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NEUROSYSTEMS

Facilitation of ipsilateral actions of corticospinal tract neurons on feline motoneurons by transcranial direct current stimulation

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Abstract

Ipsilateral actions of pyramidal tract (PT) neurons are weak but may, if strengthened, compensate for deficient crossed PT actions following brain damage. The purpose of the present study was to examine whether transcranial direct current stimulation (tDCS) can strengthen ipsilateral PT (iPT) actions; in particular, those relayed by reticulospinal neurons co-excited by axon collaterals of fibres descending in the iPT and contralateral PT (coPT) and of reticulospinal neurons descending in the medial longitudinal fascicle (MLF). The effects of tDCS were assessed in acute experiments on deeply anaesthetized cats by comparing postsynaptic potentials evoked in hindlimb motoneurons and discharges recorded from their axons in a ventral root, before, during and after tDCS. tDCS was consistently found to facilitate joint actions of the iPT and coPT, especially when they were stimulated together with the MLF. Both excitatory postsynaptic potentials and inhibitory postsynaptic potentials evoked in motoneurons and the ensuing ventral root discharges were facilitated, even though the facilitatory effects of tDCS were not sufficient for activation of motoneurons by iPT neurons alone. Facilitation outlasted single tDCS periods by at least a few minutes, and the effects evoked by repeated tDCS by up to 2 h. The results of this study thus indicate that tDCS may increase the contribution of iPT actions to the recovery of motor functions after injuries to coPT neurons, and thereby assist rehabilitation, provided that corticoreticular and reticulospinal connections are preserved.

Introduction

Ipsilateral corticospinal actions are mediated via descending neurons projecting to the spinal cord and supraspinal relay neurons. Different strategies might thus be used to increase the contribution of ipsilateral corticospinal actions to movements when contralateral actions are deficient. Both stimulation of the ipsilateral motor cortex and more intense use of the ipsilateral extremities were demonstrated to improve actions evoked by spinally projecting cortical neurons (Raineteau *et al.*, 2002; Martin *et al.*, 2007); for the most recent references, see Nishimura & Isa (2012), Carmel *et al.* (2013, 2014), and Isa *et al.* (2013). The transmission between the corticospinal neurons and their relay neurons may also be facilitated by, for example, 4-aminopyridine (Jankowska *et al.*, 2005) or additional input to the involved neurons (Edgley *et al.*, 2004; Jankowska & Stecina, 2007; Stecina & Jankowska, 2007). Considering the possibility of further facilitation by transcranial direct current stimulation (tDCS), the aim of the present study was to examine to what extent ipsilateral actions of corticospinal neurons relayed by reticulospinal neurons may be enhanced by tDCS.

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We expected ipsilateral corticospinal actions to be enhanced by tDCS, taking into account the evidence for long-lasting effects of tDCS on not only cortical neurons (e.g. Bindman *et al.*, 1964; Purpura & McMurtry, 1965; Stagg & Nitsche, 2011) but also subcortical neurons, including reticulospinal neurons (Bolzoni *et al.*, 2013a,b). Another reason was the evidence that at least some reticulospinal neurons are excited not only by contralateral pyramidal tract (coPT) neurons but also by ipsilateral pyramidal tract (iPT) cortical neurons (He & Wu, 1985; Canedo & Lamas, 1993; Edgley *et al.*, 2004; Stecina & Jankowska, 2007; Baker, 2011). However, even if facilitation of iPT actions relayed by reticulospinal neurons by tDCS appeared to be likely, the degree and the timing of the facilitation, or experimental conditions under which the facilitation is optimized, could not be predicted.

In order to restrict the ipsilateral actions of corticospinal neurons to actions mediated by reticulospinal neurons with axons descending in the medial longitudinal fascicle (MLF), we used the technique of spatial facilitation to activate reticulospinal neurons. To this end, we used stimuli that, by themselves, failed to induce motor responses from either the ipsilateral or the contralateral medullary pyramid [pyrimidal tract (PT)] or the MLF, but became effective when applied jointly, by activating reticulospinal neurons co-excited by axon collaterals of PTs and of the MLF. The effects of iPT stimuli

were compared during and after application of tDCS with the polarity (anodal tDCS), timing and intensities previously found to facilitate activation of reticulospinal neurons (Bolzoni *et al.*, 2013b). The mutual facilitation of synaptic actions of nearly synchronous iPT and coPT and/or MLF stimuli evoked during and after tDCS made it unlikely that the effects of the iPT stimuli were relayed by other entities than reticulospinal neurons (for further arguments, see the first section of the Discussion).

Materials and methods

Ethical approval

All experiments were approved by the regional Ethics Committee for Animal Research (Göteborgs Djurförsöksetiska Nämnd), and complied with EU and NIH guidelines for animal care. The animals were bred and housed under veterinary supervision at the Laboratory of Experimental Biomedicine at Sahlgrenska Academy, where the experiments were carried out.

Preparation

The experiments were performed on 11 deeply anaesthetized cats weighing 2.7–3.5 kg. Anaesthesia was induced with sodium pentobarbital (40 mg/kg, intraperitoneal; Apoteksbolaget, Sweden or Apotek Produktion & Laboratorier AB, Stockholm, Sweden), and maintained with intermittent doses of α-chloralose [5 mg/kg administered every 1–3 h, up to 65 mg/kg, intravenous (i.v.); Rhône-Poulenc Santé, France]. Additional doses of α-chloralose were given when motor reactions were evoked during dissection and when increases in the continuously monitored blood pressure or heart rate were evoked by any experimental procedures. During recordings, neuromuscular transmission was blocked with pancuronium bromide (0.3 mg/kg i.v. supplemented with ~0.2 mg/kg per h; Pavulon; Organon, Sweden), and the cats were artificially ventilated. Mean blood pressure was maintained at 100–130 mmHg and end-tidal CO_2 at 4–4.5% by adjusting the parameters of artificial ventilation and the rate of a continuous infusion of a bicarbonate buffer solution with 5% glucose (1–2 mL/kg per h). Body temperature was kept at ~37.5 °C with servo-controlled heating lamps. The experiments were terminated by an overdose of i.v. pentobarbital and formalin perfusion.

Following the initial vein, artery and tracheal cannulation, the caudal lumbar and sacral segments of the spinal cord were exposed by laminectomy, and selected nerves in the ipsilateral hindlimb were dissected free. These included the quadriceps, gastrocnemius soleus (GS) and posterior biceps-semitendinosus (PBST) muscle nerves. The head was fixed in a stereotactic frame, and the cerebellum was exposed by craniotomy to allow insertion of electrodes into the left and right PT and the left MLF to stimulate axons of corticospinal and reticulospinal neurons, respectively. The electrodes were inserted at an angle of 20° from the vertical (tip directed rostrally), stimulation sites in PTs and MLF being aimed at Horsley-Clarke coordinates, P6-8, L1.3, H-10 and P9, L0.8, and H-5, respectively. The electrodes were left at locations from which distinct descending volleys evoked at stimulus intensities of ≤ 20 μA were recorded from the surface of the spinal cord at the C1/C2 segments. To this end, an area of 1–2 mm^2 over the lateral funiculus between the first and second vertebrae was made accessible by widening the space between these vertebrae, thereby allowing one silver ball recording electrode to be in contact with the spinal cord surface, with a reference electrode inserted in a neck muscle. At the end of the experiments, the stimulation sites were marked with electrolytic lesions, the locations of which were verified on 100-μm-thick sections, cut in the plane of the electrode insertions with a vibratome, mounted on slides, counterstained with cresyl violet, and scanned (Fig. 1).

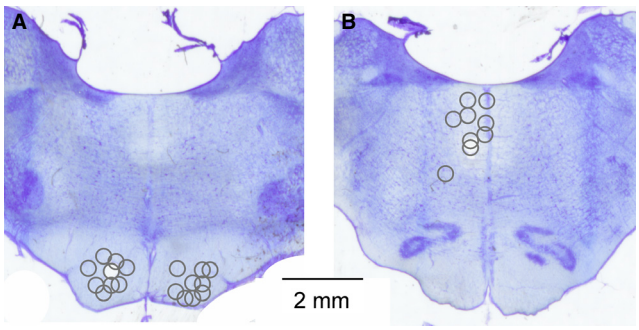

FIG. 1. Sites of brainstem stimulation. Reconstructions of the locations of the stimulating electrodes in the PTs (A) and in and lateral to the MLF (B). The stimulation sites defined by electrolytic lesions made at the end of the experiments are indicated on representative sections of the medulla at the levels of the superior (A) and inferior (B) olives respectively. The circles in (A) and (B) correspond to the centres of the lesions, and their diameters to the distances of estimated spread of current, within 0.2–0.3 mm from the electrode tip for 20–30 μA [see Fig. 11 in Gustafsson & Jankowska (1976)].

