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Direct Current Stimulation modulates the excitability of the sensory and motor fibres in the human posterior tibial nerve, with a long-lasting effect on the H-reflex

Francesco Bolzoni [1,2], Roberto Esposti [1], Carlo Bruttini [1], Giuseppe Zenoni [1], Elzbieta Jankowska [2], Paolo Cavallari [1]

[1] *Human Physiology Section of the De.P.T., Università degli Studi di Milano, Italy*
[2] *Dept. of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden*

Corresponding author:
Prof. Paolo Cavallari
Human Physiology Section of the DePT
Università degli Studi di Milano
Via Mangiagalli 32
I-20133 Milano
e-mail: paolo.cavallari@unimi.it

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Abstract

Several studies demonstrated that transcutaneous direct current stimulation (DCS) may modulate CNS excitability. However, much less is known about how DC affects peripheral nerve fibres. We investigated the action of DCS on motor and sensory fibres of the human posterior tibial nerve, with supplementary analysis in acute experiments on rats.

In forty human subjects, electric pulses at the popliteal fossa were used to elicit either M-waves or H-reflexes in the Soleus, before (15 min), during (10 min) and after (30 min) DCS. Cathodal or anodal current (2 mA) was applied to the same nerve. Cathodal DCS significantly increased the H-reflex amplitude; the post-polarization effect lasted up to ~25 min after the termination of DCS. Anodal DCS instead significantly decreased the reflex amplitude for up to ~5 min after DCS end. DCS effects on M-wave showed the same polarity-dependence but with considerably shorter after-effects, which never exceeded 5 min. DCS changed the excitability of both motor and sensory fibres. These effects and especially the long-lasting modulation of the H-reflex suggest a possible rehabilitative application of DCS, that could be applied either to compensate an altered peripheral excitability, or to modulate the afferent transmission to spinal and supraspinal structures.

In animal experiments, DCS was applied, under anaesthesia, to either the exposed Peroneus nerve or its Dorsal Root, and its effects closely resembled those found in human subjects. They validate therefore the use of the animal models for future investigations on the DCS mechanisms.

Introduction

Several studies have demonstrated that direct current stimulation (DCS) may modulate the excitability of different CNS structures in humans, including the cerebral cortex, cerebellum and spinal cord (see: Brunoni *et al.*, 2012; Ahmed, 2013; Bolzoni *et al.*, 2015; Ferrucci *et al.*, 2016). The DCS effects have been often described as polarity dependent: typically increasing the cortical output when using anodal polarity and decreasing it when using cathodal (for the most recent review see Jackson *et al.*, 2016) It has been also demonstrated that DCS evoked modulation starts during the polarisation period but may last for several minutes after its termination. (Nitsche & Paulus, 2011).

Although literature reports a large and constantly growing number of cognitive/behavioural effects of DCS, investigations on the mechanisms underlying such effects are less frequent. In addition, most of these studies focused on DCS effects on synaptic transmission (for a review on animal studies, see Jackson *et al.*, 2016 and Lefaucheur *et al.*, 2017, respectively) and neuronal networks (Reato *et al.*, 2010, 2015). As an example, long-lasting after-effects have been attributed to changes in synaptic activity (in animals: Gartside, 1968; in humans Nietsche *et al.*, 2003). Different factors may contribute to these effects: acute and/or incremental changes in membrane polarization, with a concurrent synaptic activity (Fritsch *et al.*, 2010; Marquez-Ruiz *et al.*, 2012); modulation of neuronal firing rate (Gartside, 1968; Bindman *et al.*, 1964); or interaction with LTP/LTD (Ranieri *et al.*, 2012, Kronberg *et al.*, 2017). Relatively less attention was paid to the effects of DC on presynaptic fibres and on axonal excitability in general. Effects on axonal excitability have been examined both in peripheral nerves (Bhadra & Kilgore, 2004; Ardolino *et al.*, 2005; Boërio *et al.*, 2011; Ravid *et al.*, 2011) and in the central nervous system of animals, either in vivo (Eccles *et al.*, 1962; Ahmed, 2014; Bączyk & Jankowska, 2014) or in vitro (Jefferys, 1981; Bikson *et al.*, 2004; Radman *et al.*, 2009; Kabakov, 2012). Depending on the experimental conditions, both anodal and cathodal stimulation were found to induce a block of conduction (Bhadra & Kilgore, 2004). However, when local cathodal polarization was applied in the CNS, cathodal block was only found at excessive current intensities, while at low intensities cathodal DC consistently increased the excitability of all tested fibres (Baczyk & Jankowska, 2014; Bolzoni & Jankowska, 2015; Jankowska *et al.*, 2016; Jankowska *et al.*, 2017). The effects of cathodal DC on human peripheral fibres analysed by Burke *et al.* (2001), were likewise facilitatory, as short conditioning depolarising stimuli reduced the fibres activation threshold. Similar effects of a

longer lasting cathodal polarization of peripheral motor axons were found by Ardolino *et al.* (2005). Ahmed (2014) reported facilitation of mouse peripheral nerve fibres by either cathodal or anodal DC, depending on the field orientation with respect to the nerve. As far as we know, no comparison has been made between the effects of DCS on sensory and motor fibres in peripheral nerves and their consequences for activation of motoneurons and muscles.

