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ORIGINAL ARTICLE

A 15-minute session of direct current stimulation does not produce lasting changes in axonal excitability

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Anodal/cathodal current;
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Summary
Objective. — To assess the applicability and lasting effects on axonal membrane excitability of transcutaneous peripheral nerve direct current stimulation (pDCS).
Methods. — We included 15 healthy subjects. pDCS was performed with the active electrode placed over the left forearm and the reference electrode on the back of the hand. We used 5 × 5 cm rubber electrodes and the current applied was 2.5 mA during 15 minutes. Three pDCS sessions were performed on the same day with a 20-minute interval between them: first a sham stimulation, followed by cathodal and anodal stimulations in random order. Motor nerve excitability measurements were performed immediately after each pDCS session using the TRONDNF nerve excitability protocol of the QTRAC program.
Results. — The protocol was completed and well tolerated in all subjects. There were no consistent significant differences in excitability measurements between the three sessions.
Conclusions. — No consistent long-lasting effects were noted on peripheral nerve excitability beyond the period of application of pDCS. We showed that a 15-minute session of DCS is not able to produce lasting changes in axonal excitability, supporting the hypothesis that the functional and clinical impact of DCS protocols applied to the central nervous system is related to long-term synaptic changes rather sustained local changes in axonal rest membrane potential.

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Introduction

Low-intensity direct currents produce polarizing effects on axonal membrane potential that can be used as a method of transcranial or transspinal neuromodulation of the central nervous system for physiological or therapeutic applications in humans [1,8,10].

In the last 20 years there has been a renewed interest in potential applications of transcranial direct current stimulation (tDCS) and transcerebellar or spinal DCS [5,11,13,15].

Transcranial direct current stimulation (tDCS) consists of placing two electrodes in pre-determined positions on the scalp over the target area of the cerebral cortex, and applying a weak polarizing current which is unable to generate rapid neuronal depolarizations needed for the induction of an action potential, but capable of inducing changes in the resting membrane potential of the underlying neurons; that is, producing subthreshold currents with a neuromodulatory effect [8,16].

Current density (dependent on current strength and electrode size), stimulus duration, orientation of the electric field (related to electrode position and polarity) and number of sessions are important to determine the effect of tDCS [1,17]. Several animal and human experiments have revealed that anodal stimulation results in subthreshold depolarization (excitability increase), while cathodal stimulation hyperpolarizes neuronal membranes (excitability reduction) [1]. The usual current density delivered by tDCS is between 0.029 and 0.08 mA/cm^2 [10,18,19].

Although there are several studies regarding the modulatory effect of transcranial cerebellar DCS and transcutaneous spinal DCS in terms of modifications of the original tDCS technique, so far there have been no consistent studies on the possible after-effects of long lasting transcutaneous DCS on the axonal membrane properties of peripheral nerves, especially the possibility of inducing lasting excitability changes using such currents. Other studies have assessed the effect of small depolarizing and hyperpolarizing DC currents applied to the stimulating electrodes over the median nerve; however they tended to focus on changes occurring only during the application of the DC currents [6,14]. Using threshold tracking techniques, it has been shown that depolarizing currents increase axonal excitability, reduce inward rectification, and create a 'fanning-in' effect on threshold electrotonus (indicating the activation of internodal K+ channels). On the other hand, hyperpolarizing DC currents increase the activation of inward rectifying K+ channels, and also reduce the conductance of the internodal membrane, creating a 'fanning-out' effect on threshold electrotonus [6].

Thus, the main purpose of this study was to assess the long-lasting effects on axonal membrane excitability by transcutaneous peripheral nerve stimulation with direct currents (pDCS).

Methods

Subject selection

We included 15 healthy subjects of different ages and both genders. Only subjects without clinical or electrophysiological evidence of peripheral nerve disorders and not taking drugs that could affect nerve excitability were selected for the study. Both pDCS and threshold tracking studies were performed on the left arm of every subject.