Stimulation and recording

PTs and the MLF were stimulated with constant-current stimuli. Trains of two to six monopolar stimuli were delivered via tungsten electrodes (impedance, 30–150 kΩ) against the reference electrode in one of the neck muscles. The stimulus parameters (duration, 0.2 ms; intensities, most often 50–100 μA) and inter-stimulus intervals of 2.5 or 3.3 ms were chosen to ensure that these stimuli induced not only direct but also indirect activation of descending fibres. Current intensities of ≤ 100 μA were previously verified to be below the critical intensities at which spread of current occurs between the left and right PTs or between the MLF and PTs (Jankowska *et al.*, 2006). Peripheral nerves were stimulated with constant-voltage stimuli of duration 0.2 ms at intensities expressed in multiples of threshold stimuli, i.e. stimuli inducing barely visible afferent volleys in records from the dorsal root entry zone.

Records of the activity of hindlimb motoneurons were obtained in two ways. In six experiments, postsynaptic potentials (PSPs) of PT and MLF origin were recorded from motoneurons within the GS and PBST motor nuclei in the caudal part of the L7 segment. The motoneurons were identified by antidromic activation following stimulation of the GS or PBST nerves. The records were obtained with glass micropipettes broken to an external diameter of ~1.5 μm ('sharp electrodes') and filled with 2 M potassium citrate (resistance, 3–7 MΩ), and a conventional high input resistance amplifier. In five experiments, the degree of activation of lumbar motoneurons was estimated from records of spike discharges of motoneuron axons running in the L7 or S1 ventral roots (VRs), which allowed comparisons of responses of populations of motoneurons. To this end, the VRs were transected distally and mounted on pairs of silver wire electrodes in a paraffin oil pool.

Descending volleys evoked by MLF or combined PT and MLF stimulation were recorded at the level of the L7 segment. The volleys were recorded monopolarly with one electrode in contact with the intact dura mater over the dorsal columns within the L7 segments, and the reference electrode in contact with one of the neck or back muscles.

Both single records and averages of 10–50 records were stored on-line (with a time resolution of 30 μs per address) and analysed off-line.

Parameters of tDCS

Transcranial polarization was applied as described by Bolzoni *et al.* (2013b) to the area over the contralateral pericruciate region approximately 3–10 mm from the midline, corresponding to the human sensorimotor cortex. Anodal current was routinely used, except in a few control polarization series, as anodal tDCS facilitates activation of reticulospinal neurons in the cat (Bolzoni *et al.*, 2013b), in contrast to the rat, in which the cathodal tDCS is facilitatory and anodal tDCS is depressive (Bolzoni *et al.*, 2013a). The polarizing current was applied via 3% agar-agar in saline contained in a chamber attached to the skull. The bottom of the chamber was fitted to the shape of the skull after the frontal sinus has been opened and sealed. It was shaped as an elipse with diameters of 14.8 and 18 mm, and a contact area of ~200 mm^2. The reference electrode was in contact with about twice that area, between the ipsilateral (left) lateral aspect of the skull and the temporal muscles, approximately 20 mm caudal and 20 mm lateral from the electrode over the sensorimotor cortex.

Current intensities were continuously monitored. Stimuli of 0.2 mA were routinely used, but they were sometimes increased to 0.5 mA in order to verify that the conditions of polarization were optimal. The intensity of 0.2-mA stimuli corresponded to ~1 $\mu A/mm^2$. This intensity exceeds the current intensity used in humans (0.3 $\mu A/mm^2$; in total, 1 mA over 35 cm^2), but falls within the range of intensities used in acute experiments on anaesthetized rats [up to 57 $\mu A/mm^2$; see Table 1 in Brunoni *et al.* (2011)]. The use of a higher current intensity than that used in humans was warranted by the significant drop in current density within the target area at depth as the size of the electrode is decreased (Miranda *et al.*, 2009) [see also Rahman *et al.* (2013)].

Polarizing current was routinely applied for six to seven periods of 5 min separated by 5-min intervals, to allow a comparison of direct effects of the polarization and the development of effects seen during the post-polarization periods. In the following, we will refer to the effects of tDCS evoked during tDCS without specifying the period of application of tDCS (unless otherwise indicated), and to the effects seen at least 10–30 min after the last tDCS as the post-polarization effects.

Analysis

The effects of tDCS were estimated from changes in PSPs evoked in individual motoneurons or in VR responses evoked by submaximal stimuli applied within PTs and the MLF. Repeated series of 10 or 20 stimuli were delivered at 2–3 Hz, alternately to PTs and the MLF, or by using overlapping short trains of PT and MLF stimuli. Responses to these stimuli were recorded during and after tDCS, and compared with those evoked before tDCS. The comparison included effects of a brief train of stimuli, and it was noted after which stimuli the responses appeared and how their areas and latencies were affected by tDCS. Software for sampling and analysis developed by E. Eide, T. Holmström and N. Pihlgren (University of Gothenburg) was used for measurements of latencies and areas, and to calculate the differences between the areas of the tested potentials from their averaged records.

Differences between data sets were assessed for statistical significance with Student's *t*-test, the Mann–Whitney rank sum test, the *z*-test, or ANOVA, with adequate (Holm–Sidak or Dunn) *post hoc* tests for determining differences from control (with STATISTICA 5.1, STATSOFT, or SIGMAPLOT 12.5; Systat Software). For all tests, the overall significance level was set at $P < 0.05$.

Results

Experimental strategy

Facilitation of activation of reticulospinal neurons by iPT neurons during and after tDCS was estimated from the effects of spacial facilitation at the level of reticulospinal neurons resulting in postsynaptic actions on hindlimb motoneurons or in activation of these motoneurons. The principles of spacial facilitation as applied to the organization of neuronal networks in animals (Lundberg, 1975; Burke, 1999; Jankowska, 2012) and humans (Pierrot-Deseilligny & Burke, 2012) have been repeatedly outlined. We will therefore point out only two consequences of the use of this indirect technique for the analysis of the results of this study. First, it is highly sensitive, i.e. the generation of action potentials in neurons in which spatial facilitation occurs may depend on minute changes in input to these neurons occurring at the critical level of transition from subthreshold to suprathreshold membrane potentials generating action potentials. Second, changes in the input critical for spatial facilitation have to be optimized in each individual case, because the use of too weak and/or not properly timed stimuli may fail to bring the postsynaptic neurons to the threshold for generation of action potentials, whereas the use of too strong stimuli will prevent converging presynaptic actions from being revealed. Facilitation of converging synaptic actions of corticospinal and reticulospinal neurons on reticulospinal neurons could thus not be examined under strictly standardized conditions. For these reasons, we chose the strategy of examining as many as possible effects of tDCS in order to verify our hypothesis, rather than one or two strictly repeatable and easiest-to-quantify effects. We considered that consistent findings of different manifestations of facilitation of synaptic actions of the iPT on reticulospinal neurons by tDCS would considerably increase the confidence in its occurrence, even if they were found in different combinations. These manifestations included larger PSPs recorded in individual motoneurons, shorter latencies of these potentials, the appearance after fewer stimuli in a train and in a higher percentage of motoneurons, and the corresponding changes in responses of populations of motoneurons recorded in the VRs.

Effects of tDCS on intracellularly recorded motoneurons

Weak facilitation by tDCS of postsynaptic actions of selectively activated iPT neurons

During and after application of tDCS, excitatory PSPs (EPSPs) evoked by selective stimulation of the iPT were found in only 12 of 66 (18%) motoneurons. This proportion was slightly larger than the proportion of motoneurons tested under control conditions (3/42; 7%), but the difference was not found to be statistically significant (Table 1). The most convincing evidence for facilitation by tDCS was obtained in three of the 12 motoneurons that were recorded both before and during application of tDCS. In all three of these motoneurons, the earliest iPT stimuli in the train evoked EPSPs during or after but not before tDCS, as shown in Fig. 2A–C, with EPSPs appearing after the first and second stimuli only during tDCS. Furthermore, EPSPs evoked by later stimuli were larger after than before tDCS, which is illustrated by the larger EPSPs after the fourth stimulus, and may be best appreciated from the superimposed

TABLE 1. Proportions of motoneurons in which EPSPs from the iPT were found before and after tDCS

Motoneurons with EPSPs from	Proportions of motoneurons, no. (%)		
	Before	After	*P*
iPT alone	3/42 (7)	12/66 (18)	≥ 0.05, NS
coPT alone	14/42 (33)	18/66 (27)	≥ 0.05, NS
iPT and coPT	14/40 (35)	36/58 (55)	≥ 0.05, NS
iPT and MLF	11/34 (32)	42/61 (69)	< 0.01*
coPT and MLF	12/36 (33)	39/61 (64)	< 0.01*

First row: data for motoneurons in which iPT stimuli were evoking oligosynaptic, most likely disynaptic, EPSPs by themselves, as in Fig. 2A. Second row: similar data for EPSPs evoked from the coPT. Third row: data from motoneurons in which excitatory effects from the iPT were deduced from mutual facilitation of oligosynaptic EPSPs evoked from the iPT and coPT, i.e. when joint actions from the iPT and coPT exceeded the sum of their separate actions, as in Figs 2G and 4C. Fourth row: data from motoneurons in which excitatory effects from the iPT were deduced from facilitation of EPSPs evoked from the MLF, as in Fig. 5A,B,D, and E. Fifth row: as in the fourth row, except for excitatory effects from the coPT, illustrated in Fig. 5C and F. The facilitation is attributed to spatial facilitation occurring at a premotoneuronal, most likely reticular, level. Differences between proportions of motoneurons were tested for significance with the *z*-test. They are indicated in the last column: *significant; NS, not significant.

records in Fig. 2C. Unfortunately, in view of the longlasting effects of tDCS, this kind of comparison could only be made in the first motoneuron tested in an experiment, but the proportion of motoneurons in which EPSPs were evoked by the first, second or third stimulus during and after rather than before tDCS was much greater in the whole sample of 15 motoneurons, as summarized in Fig. 2L. Figure 2L also shows that EPSPs evoked under control conditions and either during or after tDCS were evoked within the same range of latencies (open symbols in Fig. 2L).