Given the above premises, we systematically investigated the effects of anodal and cathodal DCS on human posterior tibial nerve. Specifically, we tested: i) whether DC stimulation modulates the excitability of sensory and/or motor fibres, by comparing how DCS affected the direct and monosynaptic reflex motor responses (M-wave and H-reflex, respectively); ii) whether this modulation is polarity dependent and iii) the duration of after-effects of DC polarization of peripheral nerves. Should an after-effect be observed on these responses, it would open the possibility of using DCS as a rehabilitation tool. DC could be applied either to compensate an altered peripheral excitability, or to modulate the afferent transmission to spinal and supraspinal structures.

We also collected preliminary data on rats, so as to validate a model allowing further investigations on the DCS mechanisms.

Materials and methods

Forty healthy volunteers (16 female, mean age = 26, SD = 3; 24 male, mean age = 24, SD = 3) participated to the study. Exclusion criteria were any history of neurological or orthopaedic disease, and intake of drugs acting on the CNS. Naïve participants gave their informed consent, but were kept completely unaware of the stimulation condition. The experiments were conducted in accordance with the policies and principles outlined in the Declaration of Helsinki and were approved by the Ethical Committee of the De.P.T., Università degli Studi di Milano.

Experimental setup

Subjects sat comfortably in a reclining chair with the right leg resting on a fixed support, as shown in Fig. 1. The subjects were asked to stay relaxed, with the hands resting on the

thigh, head laying on a headrest and gaze toward a fixed point in front of them. The setup was adapted to each subject's body size.

The experiment aimed at conditioning the M-wave and H-reflex responses, elicited in the resting Soleus muscle by electrical pulse (*Test Stimulus*) to the posterior tibial nerve, by means of DCS applied to the same nerve (C*onditioning Stimulus*). Therefore, two electrodes were mounted on a rigid plastic plate (3.5 cm apart): the *Active Electrode* for the *Test Stimulus* (Ag/AgCl circular electrode, 1 cm^2, connected to a Digitimer® DS7A current-controlled stimulator), and the *Active Electrode* for the C*onditioning Stimulus* (an hemisphere of conductive rubber, covered by a 10 cm^2 sponge soaked with saline solution and conductive gel, connected to a Neuroconn® DC-Stimulator Plus, model 0021). With this configuration, any voltage drop across the body, due to the DCS, should not affect the test stimulus current, as the higher or lower voltage drop necessary to inject such current should be immediately and automatically compensated by the internal circuitry of the DS7A stimulator. The plastic plate was placed on the skin of the right popliteal fossa and kept in place with a Velcro® band, so the two active electrodes were both placed on the tibial nerve. This was verified by checking that both electrodes, if alternatively connected to the square-pulse stimulator, were capable of eliciting an H/M response. The larger *Active Conditioning Electrode* required a current no more than twice that required by the *Active Test Electrode*.

In the H-reflex study (see Fig. 1A), the *Active Test Electrode* was distal to the active *Conditioning Electrode*, while the *Reference Test Electrode* (gel-coated conductive rubber, 20 cm^2) was placed on the patella, and the *Reference Conditioning Electrode* (square sponge soaked with saline solution and gel, 50 cm^2 so that the current density was functionally inefficient) was positioned 5 cm proximal to it, over the quadriceps muscle. In the M-wave study (see Fig. 1B), the *Active Test Electrode* was proximal to the *Active Conditioning Electrode*, while the *Reference Test Electrode* was placed on the patella and the *Reference Conditioning Electrode* was positioned 5 cm distal to it, over the tibia. With this setup the DCS preferentially polarized the proximal portion of the nerve when testing the H-reflex and the distal portion when testing the M-wave. To grant stable stimulating and recording conditions, the foot was left free to move in order to exclude any mechanical effects of the muscle contractions. Additionally, the subjects were asked to stay as still and relaxed as possible, to avoid changes in the motoneuronal excitability.

The H-reflex and M-wave responses were recorded by two pre-gelled surface electrodes placed 25 mm apart (H124SG, Kendall ARBO, Tyco Healthcare,

Neustadt/Donau, Germany) on the distal part of the leg where the two bellies of the Gastrocnemius diverge to reveal the Soleus. To minimize the stimulation artefact, a bracelet ground was put on the shin proximal to the recording electrodes. The most selective place for recording was achieved both by a careful positioning of the electrodes and by checking that the activity from the Soleus muscle, during voluntary phasic contractions, was not contaminated by signals from other sources. Recorded signals were AC amplified (IP511, Grass Technologies®, West Warwick, Rhode Island, USA; gain 0.5–2 k) and band-pass filtered (3–3000 Hz), then digitized at 10 kHz with 12 bit resolution (PCI-6024E, National Instruments®, Austin, Texas, USA) visualized on a computer screen and stored for further analysis.

Experimental design

The effects of either cathodal or anodal DCS (60 s fade-in, 10 min at 2 mA, 15 s fade out) on the H-reflex (10 subjects for each polarity) and M-wave (10 subjects for each polarity) response were tested on separate days, in random order. Current density (2 A/m^2) was more than one order of magnitude lower than the safety limit (25.46 A/m^2) reported by Bikson *et al.* (2009) on humans and much lower than the minimal current density (142.9 A/m^2) that Liebetanz et al. (2009) reported to induce brain lesion in the rat, if applied for more than 10 min. None of the subject reported unpleasant sensations related to the polarization or square-pulse stimulation. Moreover, none of them could recognize the DC polarity.

