and to a variety of neurochemical substances involved in spinal cord stimulation (Linderoth et al. 1992), a-tscCCS and c-tscCCS may have affected differently spinal inhibitory/excitatory interneurons. This is reflected by the opposing effects of DC stimulation on reciprocal inhibition directed from flexors to extensors and from extensors to flexors (Lackmy-Vallée et al. 2014). Additionally, current is known to induce concomitantly depolarization and hyperpolarization of neurons, dendrites, and axons based on their anatomical topography with respect to the stimulating electrode (Delgado-Lezama et al. 1999), thereby affecting neuroplasticity-mediated calcium and sodium channels (Nitsche et al. 2003) differently. Thus, while neurophysiological excitability changes by cathodal DC stimulation are related to membrane depolarization and by anodal DC stimulation to membrane hyperpolarization (Nitsche et al. 2003), it is possible that tscCCS induces both depolarization and hyperpolarization regardless of polarity. These arguments are supported by the differential effect of c-tscCCS on the motor output of knee and ankle muscles (Fig. 5).

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Plasticity After Transspinal Stimulation

Long-lasting tsCCS induced cortical, corticospinal, and spinal plasticity in healthy human subjects. The neurophysiological changes described above likely involved changes in the synaptic efficacy between cortical interneurons and descending motor axons (Fig. 1A), descending motor axons and spinal motoneurons (Fig. 1B), and IA afferents and motoneurons (Fig. 1C). Changes in the intrinsic properties of spinal motoneurons and resting membrane potentials of dendrites, axons, and afferent fibers (Armano et al. 2000; Camp 2012; Daoudal and Debanne 2003; Heckman et al. 2008) constitute plausible sites for nonsynaptic plasticity after long-lasting transspinal stimulation. Polarizing DC surface currents have been shown to produce parallel changes in spontaneous neuron activity, dendritic potentials, and membrane potentials of pyramidal tract cells (Creutzfeldt et al. 1962; Purpura and McMurtry 1965). Positive (anodal) currents increase and negative (cathodal) currents decrease these potentials (Creutzfeldt et al. 1962; Purpura and McMurtry 1965). While in this study we did not use direct current, constant-current stimulation at low frequencies can increase the probability that an EPSP will elicit an action potential, a nonsynaptic plasticity mechanism described as EPSP-spike plasticity (Daoudal and Debanne 2003). Thus it is highly likely that transspinal stimulation induced synaptic and nonsynaptic plasticity coherently, sharing common induction and expression mechanisms (Campanac and Debanne 2007).

Excitation of Neuronal Elements upon tsCCS

The neuronal elements that were excited upon transcutaneous stimulation of the spinal cord need to be considered. Stimulation was delivered for all subjects at the T10–L2 vertebral levels to counteract differences in shape and thus neurophysiological properties based on stimulation level (Roy et al. 2012). The nonuniform shape of TEPs across muscles (Fig. 7A) supports the concept of excitation of different types of fibers. Several studies have proposed that TEPs are generated by excitation of dorsal column fibers, orthodromic excitation of motor axons, and antidromic excitation of muscle afferents leading to strong facilitation of motoneurons and altered transmission in reflex pathways to motoneurons (Coburn 1985; Hunter and Ashby 1994; Maertens de Noordhout et al. 1988).

Stimulation through surface electrodes placed similarly to those in the present study induces a current flow perpendicular to the spine with the concentration of the current located near the transspinal electrode (Minassian et al. 2007). Computational models of epidural or transcutaneous spinal cord stimulation over the lumbosacral cord demonstrated that IA afferents in dorsal root fibers have significantly lower excitation thresholds compared with ventral root fibers and dorsal column fibers (Rattay et al. 2000), with the latter requiring triple the stimulation intensity (Danner et al. 2011). Furthermore, dorsal column fibers are insensitive to excitation within the clinical range of 10 V (Rattay et al. 2000). Therefore, dorsal column fibers along with their collaterals could have been excited only when stimulation was delivered at high intensities, although this is unlikely (Danner et al. 2014). Based on differences between indirect (spinal stimulation) and direct (F wave) latencies, scan measurements of the distance between the dura and intervertebral foramina, and simulation studies, transspinal stimulation excites the nerve roots near their exit from the spinal column, near the emergence of the axons from the anterior horn cells, or close to the entry point of the dorsal root fibers (Danner et al. 2011, 2014; Ladenbauer et al. 2010; Mills and Murray 1986; Struijk et al. 1993). Although the exact excitation site cannot be determined from the present experiments, impulses following transcutaneous stimulation of the spinal cord traveled caudally and rostrally, affecting both descending motor inputs and ascending afferent inputs through synaptic and nonsynaptic actions changing motor cortex output at its origin site.

Limitations

Limitations of this study are that the number of tested subjects was small and the time course of excitability changes was not examined. However, the main scope of this exploratory research work was to assess prospective excitability changes of the CNS, allowing appropriate power analysis for future studies. Larger-scale neurophysiological research studies are needed to comprehensively characterize neuroplasticity after long-lasting transspinal stimulation in humans. In this study, no sham stimulation was applied. In our opinion, it is unlikely that the observed neurophysiological changes were due to a placebo effect, since the subjects were blind to the stimulation polarity and the effects of stimulation, while any change in muscle activity would have been identified by the surface EMG electrodes. In future studies, assessment of somatosensory and cervicomedullary evoked potentials, intracortical inhibition, intracortical facilitation, interhemispheric inhibition, and pre- and postsynaptic inhibition exerted on spinal motoneurons (Knikou 2008; Rossini et al. 2015) will contribute to a better understanding of the physiological changes underlying this intervention.

Conclusions

This is the first report on neurophysiological excitability changes after 40 min of cathodal and anodal transspinal stimulation in healthy human subjects. Our findings demonstrate that c-tsCCS and a-tsCCS can induce changes in intracortical and corticospinal excitability and presynaptic inhibition of IA afferents. We suggest that brain plasticity may be achieved through transspinal stimulation, an intervention that is ideal for people with neurological disorders due to brain or spinal lesions.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.K. conception and design of research; M.K., L.D., D.S., and M.M.I. performed experiments; M.K., L.D., D.S., and M.M.I. analyzed data; M.K. interpreted results of experiments; M.K. prepared figures; M.K. drafted manuscript; M.K. edited and revised manuscript; M.K., L.D., D.S., and M.M.I. approved final version of manuscript.

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