Latencies of EPSPs evoked by iPT stimuli were measured as shown in Fig. 3. When an EPSP appeared after the second but not after the first stimulus in a train, its latency was measured from the second stimulus. Likewise, when another EPSP appeared when the number of stimuli was increased from three to four, its latency was measured from the fourth stimulus. Measured in this way, the latencies of EPSPs evoked by iPT stimulation fell within the same range (4.29–6.98; mean ± standard error of the mean, 5.38 ± 0.16 ms; $n = 19$) as the latencies of EPSPs evoked by coPT stimulation (4.54–6.66; mean, 5.54 ± 0.09 ms; $n = 29$). No statistically significant differences were found between them with Student's *t*-test for two samples, assuming equal variances.

Latencies within these ranges are fully consistent with the disynaptic coupling indicated in Fig. 2. Latencies of the earliest disynaptic actions of PT neurons on hindlimb feline motoneurons were previously found to be 4.70 ± 0.06 ms (Stecina & Jankowska, 2007), and synaptic actions involving one or two additional synaptic delays would be evoked with approximately 1- and 2-ms-longer latencies respectively.

Joint actions from the iPT and coPT are stronger after tDCS

Joint actions of the iPT and coPT should increase the probability of facilitatory actions of tDCS being revealed, in view of previous evidence that reticulospinal neurons relay indirect synaptic actions of the two PTs on motoneurons (Edgley *et al.*, 2004; Stecina & Jankowska, 2007). Spatial facilitation of synaptic actions of iPT and coPT neurons on reticulospinal neurons, as indicated in the

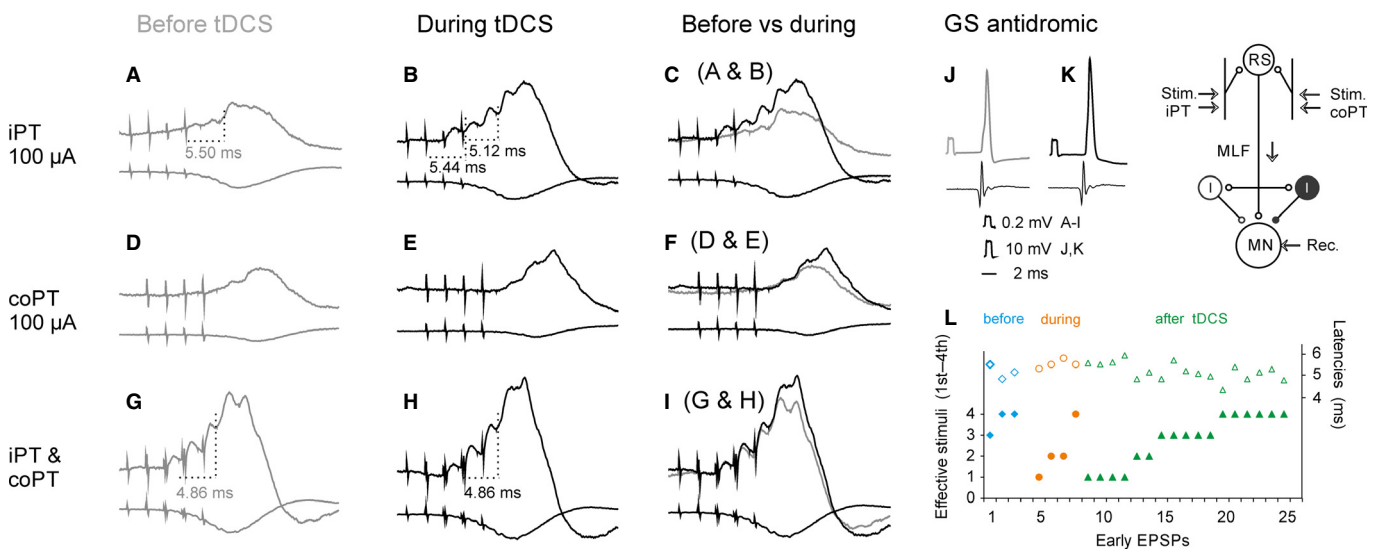

FIG. 2. Facilitation of EPSPs evoked from the iPT by tDCS. (A–I) Intracellular records from a GS motoneuron before (left column) and during (middle column) anodal tDCS. In each panel, the upper traces are averaged records ($n = 20$) from the motoneuron, and the lower traces are averaged records from the cord dorsum. The upper, middle and lower row records show the effects of stimulation of the iPT, of the coPT, and of overlapping trains of stimuli applied in both the iPT and coPT, as indicated. (C, F, and I) Superimposed records from periods before and during application of tDCS. (J and K) Antidromic action potentials from the motoneuron obtained just before the records in A and B, respectively. The records in A were taken at a membrane potential that was 6 mV lower than in B. The rectangular voltage calibration pulses in J and K are for intracellular records, as indicated. Time calibration is for all records. In this and in all of the following figures, the negativity is down in intracellular records and up in records from the cord dorsum. (L) A plot summarizing the higher efficacy of iPT stimulation during and after than before tDCS, as reflected in the appearance of EPSPs following earlier stimuli in the train (left ordinate, filled symbols). These were evoked at similar latencies from the effective iPT stimuli (right ordinate, open symbols). Data for three motoneurons recorded before and during tDCS and for 12 motoneurons recorded during or after tDCS (with more than one early EPSP evoked in individual motoneurons) are shown. The diagram indicates the stimulation and recording sites and the underlying convergence of the iPT and coPT on reticulospinal neurons providing input to motoneurons, either directly or via spinal excitatory (white) and inhibitory (black) interneurons. MN, motoneuron; RS, reticulospinal neuron.

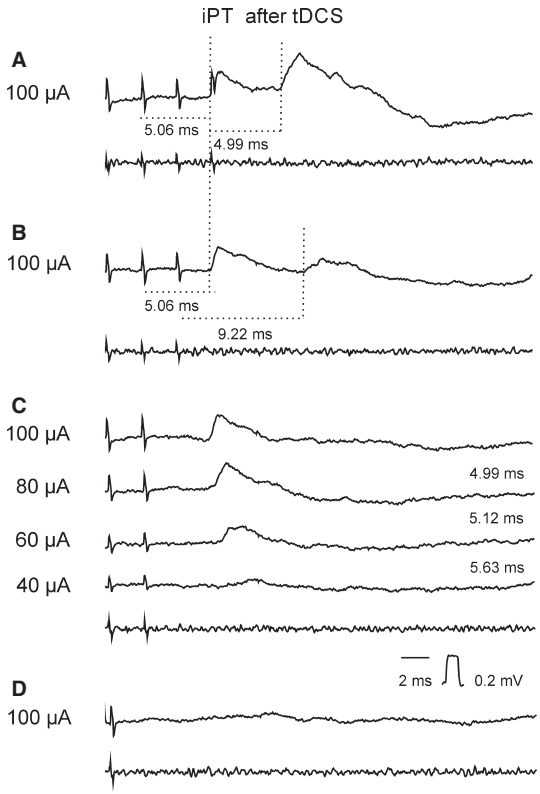

FIG. 3. Examples of EPSPs linked to the early iPT stimuli. Intracellular averaged records of EPSPs evoked from the iPT in a GS motoneuron after tDCS (upper traces; $n = 20$) and records from the cord dorsum (bottom traces). (A–D) Effects of decreasing numbers of stimuli at intensities indicated on the left. Calibration in C is for all intracellular records.