Before testing the reflex, the maximal peak-to-peak amplitude of the H-wave (H-max) and of the M-wave (M-max) were measured after delivering square-pulse stimuli (0.5 ms duration) of progressively increasing current. When testing the H-reflex, the square-pulse current was set at the intensity eliciting a stable H-reflex (in its rising phase) of about 50% H-max amplitude. Stimuli were repeated every 8 sec throughout the experiment. When testing the M-wave, in separate sessions, the square-pulse current was set to elicit an M-wave of about 50% M-max.

Four packets ($C_1 \ldots C_4$), each containing 10 contiguous H or M responses, were collected evenly spaced in time in the 15 min control period (CTRL); three further packets ($D_1 \ldots D_3$) were collected during the 10 min DCS period (DURING) and seven packets ($A_1 \ldots A_7$) over the following 30 min period after the DCS switch-off (AFTER). All data were stored for offline analysis.

Analysis

The 10 responses of each packet were first averaged, then the *peak-to peak amplitude* of the mean signal was measured and expressed in %M-max. By combining the DC polarities (cathodal and anodal) and the muscular response (H-reflex and M-wave), four experimental conditions were obtained. In each condition, the data recorded in the CTRL packets were compared in the whole population by one-way repeated measures ANOVA (C_1 vs. C_2 vs. C_3 vs. C_4). Having found no significant difference, data from the four packets were then pooled, to obtain a single CTRL value (C_{MEAN}) for each subject. C_{MEAN} data were then compared to data from all other packets of the DURING and AFTER periods by one-way repeated measures ANOVA (C_{MEAN} vs. D_1 vs. D_2 vs. … A_7). Dunnett's post-hoc tests were then applied to compare each DURING and AFTER value to C_{MEAN}.

Experiments on rats

These experiments followed the main procedures described in Jankowska *et al.* (2016). All experiments were approved by the Regional Ethics Committee for Animal Research (Göteborgs Djurförsöksetiska Nämnd), and followed NIH and EU 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. The animals (10 Wistar rats, 250-450g of both sexes) were anaesthetised first by isoflurane (4% in air) (Baxter Medical AB, Kista, Sweden) and then by α-chloralose (Acros organics, Geel, Belgium) at a dose of 30-40 mg/kg i.p. together with pentobarbital sodium (Apoteksbolaget, Göteborg, Sweden) at a dose of 20-25 mg/kg. During the experiment the neuromuscular transmission was blocked by Gallamine triethiodide (Sigma-Aldrich, G8134-5G), artificial ventilation was applied using a respiratory pump (CWE; 65-80/min and 0.2-0.4 ml/min volume depending on animal weight), maintaining the expired CO_2 level at 3-4%. The CO_2 level and the heart rate were continuously monitored. The experiments were continued only for as long as these remained within physiological ranges.

In order to test the DCS effect on peripheral axons excitability, experiments were conducted in two different setup.

In eight experimental sessions on 6 rats (setup 1), the Common Peroneal nerve and the corresponding dorsal roots (L4-L6 DRs) were dissected free in continuity and mounted on stimulating and recording electrodes in two separate paraffin oil pools. In the first pool, the dorsal roots were cut proximally; an Ag/AgCl electrode pair was put in contact with the end of the L6 DR and used to deliver the *Test Stimulus*. In the second pool, the peroneus nerve was cut distally, and placed on another pair of electrodes, for recording the antidromic volley elicited in the afferent fibres. DC conditioning was applied via the *Active Electrode* (made of silver) in contact with the DR over a distance of 4 mm, immediately distal to the *Test Electrodes*; the *Reference DC Electrode* (a 3x3 cm sponge moistened with saline) was instead on the rat belly.

In six experimental sessions on 4 rats (setup 2), the Common Peroneal and the Sciatic nerves were dissected free and mounted on stimulating and recording electrodes in the same paraffin oil pool. The Peroneal nerve, cut distally, was placed on an electrode pair that delivered the *Test Stimulus,* while a second pair under the Sciatic nerve was used to record the compound volley elicited in both sensory and motor fibres. The *Active DCS Electrode* was placed on the Peroneal nerve, immediately proximal to the *Test Electrodes*, while the *Reference DC Electrode* was on the rat belly.

For both experimental setups, the *Test Stimulus,* eliciting the volley, was a constant current pulse of 0.2 ms duration, not exceeding twice the threshold intensity. After a control period of 15 min, cathodal DCS (0.3 μA) was applied for 10 minutes; then the recordings continued for 30 further minutes. The volley sampling followed the same procedure as in the human experiments.

The effects of the polarizing current were estimated from changes in the size (as determined from the area) of the first component of the volleys evoked by the *Test Stimulus* DURING and AFTER DCS, as compared with those evoked prior to the polarization period (CTRL). The volley areas were then assessed with a software for sampling and analysis developed by E. Eide, T. Holmström, and N. Pihlgren (University of Gothenburg). Volley areas referring to DURING and AFTER DC were normalized with respect to the mean value of the four CTRL volleys. The same statistical analysis was applied as for the human experiments.