Peripheral nerve direct current stimulation (pDCS)

The authors hypothesized that by setting up an active DCS electrode over the distal forearm (the distance between the center of the electrode and the center of the stimulating and recording electrodes used in the threshold tracking studies was 4 and 6 cm, respectively, as shown on Fig. 1) and a reference electrode located at a distance, on the back of the hand, we could generate a current density focused on the distal part of the median nerve. Two 5×5 cm rubber electrodes were used and a large amount of gel was applied between skin and electrode surface to maintain a low impedance. The reference electrode was placed on the back of the left hand and the active electrode placed distally on the forearm, 5 cm proximal to the wrist crease. We used a current setting of 2.5 mA during 15 min, which was chosen arbitrarily within previous safety parameters described. Three different settings were used, which reflected the polarity change in the active electrode: sham pDCS, cathodal current and anodal current.

Peripheral nerve excitability assessment — Threshold tracking

Excitability studies were performed according to previously published standard protocols [2—4,7]. Every subject was seated in a relaxed position, with the left arm resting on a pillow, and when needed, heated either by a heater device or a hot water bag placed underneath the left arm. The temperature was kept at a minimum of 31 °C during the procedure, with regular checks after each step of the protocol. Whenever the temperature dropped below the established minimum, the test was interrupted and the hand warmed again [4]. Excitability measurements were performed using the TRONDNF nerve excitability protocol of the QTRAC program (Professor Hugh Bostock, Institute of Neurology, Queen Square, London, UK). The EMG signal was recorded through a D440-2 — Two Channel Isolated Amplifier (Digitimer, Welwyn Garden City, UK) connected to a NeuroLog System (Digitimer, Welwyn Garden City, UK) and filtered between 2 Hz and 10 kHz. The active electrode was placed overlying the motor point of the abductor pollicis brevis (previously mapped with a conventional nerve conduction study) and the reference on the proximal phalanx of the first finger (20 mm diameter disk, E. K50430-001, Digitimer, Welwyn Garden City, UK). Stimulus waveforms were generated by the test computer and converted to current by a DS-5 isolated linear bipolar constant-current source (Digitimer, Welwyn Garden City, UK) with a maximal output $\pm$ 50 mA. The stimulus currents were applied via nonpolarizable electrodes (20 mm diameter disk, E.K50430-001, Digitimer, Welwyn Garden City, UK) with the active electrode over the nerve at the wrist and the reference electrode $\sim$ 10 cm proximal at the medial region of the forearm. The amplitude of the compound muscle action potential (CMAP) was measured from baseline to negative

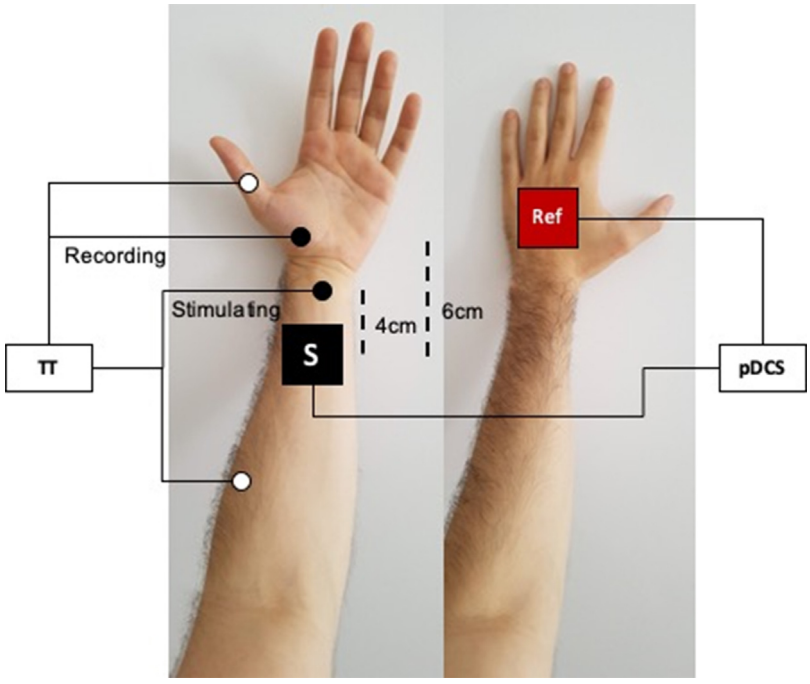

Figure 1 Electrode setup used for pDCS and threshold tracking studies. TT: threshold tracking; S: stimulating electrode; Ref: reference electrode.

peak. For all tracking studies, the target CMAP was set to 40% of the peak response [2—4,7,12].