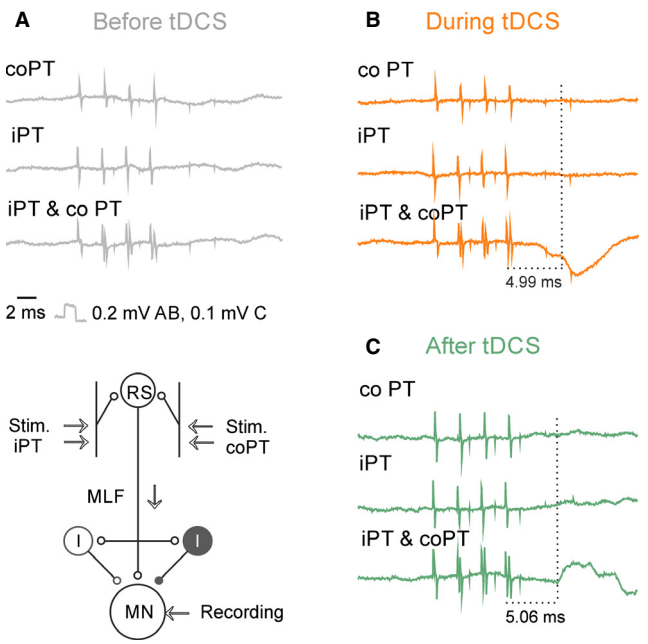

FIG. 4. Examples of PSPs evoked by combined stimulation of the iPT and coPT. (A and B) Records from a GS motoneuron before and during application of tDCS, respectively. (C) Records from a PBST motoneuron after application of tDCS. In each panel, the records were obtained upon stimulation of the coPT, iPT and both of them conjointly at 100 μA. The diagram indicates the stimulation and recording sites and the underlying convergence of the iPT and coPT on reticulospinal neurons providing input to motoneurons, either directly or via spinal interneurons, excitatory (white) and inhibitory (black). Note that in B an IPSP appears upon combined stimulation of the iPT and coPT during but not before tDCS (and not upon iPT or coPT stimuli alone). Note also that in C an EPSP is evoked by combined but not by separate iPT and coPT stimuli. MN, motoneuron; RS, reticulospinal neuron.

diagram in Fig. 2, should thus bring reticulospinal neurons closer to the firing threshold. Any additional facilitation induced by application of tDCS (Bolzoni *et al.*, 2013a,b) should therefore contribute to this threshold being reached. Consequently, EPSPs and/or inhibitory PSPs (IPSPs) should be evoked in greater proportions of motoneurons by combined stimulation of the iPT and coPT. They should also be larger than the sum of EPSPs/or IPSPs evoked from each of the PTs separately, and be evoked at shorter latencies.

The proportion of motoneurons in which EPSPs were evoked by joint actions of the iPT and coPT was indeed greater than that of motoneurons in which EPSPs were evoked from the iPT alone, and the proportion of activated motoneurons further increased following tDCS. It increased from 35 to 55%, although this increase was not found to be statistically significant (Table 1). The degree of facilitation varied both before and after tDCS; in extreme cases, EPSPs appeared upon combined iPT and coPT stimulation, even though separate iPT and coPT stimuli failed to evoke EPSPs by themselves, as shown in Fig. 2G for EPSPs evoked by the first three stimuli in the train and in Fig. 4C. Such combined actions further increased during tDCS (Fig. 2H, for EPSPs evoked by the fourth stimulus), and appeared at shorter latencies, which is another manifestation of facilitation of iPT actions by tDCS.

The latencies of EPSPs evoked by joint actions of the iPT and coPT decreased by 0.2–0.4 ms when measured from the effective stimulus, e.g. 4.86 ms in Fig. 2H as compared with 5.12 ms in Fig. 2B. In addition, such EPSPs were evoked by the fifth or fourth coPT and iPT stimuli before tDCS, but by the second, third or fourth stimulus after tDCS. On average, applying coPT stimuli before iPT stimuli, or vice versa, reduced the latencies of EPSPs from the first stimulus in the train by 2.42 ms before tDCS but by 4.08 ms during or after tDCS ($P < 0.001$; *t*-test for two samples on the assumption of equal variances; data for 10 and 15 motoneurons respectively).

Inhibitory PSPs evoked by joint stimulation of the iPT and coPT during and after tDCs were usually more distinct than IPSPs evoked by separate iPT or coPT stimuli under control conditions; sometimes, IPSPs appeared only in response to joint iPT and coPT stimuli (Fig. 4A and B). IPSPs with distinct onset appeared at latencies of 6.08 ± 0.15 ms from the effective stimulus ($n = 25$), which were, on average, 0.9 ms longer and significantly different ($P < 0.01$, *t*-test) from latencies of EPSPs of PT origin. Latencies of EPSPs and IPSPs of PT origin were approximately 1 and 2 ms longer than latencies of disynaptic EPSPs evoked from the MLF (4.10 ± 0.08 ms), indicating only one additional synaptic delay for the EPSPs and two additional synaptic delays for the IPSPs, as indicated in the diagram of the most direct pathways between the PTs and motoneurons in Fig. 2.

Joint actions from the iPT and the MLF are stronger after tDCS

In a further attempt to increase the probability of detecting facilitatory effects of tDCS in pathways between the iPT and motoneurons, we compared synaptic actions from the iPT stimulated conjointly not only with the coPT but also with the MLF. As collaterals of fibres descending in the MLF provide strong input to reticulospinal neurons, these neurons should be brought even closer to the

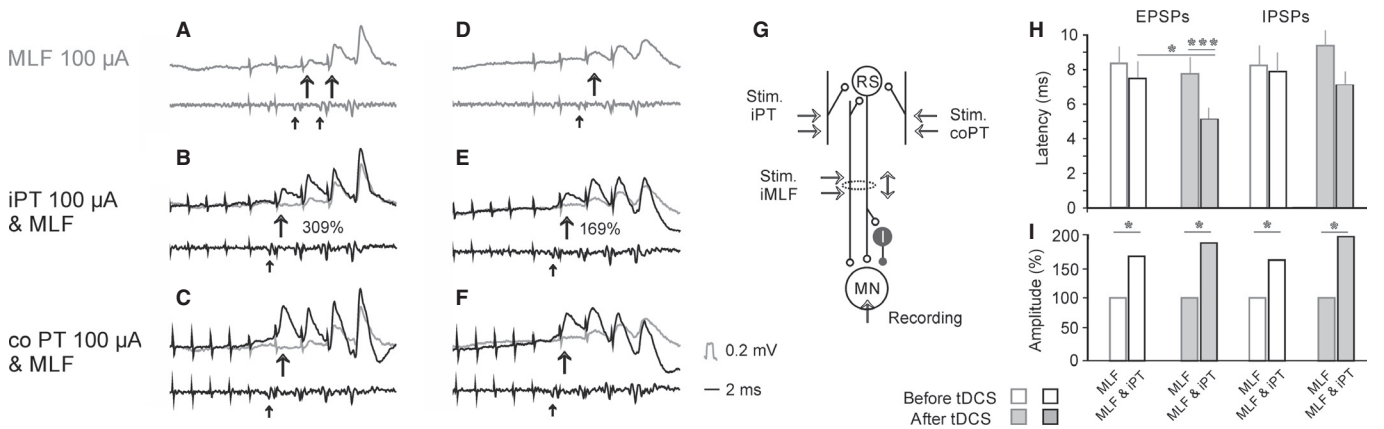

FIG. 5. Comparison of PSPs evoked by stimulation of the MLF alone and when preceded by stimulation of the iPT or coPT after tDCS. (A–C and D–F) Intracellular records from two GS motoneurons penetrated in the same experiment after tDCS (upper traces; averages of 20 records) and the corresponding records from the cord dorsum. Upper row: effects of a train of four stimuli applied in the MLF alone. Middle and bottom rows: effects of the same stimuli preceded by four stimuli applied to either the iPT or coPT, as indicated, with EPSPs evoked by the MLF alone (grey) superimposed. Large arrows indicate the earliest EPSPs. Small arrows indicate MLF volleys followed by these EPSPs. The increases in EPSPs evoked by the second stimulus as a percentage of control are indicated in B and E. Calibration in C is for all intracellular records. (G) Diagram showing the stimulation and recording sites and the involved inhibitory interneurons. (H) Decreases in the latencies (mean latencies and standard error of the mean; from the first MLF stimulus) of EPSPs and IPSPs evoked by MLF or by joint iPT and MLF stimuli. After tDCS, the decreases in latencies of the joint iPT–MLF actions (shaded columns) were significantly larger with respect to EPSPs evoked by both iPT and MLF stimuli under control conditions and only MLF stimuli after tDCS (*$P < 0.05$, *t*-test paired data; ***$P < 0.001$, *t*-test two samples). (I) Increases in amplitudes of EPSPs and IPSPs evoked by MLF stimuli when combined with iPT stimuli (*$P < 0.05$, *t*-test for either paired data or two samples). In H and I, data are shown for 10 and 12 motoneurons in which EPSPs were recorded before or during/after tDCS, and for nine and 14 motoneurons in which the effects of tDCS on IPSPS were compared. MN, motoneuron; RS, reticulospinal neuron.

threshold for activation when the MLF is stimulated than when only the two PTs are stimulated.

The net effects of iPT stimuli combined with MLF stimuli were assessed from differences between disynaptic EPSPs evoked by combined stimuli (Fig. 5B and E) and those evoked when the MLF was stimulated alone (Fig. 5A and D). More marked differences following tDCS, or a higher proportion of activated motoneurons, were taken to indicate a larger contribution from the iPT after tDCS, and thus a higher degree of facilitation of activation of reticulospinal neurons by the iPT.