Results

Human study

M/H responses

The *Test Stimulus* (0.5 ms square electrical pulse to the Posterior Tibial nerve) was sufficient to elicit M-wave and H-reflex in all subjects. In full agreement with the well-known H-M recruitment curve (Pierrot-Deseilligny & Mazevet, 2000), the current intensity required for evoking an H-reflex of about 50% H-max was regularly lower than that required for an M-wave of about 50% M-Max. The H-reflex had a latency of about 25-30 ms while the M-wave latency ranged about 7-9 ms, in accordance to the conduction velocity of Ia afferent axons and of motor fibres.

Effects of DC stimulation

Figure 2 shows the M and H responses elicited, in different sessions, in representative subjects, CTRL (before), DURING and AFTER anodal and cathodal polarization. It is apparent that the plotted responses were very stable in the CTRL periods, while application of DC stimulation had a clear and polarity-dependent effect on each response. The top panels illustrate DCS effect on H-reflex. As shown, anodal DCS gradually reduced the amplitude of the reflex (shaded area in Fig. 2A) while the cathodal polarisation increased it (Fig. 2B). In both cases, there was an apparent after effect that lasted several minutes after DCS was switched-off.

The DCS effects on M-waves showed a similar polarity-dependence. Indeed, M-wave amplitude decreased during anodal DCS (Fig. 2C) and increased with cathodal DCS (Fig. 2D). For both polarities, the M-wave recovered close to the CTRL level well before 30 min, after DCS was turned off. DCS has not been found to change the latency of either M-wave or of H-reflex responses.

Figure 3 reports data from the whole population (mean values ± SE), following the same graphical layout as in Fig. 2. Again, it demonstrates the high stability of the responses during the CTRL periods. Statistical analysis confirmed this observation. Indeed, one-way repeated measures ANOVAs did not find any significant difference among the four CTRL packets ($C_1 \ldots C_4$; P always > 0.5). Therefore, the four measurements were averaged and compared, by one-way repeated measures ANOVAs and Dunnett post-hoc (see asterisks in the figure), to the packets taken DURING and

AFTER polarisation. DCS deeply affected the H-reflex (Fig. 3A-B), both DURING and AFTER its application. Anodal DCS induced a significant decrease of the reflex response ($F_{10,90}$ = 5.33 , P < 0.0001), which lasted about 5 min in the AFTER period. Cathodal, instead, elicited a significant increase of H-reflex amplitude ($F_{10,90}$ = 6.25 , P < 0.0001). In this case the after effect lasted longer (about 25 min). Panels C-D show that DCS affected the M-wave DURING its application, with the same polarity dependence as on the H-reflex, whereas AFTER its application was very weak and short lasting. Indeed anodal DCS induced a significant decrease ($F_{10,90}$ = 3.49 , P = 0.0006) in M-wave amplitude DURING its application, but with no statistically significant after effect. Cathodal DCS, conversely, produced a significant M-wave increase ($F_{10,90}$ = 4.95 , P < 0.0001) which lasted about 5 min after DCS was switched off.

Animal experiments

Figure 4 shows the effect of cathodal polarisation on fibre excitability in rats. For both setups, ANOVA confirmed the stability of the response in the CTRL periods (P always > 0.75). Panel A shows that DCS, applied at the level of the dorsal root, affected the antidromic volley elicited by applying the *Test Stimulus* on the same root ($F_{10,70}$ = 2.23 , P = 0.026). DCS clearly increased the excitability of afferent fibres in the dorsal root DURING its application. The mean AFTER-effect was of about 10% and lasted about 10 minutes. Panel B shows the effects of DCS of the Peroneal nerve on the compound afferent volley elicited by applying the *Test Stimulus* to both afferent and motor fibres. DCS increased the nerve excitability both DURING and, for some minutes, also AFTER its application. However, no observed changes reached the level of statistical significance ($F_{10,50}$ = 1.60 , P = 0.13).

Discussion

The reported experiments demonstrate the ability of DCS to modulate the amplitude of both the direct (M-wave) and reflex (H-reflex) responses: the cathodal polarisation increased their amplitude while the anodal polarisation reduced it. Such modulation persisted after the DCS was switched-off (after-effect). However, it lasted longer on the H-reflex than on the M-wave. Animal experiments led to similar results, thus validating a possible rat model and allowing to plan future studies exploring mechanisms underlying the DCS effect. In this model, differentiating the motor from the sensory effects would be possible by comparing DCS effects on the dorsal and ventral roots.

In the literature, studies regarding the effects of polarising current on fibres are much less frequent than those regarding the central effects, and were collected under very different experimental conditions.

High intensity DCS, of both polarities and for periods from seconds to minutes, was reported to block the nerve conduction (Manfredi, 1970; Sassen & Zimmermann, 1973; Bahdra & Kilgore, 2004; Ackermann *et al.*, 2011). Depolarising DCS required less current than the hyperpolarising ones, and the block mainly involved large axons, without appreciable effects on the conduction of small fibres (Whitwam & Kidd, 1975). In our study, which used a much lower current density, block of conduction was never observed.