The overall excitability variables used are described below.

Test sequence

Three different pDCS sessions were tested on each subject. The first stimulation was always the sham stimulation, and the order of the remaining ones (cathodal or anodal) was randomized and blinded to the investigator (AC) and subjects. After each stimulation protocol there was a resting period of at least 20 minutes, to avoid carry-on effects. Threshold tracking studies were performed at the beginning of the protocol and immediately after each pDCS session. Each threshold tracking study had a duration of 11 to 12 minutes.

Variables and statistical analysis

Using previously published protocols [2—4,7,12], we obtained the following excitability variables:

- strength-duration time constant (SDTC), inferred from the relationship between threshold current and stimulus duration; and Rheobase, the threshold for a current of infinitely long duration. Both (SDTC and rheobase) are calculated by measuring threshold for stimuli from 0.2 to 1 ms and plotting stimulus charge versus duration;
- threshold electrotonus (TE), which measures the threshold changes produced by subthreshold depolarizing or hyperpolarizing currents of 100ms duration and 20% and 40% (depolarizing [TEd]) and —20% and —40% (hyperpolarizing [TEh]) of the control threshold current; subsequently the threshold is tested at different time points during and after the polarizing currents;
- recovery cycle, which is investigated by a double stimulation technique where a supramaximal conditioning stimulus is followed by a submaximal test stimulus, with a variable interstimulus interval (2 to 200 ms), to evaluate the refractory, supernormal and late subnormal periods;
- current-threshold relationship (I/V), which describes the maximal extent of threshold changes from 200ms polarizing currents, with a strength from + 50 to —100% of the resting threshold current.

Using these variables, the following excitability parameters were used for statistical analysis:

- stimulus-response and Strength-duration parameters:
  - peak CMAP (mV),
  - stimulus for 50% CMAP (mA),
  - stimulus response/slope,
  - rheobase (mA),
  - SDTC (ms);
- current-threshold relationship—I/V parameters:
  - resting I/V slope,
  - minimum I/V slope,
  - hyperpolarizing I/V slope;
- threshold electrotonus (TE) parameters:
  - TE d (peak),
  - TE d20 (peak),
  - accommodation half time (ms),
  - TE d (90—100 ms),
  - TE h (90—100 ms),
  - TE d (undershoot);

- recovery-cycle parameters:
  - relative refractory period (RRP) (ms),
  - refractoriness at 2.5 ms (%),
  - superexcitability (%),
  - subexcitability (%).

Changes in the excitability measures across the three stimulation conditions (sham, cathodal and anodal) were searched for using Friedman test (given non-normal distribution). For post-hoc testing to find differences between groups, when relevant, Dunn's multiple comparison test were used, according to non-normal distribution. Significance was set at $P < 0.05$, after correcting for multiple comparisons. Analysis was performed using IBM SPSS version 23.0 (IBM, Armonk, NY, USA).

Considering the reliability of threshold tracking studies, we hypothesized that any changes in excitability measurements found during the first set of tests should remain consistent when repeating the protocol after a time gap. For this reason we randomly recruited 5 controls who agreed to undergo a second assessment using sham and cathodal pDCS (> 1 month after the first assessment to allow for a full 'washout' of the pDCS effect).

The study protocol was approved by the ''Centro Académico de Medicina'' Ethics' Committee.