As shown in Table 1, stimulation of the iPT after tDCS was found to facilitate synaptic actions evoked from the MLF in 32% of motoneurons before tDCS but in 69% of motoneurons after tDCS (difference significant at $P < 0.01$, *z*-test). EPSPs evoked by joint actions of the iPT and MLF, or the coPT and MLF, were facilitated in similar proportions of motoneurons, i.e. 32 vs. 33% before tDCS and 69 vs. 64% after tDCS, respectively; both differences were statistically significant. Similar degrees of facilitation of the effects of stimulation of the iPT and of the coPT after tDCS are further illustrated in Fig. 5B,C,E, and F. The facilitatory effects are reflected by larger amplitudes of EPSPs evoked from the iPT or coPT stimulated jointly with the MLF than of EPSPs of MLF origin. In particular, they show that both the iPT and coPT could cause EPSPs to appear following the earlier, originally ineffective, MLF stimuli after tDCS (arrows in Fig. 5B,C,E, and F). On average, iPT stimuli shortened the latency of EPSPs of MLF origin from the first stimulus by 0.85 ms before tDCS and by 2.63 ms during and after tDCS ($P < 0.05$; *t*-test paired data from 10 and 12 motoneurons, respectively; Fig. 5H). The amplitudes of EPSPs from the MLF were increased by PT stimuli to different extents depending on their amplitude, the medium-size EPSPs within the range 150–200%, but the smallest EPSPs several-fold (Fig. 5I).

Facilitation of IPSPs evoked from the MLF by iPT stimulation was also more effective during and after than before tDCS. In the total sample of nine and 14 motoneurons recorded before and during or after tDCS, the difference was most marked in the latencies of IPSPs measured from the first MLF stimulus (Fig. 5H). These were shortened by iPT stimuli by 0.36 ms in the control sample but by 2.26 ms after tDCS (although the difference was not statistically significant; $P > 0.5$). The peak amplitude of IPSPs evoked by the first effective stimuli in these two samples increased to a similar extent (to 187 and 197%, respectively; statistically significant; $P < 0.5$). However, IPSPs evoked in the same samples of motoneurons by the second effective stimulus showed almost twice as much enhancement after tDCS as those evoked by the first stimulus. The estimates of the degree of facilitation of the IPSPs also depended on the state of the motoneurons after the penetration. Therefore, parallel changes in the latency and the size of the IPSPs found in three motoneurons recorded before and during tDCS might be more representative. Thus, in the motoneuron illustrated in Fig. 6, IPSPs following the third MLF stimulus from the end were only weakly facilitated by iPT and/or coPT stimulation before tDCS and during the first periods of tDCS (Fig. 6B), but they substantially increased during the third and fourth periods of tDCS and after tDCS, when the first IPSPs appeared following an earlier stimulus (arrows in Fig. 6C–E). Stimulation of the iPT combined with stimulation of the coPT and MLF resulted in the appearance of IPSPs following two earlier stimuli (indicated by the two arrows in Fig. 6D), and these IPSPs were also much larger. As joint actions of the coPT and MLF (third column) were not much stronger than that of the MLF alone, the facilitatory effects in the last column must have been linked to the additional actions of the iPT.

Effects of tDCS on VR discharges

Because a comparison of PSPs evoked in the same motoneurons before and after tDCS was only possible in a small sample of motoneurons, we supplemented records from individual motoneurons

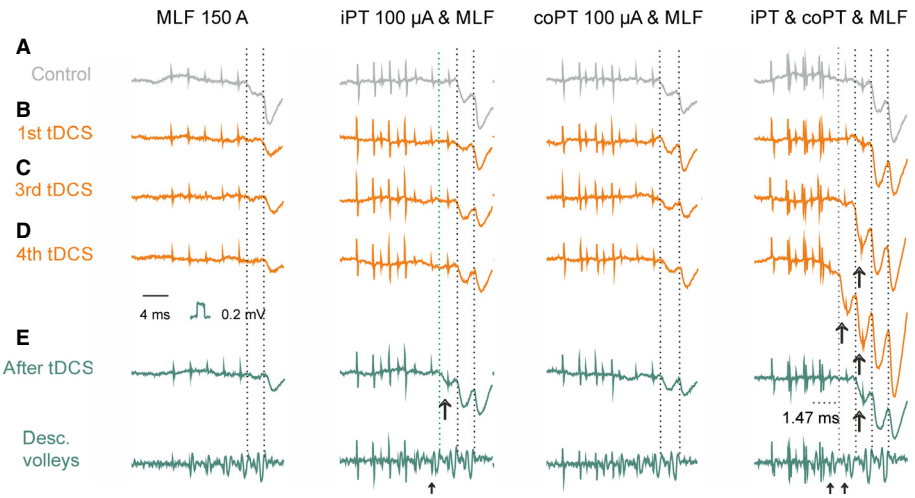

FIG. 6. Examples of facilitation of IPSPs evoked from the MLF during and after tDCS. Records from a GS motoneuron held at approximately the same level of membrane potential through tDCS application. (A) Control records obtained before tDCS with various combinations of the stimuli as indicated. Note facilitation of IPSPs evoked by the last two MLF stimuli by either the coPT or iPT stimulated separately or jointly. (B–E) Records of IPSPs evoked by the same combinations of stimuli during the first, third and fourth periods of tDCS and 10 min after the last period of tDCS, as indicated. Note facilitation of IPSPs evoked by not only the last two MLF stimuli but also an earlier stimulus after the third period of tDCS and two earlier stimuli during the fourth period of tDCS. Large arrows indicate the earliest IPSPs facilitated by the iPT. Small arrows indicate MLF volleys followed by these IPSPs. Vertical dotted lines indicate the onset of IPSPs evoked by MLF stimuli. Voltage calibration is for all intracellular records. The sites of recording and stimulation and the underlying convergence of iPT, coPT and MLF fibres on reticulospinal neurons were as indicated in the diagram in Fig. 6.

with records of discharges of populations of motoneurons with axons in one of the VRs.

Facilitation of iPT actions by tDCS was found in all five experiments in which it was assessed from changes in VR discharges. Prior to tDCS, VR discharges failed to appear in these experiments upon stimulation of either the iPT or coPT, or even both the iPT and coPT. MLF stimuli alone also failed to evoke any discharges in two of these experiments, although weak discharges appeared when MLF stimuli were combined with PT stimuli. However, in all five experiments, the situation changed dramatically during tDCS. As illustrated in Fig. 7E (middle column) MLF stimuli combined with coPT stimuli became much more effective during the first period of tDCS, and the discharges evoked by these stimuli increased and appeared at shorter latencies during and after the subsequent tDCS periods (Fig. 7E, right column). Maximal facilitation was evoked when iPT stimuli were added to a combination of coPT and MLF stimuli (Fig. 7F and G, middle and right columns), indicating that jointly applied MLF, iPT and coPT stimuli activated a considerably greater number of motoneurons. In the total sample of discharges evoked in five experiments, PT stimuli decreased the latencies of MLF discharges from the first stimulus by 2.5 ms before tDCS, by 4.5 ms during tDCS, and by 3.5 ms after tDCS.

With changes in the area of VR discharges illustrated in Fig. 7A–G as a measure of facilitation, the time course of these changes is plotted in Fig. 7H. The plot shows that the degree of facilitation tended to increase between the second and seventh periods of tDCS and during the immediately following 5-min periods. The increase continued during the first 20 min of the post-polarization period before declining to some extent, but it remained at a level exceeding that of control records for at least 30 min. When the data from all polarization and post-polarization periods were pooled together and compared with controls, they were found to be significantly different (ANOVA, $F_{2,12} = 7.31$, $P < 0.01$; Holm–Sidak *post hoc* test, $P < 0.05$).

The contribution of iPT effects is indicated by subtracting VR discharges evoked by coPT and MLF stimuli from those evoked by iPT, coPT and MLF stimuli (Fig. 7G). Another indication of the facilitatory effects of tDCS on iPT actions was the shortening of latencies of the discharges (arrows at the level of the first dotted vertical lines) evoked when iPT stimuli were added to coPT and MLF stimuli in Fig. 7F.

In three of the five experiments in which MLF stimuli already evoked weak responses before tDCS, although not from PTs (Fig. 8A–D), iPT stimuli enhanced them to a greater extent during and after than before tDCS (Fig. 8E) and to a similar extent as coPT stimuli (Fig. 8F). The superimposed records in Fig. 8H show that the facilitation of discharges following the first and second MLF stimuli was particularly marked (arrows), whereas facilitation of EPSPs evoked by the third stimulus in a train was less potent. This is also reflected in the plots of timing of tDCS effects on VR discharges examined during post-polarization periods of 2 h in two experiments (Fig. 9A–F and G–L). When data from all polarization or post-polarization periods were pooled together and compared with control data, only the effects of the second stimulus in a train were found to be significantly different (ANOVA, $F_{3,28} = 19.328$, $P < 0.01$, Fig. 9B; ANOVA, $F_{3,26} = 4.522$, $P = 0.011$, Fig. 9E; ANOVA, $F_{3,34} = 18.065$, $P < 0.01$, Fig. 9H; ANOVA, $F_{3,32} = 5.036$, $P = 0.006$, Fig. 9K; all with *post hoc* tests to control at $P < 0.05$, Holm–Sidak method).