Other studies investigated the effects of brief stimuli (200-300 ms), reporting a polarity dependent effect on the firing threshold of nerve fibres (Bostock *et al.*, 1998; Kiernan & Bostock, 2000). These authors suggested that the initial strong facilitation, associated to the depolarising DC, could be attributed to an interaction with Na^+ channels, while the following partial repolarisation to the activation of a slow potassium conductance. The hyperpolarising DC was instead presumed to reduce the excitability by acting on K^+ internodal channels. At both polarities, a rebound effect was observed after the short DC application: when the depolarising DC was switched-off, the nerve excitability became lower than before DC application. Thereafter it returned to the “before DC” level in about 100 ms; the opposite phenomenon was observed with hyperpolarising DC. When compared with the effects of brief polarizing pulses, the effects of longer lasting polarization used in the present study showed the same polarity dependence but with additional after-polarization effects. Changes found during the after-polarization period were in the same direction as during the DC application. The after-polarization changes were also longer lasting than those reported by Ardolino *et al.* (2005), especially with

respect to the increase in the excitability of sensory fibres that were not differentiated from motor axons in the study of Ahmed and were not examined by Ardolino. A very long lasting after-polarisation DC effect was recently found on fibres stimulated within the dorsal columns, following weak epidural polarization in anaesthetised rats (Jankowska *et al.,* 2017). In this preparation, the number of dorsal column afferent fibres stimulated epidurally was greatly increased by epidural polarization, and such after-effect lasted for more than an hour.

Possible origin of the DC after-effects on peripheral nerve fibres

The most direct explanation may be that the long lasting current used in our experiments may have triggered a self-sustained opening of “persistent” sodium channels. It is, in fact, known that these channels open at more negative potentials than the classical “transient” sodium channels, producing an inward leak current that, once stabilized, may keep the membrane potential steadily depolarized (Bostock & Rothwell, 1997). Another possibility is that the after effects are the manifestation of an underlying plasticity process. Indeed, Debanne *et al.* (2003) reported cases in which the plasticity was induced by a synergic interaction between the “classical” synaptic plasticity and the regulation of ionic conductance in specialized neuronal areas (e.g. the dendrites, the cell body and also the axon). In this respect, it has been observed that a constant electric field could interfere with the distribution of proteins on the plasmatic membrane (Jaffe, 1977; Stollberg & Fraser, 1988). Moreover, a relatively small electric field, even like that induced by the spontaneous neural activity, could change the H^+ concentration, and thus influence the activity of several ion channels. This could in turn lead to a modulation of the protein functionality and generally of the cell activity (Chesler, 2003). Although we are not in position to determine what causes the reported after-effects, it is possible that one or many of the above mentioned mechanisms contribute to it. Finally, we cannot exclude the possibility of an interaction between the DCS and the myelin. Such interaction was concluded to occur by Zheng & Schlaug (2015) who observed that a rehabilitation protocol associating tDCS to physical therapy favoured an increase in fractional anisotropy, most likely due to modifications of white matter. Indeed, higher fractional anisotropy values are thought to be associated with the changes in myelination level that accompany variations in fibres alignment, while lower ones could indicate the opposite effect (Hoeft *et al.*, 2007;

Sidaros *et al.*, 2008). It is however, difficult to conclude that just one period of peripheral DCS could increase myelination so as to improve the efficiency of signal conduction.

The longer duration of the after-effects on H-reflex than on M-wave responses also merits a comment. As all of the above mentioned mechanisms concerned the peripheral fibres, they may explain the changes in the excitability of both Ia afferents and motor axons, but they cannot explain the longer after effect on H-reflex than on M-wave unless postulating differences in properties of these fibres. Some additional factors might therefore contribute to prolong the after effect in the reflex arc, maybe a central modulation of motoneuronal excitability. This aspect will be discussed below.

Differences in polarity dependence

Most literature on transcranial cortical and cerebellar DCS reports a polarity dependence of the effect that is opposite in sign as compared to that found in the present study, being usually facilitatory when anodal and inhibitory when cathodal (see Brunoni *et al.*, 2012). The same effect was also found by Di Lazzaro *et al.* (2013) on the direct and indirect responses of the cortico-spinal tract. In contrast, our results clearly showed that cathodal DCS increased peripheral fibres excitability, while anodal DCS decreased it, a finding more in agreement with most of the studies on trans-spinal DCS (see Cogiamanian *et al.*,2012; Jankowska 2017). It is plausible that the effect of DCS on central neural structures, which are more complex than the peripheral nerve, results from a balance between the DC effects on neuronal substructures (synapse, soma, dendrites and fibres) at various distances from the source of DC, thus indicating that the overall modulation comes from a sum of actions that may also be of opposite sign, as discussed by Jackson *et al.* (2016). There are, obviously, differences between our simple model and the complex cytoarchitecture of the cortical system. One crucial factor determining DCS effect could be the orientation of the electric field with respect to the neural tissue, as already observed by Terzuolo and Bullock (1956), Maccabee *et al.* (1998) and also Bikson *et al.* (2004). Also a study by Jefferys (1981) led to the conclusion that the axonal orientation relative to the electric field vector determines whether the regional neuronal excitability is increased or lowered, a result further confirmed on nerve fibres by Ahmed *et al.* (2014). Finally, cortical neuron morphology relative to electric field and also cortical cell type play a role in determining sensitivity to sub- and supra-threshold brain stimulation (Radman *et al.*, 2009).