To explain the weaker facilitation of VR discharges evoked by the last MLF stimulus in the stimulus train, we would favour the possibility that facilitation of excitation of motoneurons by these stimuli after tDCS was counteracted by an increase in facilitation of IPSPs evoked in motoneurons, which would reduce the EPSPs that preceded them.

Time course of facilitation of actions of iPT neurons by tDCS

The time course of the enhancement of intracellularly recorded PSPs of iPT origin by tDCS could not be defined, because the size of these PSPs greatly depended on changes in the state of the penetrated motoneurons. However, this problem was obviated by using

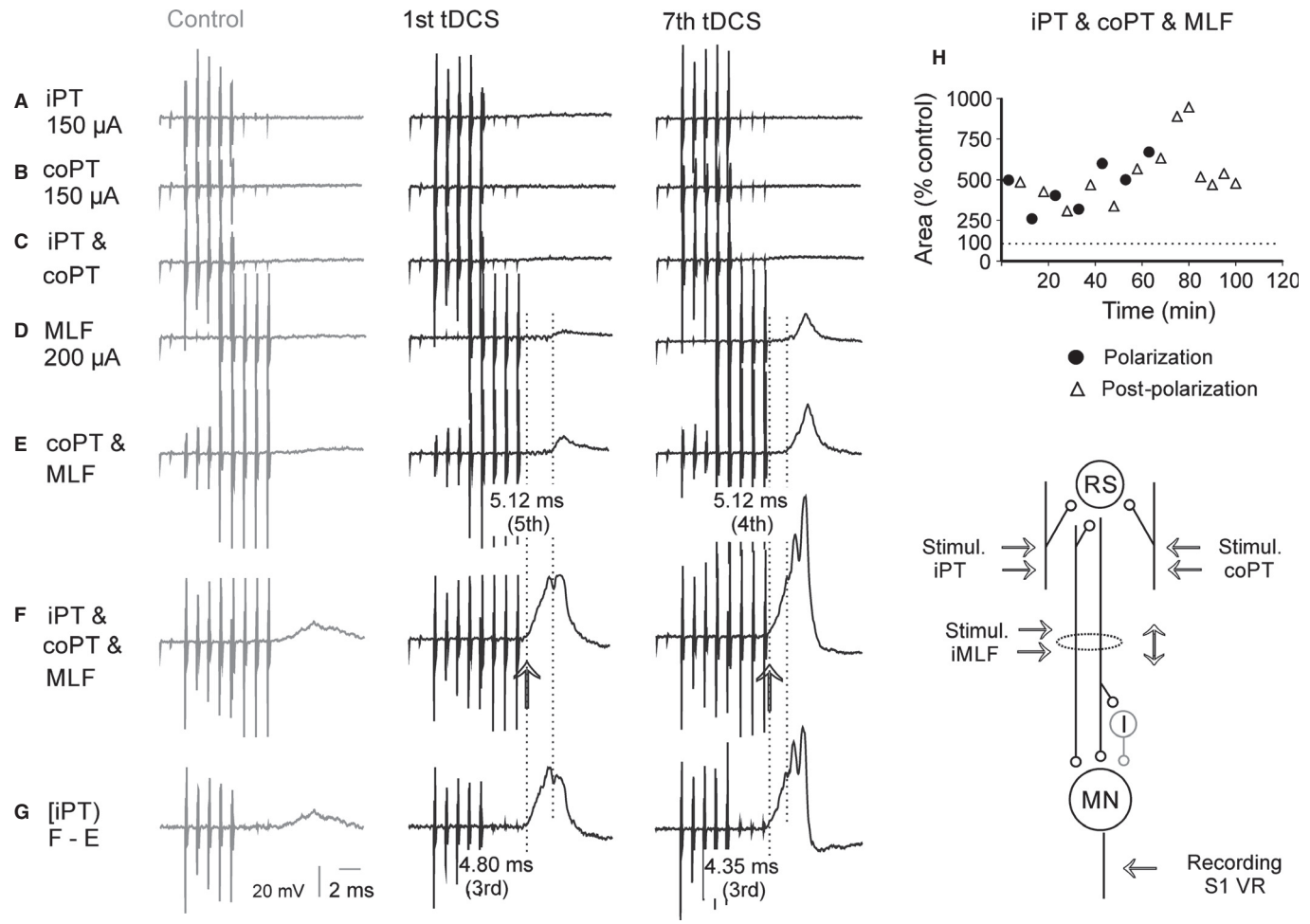

FIG. 7. Examples of activation of motoneurons (VR discharges) by iPT, coPT and MLF stimulation after tDCS. (A–F) Averaged records (n = 20) of discharges of motoneurons in the S1 VR evoked by different combinations of iPT, coPT and MLF stimuli, as indicated to the left, after but not before tDCS. Left panels: control records. Middle and right panels: records obtained during the first minute of the first and seventh periods of tDCS. (G) Addition of effects from the iPT when stimulated together with the coPT and MLF (records from row F minus records from row E). Dotted vertical lines indicate the onset of discharges evoked at the shortest (in F and G) and longest (in D and E) latencies. Note the increase in amplitude of both the earliest and later components of VR discharges and shortening of latencies of discharges evoked by the most efficient combinations of the stimuli during tDCS. Note also that the earliest discharges after tDCS were evoked during the time window between the two dotted lines in D–G, during which no discharges were evoked by either MLF or coPT stimuli; iPT stimulation must have been responsible for these early responses. (H) Time course of changes in the areas of records of VR discharges following stimulation of the MLF preceded by stimulation of both the iPT and coPT, i.e. the records illustrated in row F. The measurements were made during the time window between the two dotted lines in D–G. Ordinate: areas as percentage of control areas. Abscissa: time in minutes from the beginning of the first period of tDCS. The dotted horizontal line indicates the original area. The diagram indicates the sites of recording and stimulation and the underlying convergence of iPT, coPT and MLF fibres on reticulospinal neurons discharging the motoneurons, either directly or via spinal interneurons. MN, motoneuron; RS, reticulospinal neuron.

facilitation of VR discharges evoked by iPT stimuli as a measure of changes in the excitatory input from the iPT to motoneurons.

The plots in Figs 7 and 9 show that the facilitation of VR discharges developed either from the actual onset of tDCS application or only during the third to fourth periods of tDCS. They also show that the facilitation outlasted tDCS, not only during the 5-min periods immediately following the termination of tDSC, but also by up to 2 h, with a facilitation of at least 20% remaining for 30 min or more in all of the five experiments in which it was tested on VR discharges.

Taken together, the results of this study lead to the conclusion that the time course of facilitation of iPT-related excitation of motoneurons by tDCS replicates the time course of similarly prolonged facilitation of descending volleys evoked by stimulation of axons of corticospinal neurons and of reticulospinal neurons within the MLF (Bolzoni *et al.*, 2013a,b).

Control experiments and additional observations for defining the mechanisms underlying enhanced activation of motoneurons

As tDCS applied above the pericruciate cortex is unlikely to have had any direct effects at the spinal level, the reported results leave us with supraspinal facilitatory effects of tDCS. Furthermore, as input from the coPT or the MLF stimulated separately did not suffice to activate reticulospinal neurons as efficiently as when iPT stimuli were also applied, the facilitatory effects must, to a great extent, have involved transmission from the iPT.

However, the degree to which a combination of iPT, coPT and MLF stimuli was effective might have also depended on the degree of excitability of motoneurons and any spinal relay neurons. The appearance of the facilitation from the onset of tDCS links it to tDCS, but does not exclude tonic excitatory actions on spinal

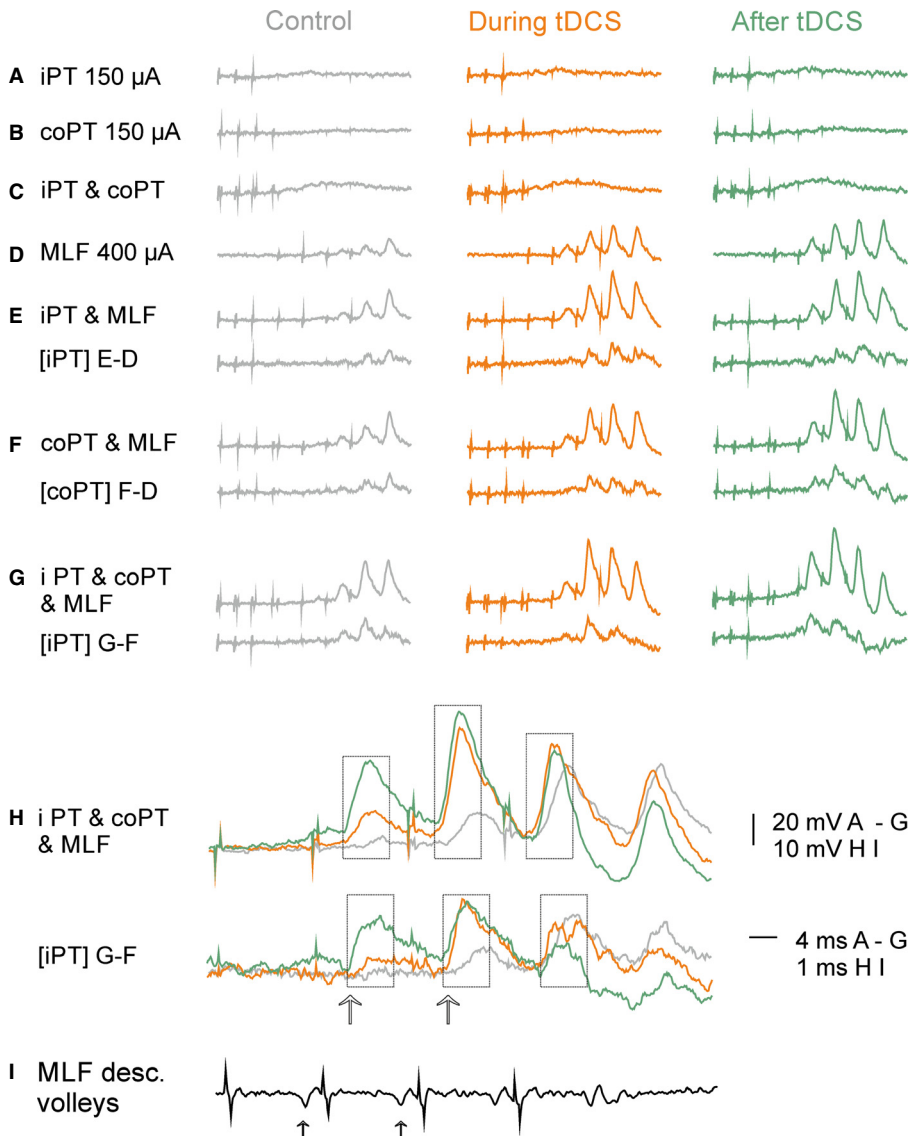

FIG. 8. Facilitation of activation of a population of motoneurons (VR discharges) by iPT stimulation combined with coPT and MLF stimulation during and after tDCS. (A–G) Averaged records (n = 20) of discharges of motoneurons from the S1 VR evoked by different combinations of iPT, coPT and MLF stimuli, as in Fig. 7. Net contributions from the iPT are indicated by lower traces; computer-generated differences between records are indicated on the left. Left panels: control records. Middle and right panels: records during the seventh period of tDCS and 30 min after its last application. (H) Superimposed expanded records from G, illustrating both increases in the discharges and the shortening of their latencies. (I) Records of descending volleys from the cord dorsum. The arrows in H indicate the strongest effects of tDCS. The arrows in I indicate MLF volleys followed by the EPSPs shown in H. For the time course of effects of tDCS, see Fig. 9A–C.

neurons set up by tDCS in addition to facilitation of phasic actions from the MLF and/or to changes in the excitability of spinal neurons resulting from other factors. Control experiments were therefore performed to allow estimates of the contributions of some of these factors.

Enhancement of VR discharges after tDCS vs. increase in the excitability of motoneurons

In order to estimate tonic changes in the excitability of motoneurons, we compared monosynaptic reflexes recorded from the same VR before, during and after tDCS. Monosynaptic reflexes were evoked by stimulation of the GS and PBST nerves supramaximal for group I afferents. Although the amplitudes of monosynaptic reflexes varied to some extent during successive periods of recording, in all of these experiments the variations were not related to changes in VR discharges evoked by MLF stimulation. In two of five experiments, the facilitation of VR discharges coincided with an increase in monosynaptic reflexes. In two experiments, the VR discharges were facilitated while monosynaptic reflexes decreased, and in one experiment the facilitation of VR discharges coincided with both increases and decreases in monosynaptic reflexes. Hence, there are no reasons to attribute facilitation of the effects of stimulation of the iPT on hindlimb motoneurons by tDCS to a tonic increase in the excitability of motoneurons.

Enhancement of VR discharges after tDCS vs. an increase in the general state of excitability of spinal neurons

The diagrams in Figs 4 and 5 indicate that the most directly mediated actions of iPT stimuli would require disynaptic or trisynaptic coupling between PT fibres and motoneurons, disynaptic actions

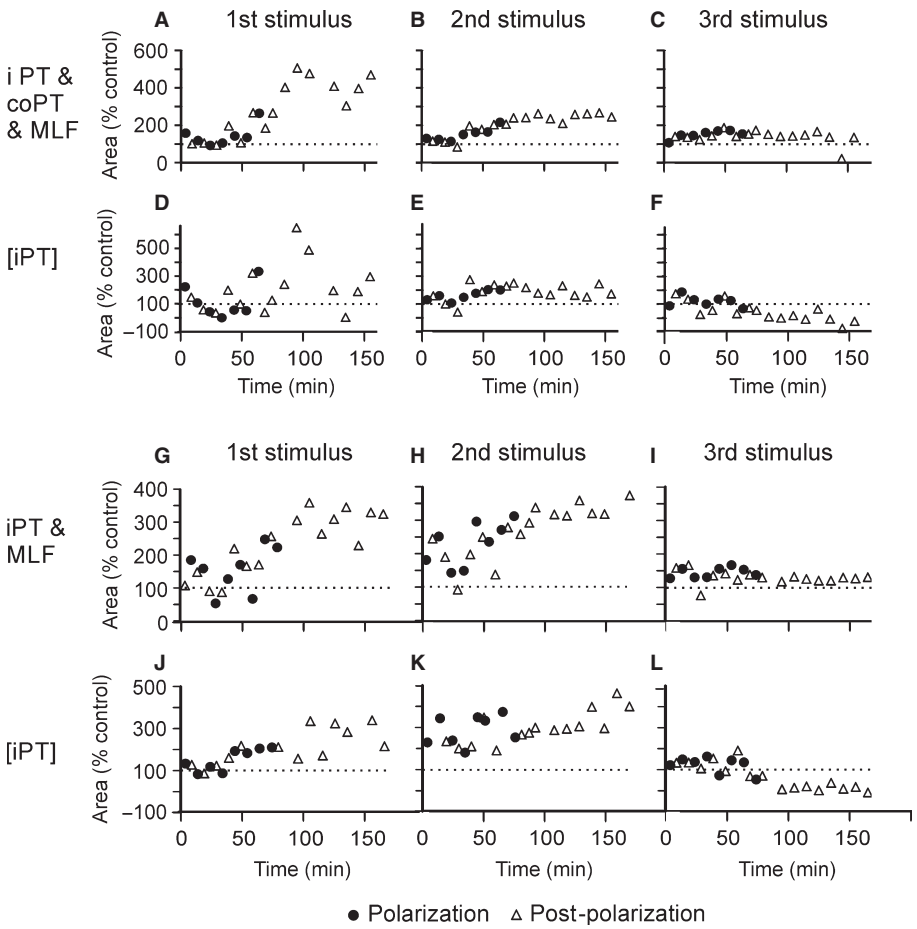

FIG. 9. Time course of facilitation of activation of motoneurons (VR discharges) by successive coPT and MLF stimuli preceded by iPT stimuli during and after tDCS. (A–C) Time course of changes in the areas of discharges exemplified in Fig. 8G and H, following the first, second and third MLF stimuli preceded by iPT and coPT stimuli, respectively; the areas were measured within the time windows indicated by dotted boxes in Fig. 8H. (D–F) Time course of the corresponding changes in the net effects of iPT stimuli. Note that they were most consistent after the second stimulus. (G–L) Time course of changes in the areas of discharges evoked following the MLF stimuli preceded by only iPT stimuli evoked in another experiment. Note the generally similar effects of tDCS. The sites of recording and stimulation and the underlying convergence of iPT, coPT and MLF fibres on reticulospinal neurons discharging the motoneurons, either directly or via spinal interneurons, were as indicated in the diagram in Fig. 7.

being relayed by reticulospinal neurons and trisynaptic actions by both reticulospinal neurons and spinal interneurons; tDCS could influence the excitability of these neurons. Effects secondary to variations in the depth of anaesthesia could not be estimated. However, the plots of the facilitatory effects of tDCS in Figs 7 and 9 show that the facilitation reached a certain plateau or started to decline during the testing periods, which does not seem to be consistent with the changes related to the decreasing depth of anaesthesia. No correlation was found between the periods of testing of the effects of tDCS and the time elapsed since the induction of anaesthesia, as marked facilitation by tDCS was also found when its application was delayed by 1, 2, or 3 h. The time course of the effects of tDCS is also not consistent with changes in the circulatory system, in view of the stability of the blood pressure and of the heart rate during periods preceding or following the plateau or decline of tDCS effects. For all of these reasons, we would tend to relate any tonic changes in the excitability of spinal relay neurons to the effects of tDCS, rather than to the state of the animal.

Discussion

The reported results reveal that the facilitatory effects of tDCS are not sufficient to increase the probability of activation of spinal motoneurons by iPT stimuli alone, at least not hindlimb motoneurons in the cat, and not under conditions of experiments under deep anaesthesia. However, we have demonstrated that tDCS potently increases the probability of activation of motoneurons by joint actions of iPT and coPT stimuli, and even more so by joint actions of iPT, coPT and MLF stimuli. The facilitated actions included both disynaptically and trisynaptically evoked EPSPs and IPSPs, and are fully consistent with the facilitation of activation of reticulospinal neurons by iPT neurons.