Differential effect on H and M responses

We observed a longer lasting after effect on H-reflex than on M-wave response. One simple explanation for such difference may be that the M-wave amplitude depends only on the number of fibres that are brought over threshold by the *Test Stimulus*, while the H-reflex amplitude also depends on the excitability of the motoneuronal pool in the spinal cord. Should the 10 min DCS modulate the communication between large afferent fibres and the motoneuronal pools, it might be possible that during the after-polarization period the Soleus motoneurons were in a different state of excitability than under CTRL condition. Another possible explanation could stem from the differences in the biophysical features of sensory and motor axons that could be observed even in the same nerve (Kuwabara *et al.*, 2000). By comparing the properties of cutaneous afferents and of motor axons of the upper limb, many dissimilarities were found in: refractoriness, supernormality and late subnormality after a single discharge (Kiernan *et al.*, 1996), the level of inward rectification (Bostock & Bergmans, 1994), Na^+ conductance (Bostock & Rothwell, 1997), rheobase, and strength-duration time constant (Panizza *et al.*, 1994; Mogyoros *et al.*, 1996). Whether or not similar differences occur between primary muscle spindle afferents and motor axons has not yet been reported.

Future applications

The possibility of applying DC to a nerve in order to modulate its excitability could be of advantage in several pathological conditions. Many nerve diseases lead to a decreased capability to conduct the nerve impulses (Kiernan & Kaji, 2013), while an increased excitability may cause pain, fasciculation and paraesthesia (see Nordin *et al.*, 1984). The spinal reflexes are also dramatically impaired as a consequence of spinal or supra-spinal damages. In these cases, modulating the strength of the proprioceptive inputs could lead to a change of the reflex outcomes, as after operant-conditioning of the H-reflex found by Thompson & Wolpaw (2015). So far, the most used treatments for spasticity include physical and occupational therapy, anti-spastic medications, and chemo-denervation (Goldstein, 2001). Spasticity is one of the most common symptoms of stroke and spinal cord injuries and is associated with hyper-reflexia, as expressed in a chronic over-activity of muscles (Sommerfeld *et al.*, 2004). In this context, it has been suggested to use DCS to decrease the peripheral nerve conduction and thus the myohypertonus (Ravid &

Prochazka, 2014). These experiments primarily aimed at simulating a nerve ablation by applying high intensity currents, so as to modify intra-axonal pH and cause a dysfunction, which is however often irreversible. The present work demonstrates that it is possible to modulate the nerve activity in a fast and reversible way. Finally, as cathodal DCS increased nerve fibres excitability should the associated increase in afferent flow facilitate spinal plasticity, as suggested by the long-lasting effects observed on the monosynaptic reflex pathway, this could open another possible application of DCS in rehabilitation.

Conclusions

The acute neuromodulation of sensory and motor fibres excitability, and the long-lasting effect on the H-reflex, suggest a potential application of DCS as a rehabilitative tool. Rat experiments led to similar results, indicating that animal model may be applied in future investigations of the DCS mechanisms.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

FB, EJ & PC conceived the study; FB, RE, GZ & CB conducted the human experiments; FB & GZ analysed the human data; FB & EJ conducted the animal experiments and data analysis; FB, RE, PC & EJ prepared the manuscript, which was approved by all authors.

Data Accessibility

Data are available from the corresponding author on request.

Abbreviations

DC, Direct Current; DCS, Direct Current Stimulation; tDCS, transcranial Direct Current Stimulation; tsDCS, trans-spinal Direct Current Stimulation; CNS, Central Nervous System; CTRL, Control period;

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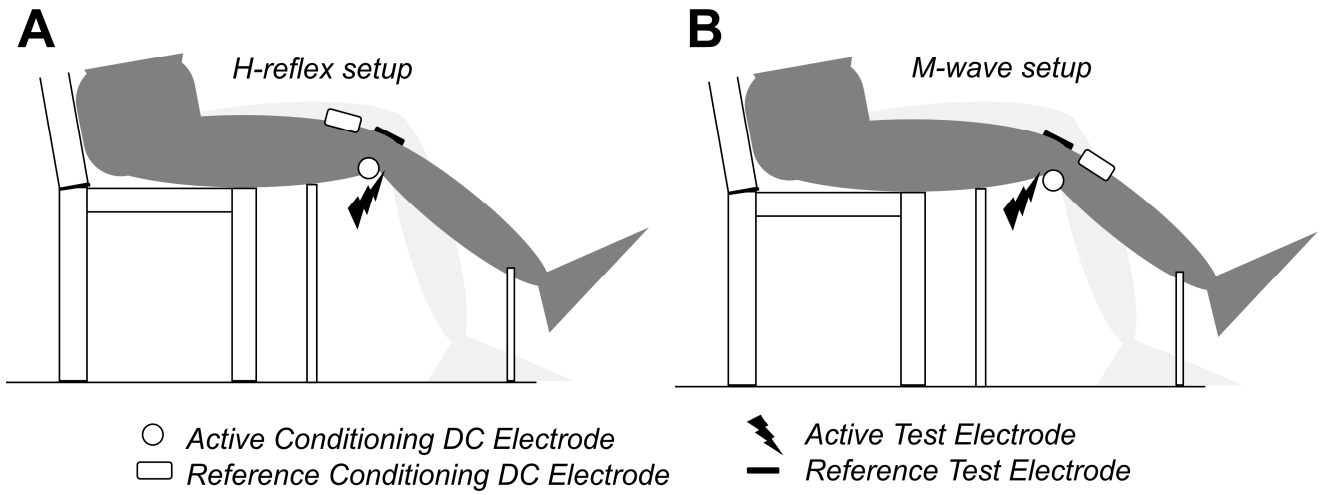

FIG. 1. Side view of the setup for human experiments. Positions of the electrodes when testing the effect of DCS *Conditioning* on H-reflex (A) and on M-wave (B), both of which were elicited by electric *Test* pulses.

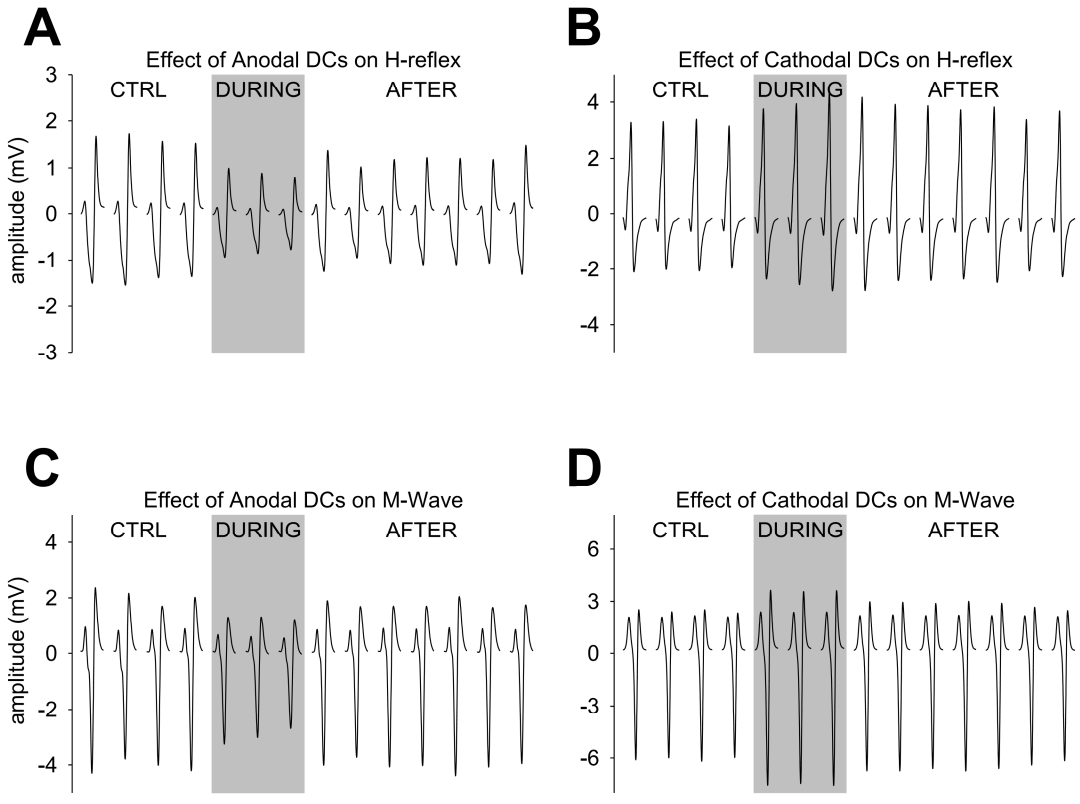

FIG. 2. Effects of DCS on H-reflex and M-wave responses, separately tested in representative subjects. DURING anodal DCS (D1…D3), the reflex amplitude decreased with respect to its average value in the control period (CTRL, C1…C4). Such decrease was still evident AFTER the end of DCS (A1…A7). Cathodal DCS exerted opposite effects (B). Similar results were observed also on M-waves (C, D).