Reasons for concluding that the facilitation of ipsilateral actions of PT neurons on motoneurons by tDCS involves activation of reticulospinal neurons by iPT stimuli

Previous studies revealed effects of tDCS on subcortical neurons. Thus, studies in humans have demonstrated that tDCS can modify actions evoked by some subcortical neurons, in particular via corticostriatal and thalamocortical connections (Polania *et al.*, 2012), and that it induces changes in regional cerebral blood flow in the brain, including in such subcortical structures as the thalamus, globus pallidus, and nucleus accumbens (Lang *et al.*, 2005), but also the red nucleus and the reticular formation [see Fig. 10 in Bolzoni *et al.* (2013b)]. In recent studies, we found that the subcortical effects of

tDCS extend to neurons located in the mesencephalon and medulla in the cat and rat (Bolzoni *et al.*, 2013a,b), but tDCS by itself is unlikely to affect spinal neurons located further away, even those in the upper cervical segments.

By testing the effects of tDCS on responses evoked by stimuli that were subthreshold when applied to either the iPT, the coPT, or the MLF, but effective when applied to both the PTs and the MLF, we could link the reported effects of tDCS to neurons co-excited by axon collaterals of the PTs and MLF. Among such neurons, we could eliminate antidromically activated cortical neurons, in view of lack of evidence for convergence of axon collaterals of iPT, coPT and reticulospinal neurons on corticospinal neurons. The lack of such convergence made it similarly unlikely that other subcortical neurons, possibly affected by MLF stimuli, are involved. This would be particularly unlikely for vestibulospinal neurons with direct input from reticulospinal but not from PT neurons, and especially not from the motor cortex (Wilson *et al.*, 1999). It would be also unlikely for propriospinal neurons in upper spinal segments, which are co-excited by coPT and reticulospinal neurons (e.g. Alstermark & Isa, 2002) but not by iPT neurons (B. Alstermark, personal communication).

In contrast, all of the available evidence is compatible with the facilitation by tDCS of the activation of reticulospinal neurons co-excited by iPT, coPT and MLF stimuli, which, in turn, would be more effective in mediating disynaptic or trisynaptic excitation of hindlimb motoneurons. Thus, increases in indirect MLF-evoked descending volleys recorded at the level of the C1–C2 spinal segments demonstrated facilitation of trans-synaptically evoked activation of reticulospinal neurons (Bolzoni *et al.*, 2013a). In lumbar motoneurons, the earliest components of EPSPs enhanced by tDCS were delayed with respect to the indirect MLF volleys only by ~1 ms, which is compatible with monosynaptic actions of these volleys, whereas latencies of IPSPs were ~1 ms longer, and indicated actions evoked via an additional spinal interneuron. The earliest components of discharges in motoneuron axons were delayed with respect to EPSPs by < 1 ms, and were therefore compatible with a marginal supplementary delay in initiating action potentials in motoneurons and the time for conduction to the VRs. Taken together, these delays were thus compatible with responses directly evoked by fibres stimulated in the MLF, but would be too short to involve actions of additional neurons activated by axon collaterals of either the MLF or PT fibres. However, they do not exclude the possibility that later components of responses facilitated by tDCS were mediated by disynaptic rather than monosynaptic reticulospinal pathways, and that the facilitation of activation of interneurons in these disynaptic pathways may be likewise enhanced by input from reticulospinal neurons.

Plasticity of coupling between iPT neurons and reticulospinal neurons

The increasingly stronger facilitatory and depressive effects of iPT stimulation on motoneurons during successive periods of tDCS replicate the time course of the effects of tDCS on descending volleys in axons of subcortical neurons (Bolzoni *et al.*, 2013b). Both might thus be compatible with accumulating background effects that outlast the previous periods of tDCS application. In addition, the slow accumulation of changes seen after the successive tDCS periods seemed to continue, at least in some experiments, after the last tDCS period, as in the material of (Bolzoni *et al.*, 2013a), and is in line with observations that the duration of the after-effects of tDCS in humans depends on the number and length of tDCS applications (Monte-Silva *et al.*, 2010; Fricke *et al.*, 2011).

The effects of tDCS may involve both postsynaptic neurons and the presynaptic fibres that provide input to them, and different aspects of synaptic transmission modified by tDCS may be subject to plastic changes (Kabakov *et al.*, 2012; Ranieri *et al.*, 2012; Edwardson *et al.*, 2013; Rahman *et al.*, 2013). Modulation of transmission between PT fibres and reticulospinal neurons by tDCS would represent only one of several cases of plasticity at subcortical levels. Functional changes secondary to use, disuse and specific training have been found at various sites, including the spinal cord (Schwab *et al.*, 2001; Wolpaw & Carp, 2006; Martin *et al.*, 2007; Schwab, 2010; Martinez & Rossignol, 2013). Nevertheless, very little is known about the degree of plasticity related to the operation of reticulospinal neurons, even though they are of major importance for many centrally initiated movements (e.g. Lundberg, 1982; Jordan *et al.*, 2008) and for motor recovery after central injuries. For example, it has been demonstrated that reticulospinal neurons contribute to the restitution of skilled digit grasping movements in the cat after transection of the rubrospinal and corticospinal tracts within the cervical spinal cord (Alstermark *et al.*, 1981; Pettersson *et al.*, 2007), whereas, in primates, the role of reticulospinal neurons in restitution of digit movements is still under discussion (Lawrence & Kuypers, 1968a,b; Zaaimi *et al.*, 2012; Isa *et al.*, 2013).

Relevance of the results of this study for enhancing the ipsilateral actions of PT neurons by tDCS in humans

Weak iPT actions may be strengthened when evoked concurrently with activation of reticulospinal neurons, both in the cat and in primates, including humans (Edgley *et al.*, 2004; Stecina & Jankowska, 2007; Baker, 2011; Fisher *et al.*, 2012; Zaaimi *et al.*, 2012). As iPT actions may be further strengthened by tDCS, the reported results lead to the conclusion that tDCS may assist in substituting actions of injured contralateral PT neurons by actions of ipsilateral PT neurons and not only facilitate the actions of surviving neurons. Even though our results were obtained in experiments on anaesthetized cats, there are no reasons to doubt that they also apply to primates, including humans.

As in the cat, primate reticulospinal neurons control not only motoneurons (Riddle *et al.*, 2009) but also interneurons in spinal premotor networks that receive input from both reticulospinal and corticospinal tract fibres (Riddle & Baker, 2010). It might thus be predicted that iPT neurons contributing to activation of reticulospinal neurons (Fisher *et al.*, 2012) would make as great a difference in primates as in the cat, and assist limb movements, together with reticulospinal neurons and other neuronal systems that are important for recovery of motor functions. It might also be predicted that any factors enhancing iPT actions, including tDCS, would play an essential role in tipping the balance when conditions for initiating motor responses via corticospinal and/or reticulospinal neurons are critical.

Knowledge of the conditions under which tDCS most effectively facilitates iPT actions should help in selection of the circumstances under which it could be used to the greatest advantage. The results of the present study indicate that tDCS would be most effective in facilitating movements mediated by reticulospinal neurons co-excited by nerve impulses from both the iPT and coPT neurons. In humans, such co-excitation would be most likely to occur during mutually supportive bimanual movements, and when PT and reticulospinal actions are combined (see Zaaimi *et al.*, 2012; Dragert & Zehr, 2013; Edwardson *et al.*, 2013). It may thus be predicted that the operation of neuronal networks activated during bimanual movements would be improved during tDCS, and hopefully also during long post-tDCS periods.

Transcranial direct current stimulation should also facilitate the iPT contribution to activation of reticulospinal neurons by any other sources of input to them. Both feline and primate spinal interneurons and reticulospinal neurons are co-excited by muscle afferents (Jankowska & Stecina, 2007; Riddle & Baker, 2010). The addition of peripheral input to other sources of input to these neurons would thus increase the probability of their activation, and several observations in humans have already substantiated such possibilities. For example, ipsilateral motor evoked potentials, which are likely to be mediated via reticulospinal tract neurons, are easier to evoke against a background of muscle contractions (Alagona *et al.*, 2001) or lengthening contractions (Howatson *et al.*, 2011), which would provide input to both spinal interneurons and reticulospinal neurons from muscle and skin receptors. It has also been demonstrated that static arm posture can modulate voluntary distal muscle activity in the paretic upper limb in both post-stroke subjects and neurologically intact subjects (Dominici *et al.*, 2005; Ginanneschi *et al.*, 2005; Hoffmann *et al.*, 2011). The results of the present study indicate that a further beneficial effect can be expected when tDCS is applied under such conditions.

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Abbreviations

coPT, contralateral pyramidal tract; EPSP, excitatory postsynaptic potential; GS, gastrocnemius soleus; IPSP, inhibitory postsynaptic potential; iPT, ipsilateral pyramidal tract; i.v., intravenous; MLF, medial longitudinal fascicle; PBST, posterior biceps-semitendinosus; PSP, postsynaptic potential; PT, pyramidal tract; tDCS, transcranial direct current stimulation; VR, ventral root.

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