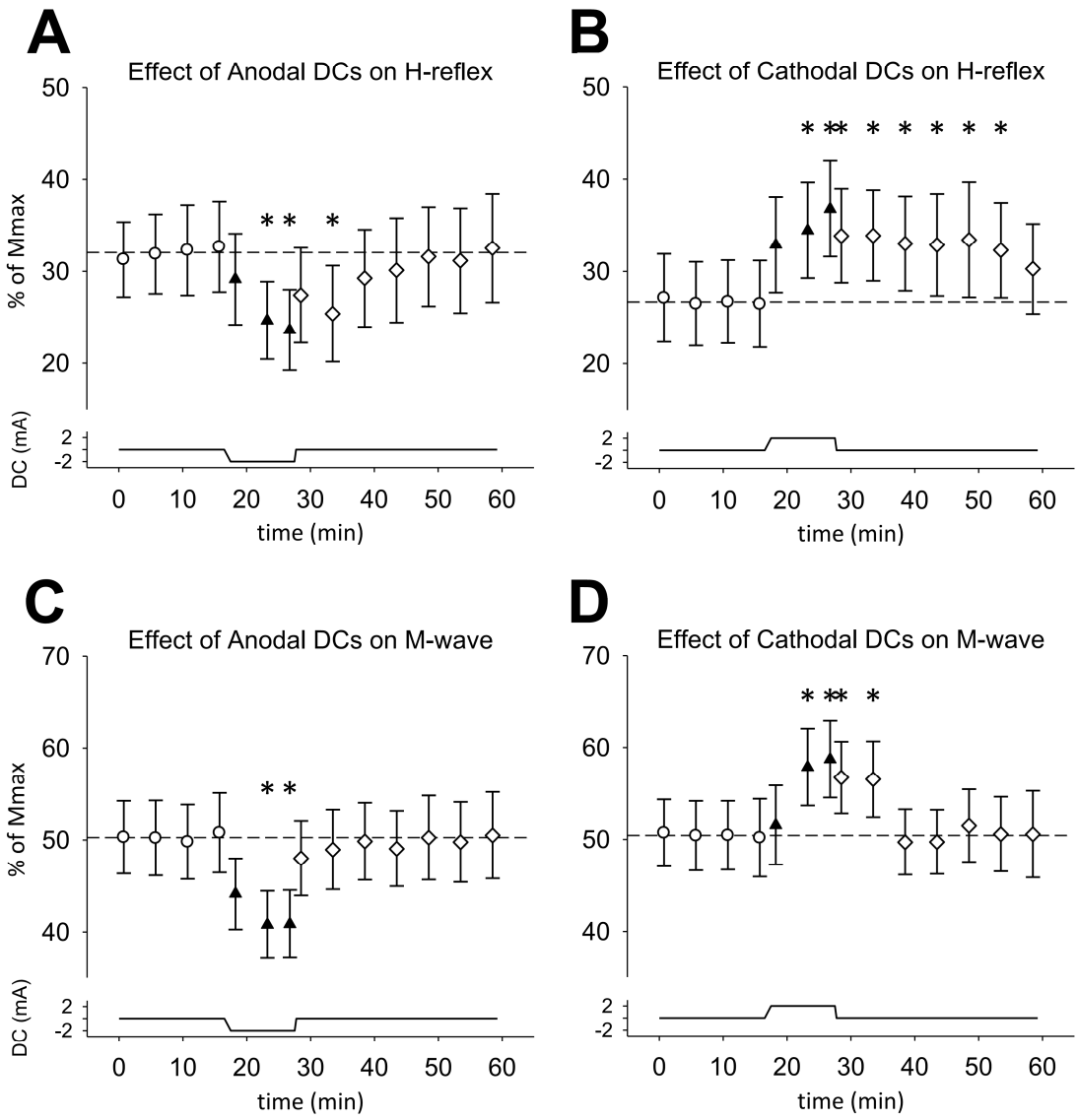

FIG. 3. Effects of DCS on H-reflex and M-wave responses in the whole population (Mean peak-to-peak amplitude ± SE). During anodal DCS (A, filled triangles) the H-reflex peak-to-peak amplitude was significantly reduced with respect to the average amplitude before DCS (open circles). Such decrease lasted for more than 5 min after DCS was switched-off (open diamonds). The opposite effect occurred with cathodal DCS (B); note, however, the longer duration of the after-effect. DCS application also exerted similar results on the M-wave (C, D) although with a much shorted after-effect. Solid line under each panel depicts the time-course of the conditioning DC. * = $P < 0.05$ with respect to the average amplitude before DCS (dashed line).

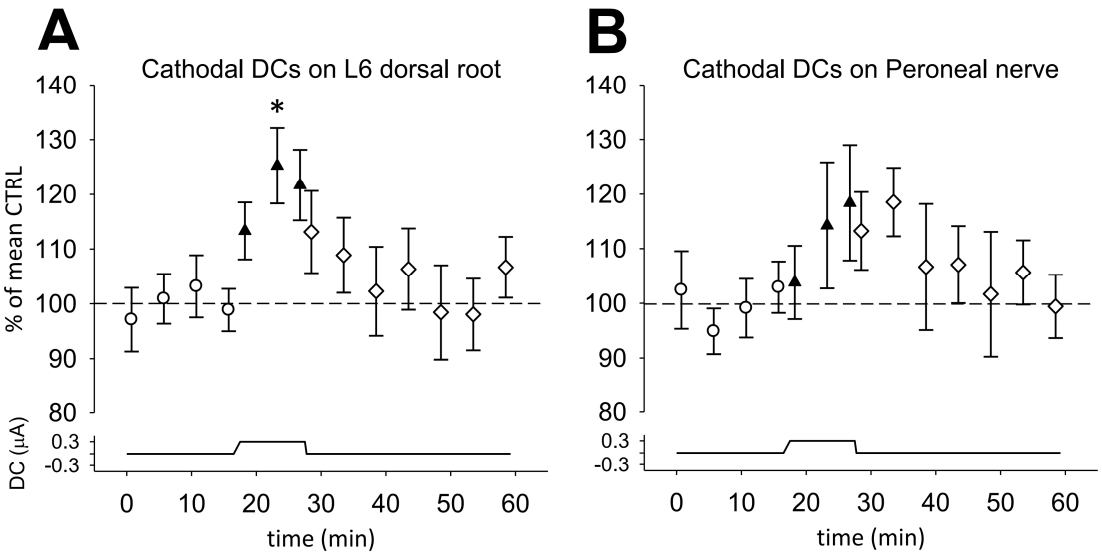

FIG. 4. Effects of DCS on the excitability of nerve fibres in L6 dorsal root and in Peroneal nerve in rats. Mean peak-to-peak amplitude ± SE against time, before (CTRL, open circles), DURING (filled triangles) and AFTER (open diamonds) DCS. Animal experiments confirm that cathodal DCS increases the axonal excitability of sensory fibres (L6 dorsal root, A), as well as the excitability of both sensory and motor fibres (Peroneal nerve, B). Although not significant, a trend for a brief after-effect occurred in both cases. The solid line underneath each panel depicts the time-course of the conditioning DC. * = $P < 0.05$ with respect to the average amplitude before DCS (dashed line).