Results

The protocol was completed and well tolerated in all controls. Main side-effects reported were redness of the skin under the stimulating electrodes and direct current electrodes. There were 6 (40%) males and median age was 37 (ranging from 21 to 64 years). There was some variability among subjects with regards to temperature (31—34 °C) although it was kept constant across tests in each subject. Excitability indices and plots are represented in Table 1 and Fig. 2, respectively. Five subjects were submitted to sham-cathodal-anodal trial order, and the remaining 10 to sham-anodal-cathodal trial order.

All excitability measures were tested for normality and given the non-normal distribution, further analysis was performed using the Friedman test and Dunn's multiple comparisons post-hoc test. There was a statistically significant difference among the three experiment protocols in regards to peak CMAP (mV), stimulus for 50% CMAP (mA), and RRP (relative refractory period). Post-hoc tests revealed that the values for peak CMAP and threshold were lower in active pDCS protocols when compared with sham protocol. On the other hand, the value for RRP was higher in anodal protocol when compared with sham ($P = 0.029$) in post-hoc tests. Among all other variables there were no statistically significant differences.

The repeated protocol performed in five random controls did not confirm any significant change regarding the measured excitability variables.

Discussion

Human studies on tDCS have concluded that the modulation of resting membrane potential results in stimulation polarity-dependent excitability and activity changes, where in cathodal stimulation decreases cortical excitability, as

Table 1 Excitability parameters across all study conditions. Statistical analysis was performed using repeated measures Friedman test ($\alpha = 0.05$).

	Sham (mean ± sd)	Cathodal (mean ± sd)	Anodal (mean ± sd)	P^*
Excitability parameter				
Peak CMAP (mV)	4.44 ± 1.07	4.36 ± 1.24	4.25 ± 1.38	0.031
Stimulus for 50% CMAP (mA)	4.14 ± 1.12	4.05 ± 1.35	3.70 ± 1.06	0.034
Stimulus response/slope	4.84 ± 0.90	4.73 ± 0.90	4.81 ± 0.89	n.s.
Rheobase (mA)	2.86 ± 0.80	2.78 ± 0.95	2.59 ± 0.82	n.s.
Strength duration time constant (ms)	0.40 ± 0.09	0.41 ± 0.10	0.41 ± 0.11	n.s.
I/V parameters				
Resting I/V slope	0.56 ± 0.17	0.62 ± 0.10	0.61 ± 0.09	n.s.
Minimum I/V slope	0.30 ± 0.26	0.24 ± 0.03	0.24 ± 0.03	n.s.
Hyperpolarizing I/V slope	0.40 ± 0.24	0.35 ± 0.06	0.34 ± 0.04	n.s.
Threshold Electrotonus parameters				
TE d (peak)	68.34 ± 4.58	66.16 ± 6.50	66.77 ± 5.99	n.s.
TE d20 (peak)	39.59 ± 3.70	38.47 ± 4.53	38.40 ± 5.28	n.s.
Accommodation half time (ms)	41.67 ± 5.81	41.58 ± 5.20	41.27 ± 5.71	n.s.
TE d (90—100 ms)	45.81 ± 3.37	44.32 ± 4.09	44.44 ± 3.82	n.s.
TE h (90—100 ms)	−123.44 ± 14.62	−117.23 ± 19.27	−119.63 ± 17.05	n.s.
TE d (undershoot)	−17.89 ± 3.11	−17.98 ± 4.24	−18.66 ± 4.24	n.s.
Recovery Cycle parameters				
RRP (ms)	2.97 ± 0.30	3.00 ± 0.18	3.02 ± 0.23	0.031
Refractoriness at 2.5 ms (%)	34.09 ± 65.86	38.22 ± 67.14	21.05 ± 14.53	n.s.
Superexcitability (%)	−26.56 ± 5.75	−25.62 ± 6.67	−25.58 ± 6.19	n.s.
Subexcitability (%)	13.16 ± 5.93	12.78 ± 5.42	15.36 ± 5.55	n.s.

* Although the mean values ± standard deviation are represented, the *P*-values refer to Friedman test ($\alpha = 0.05$).

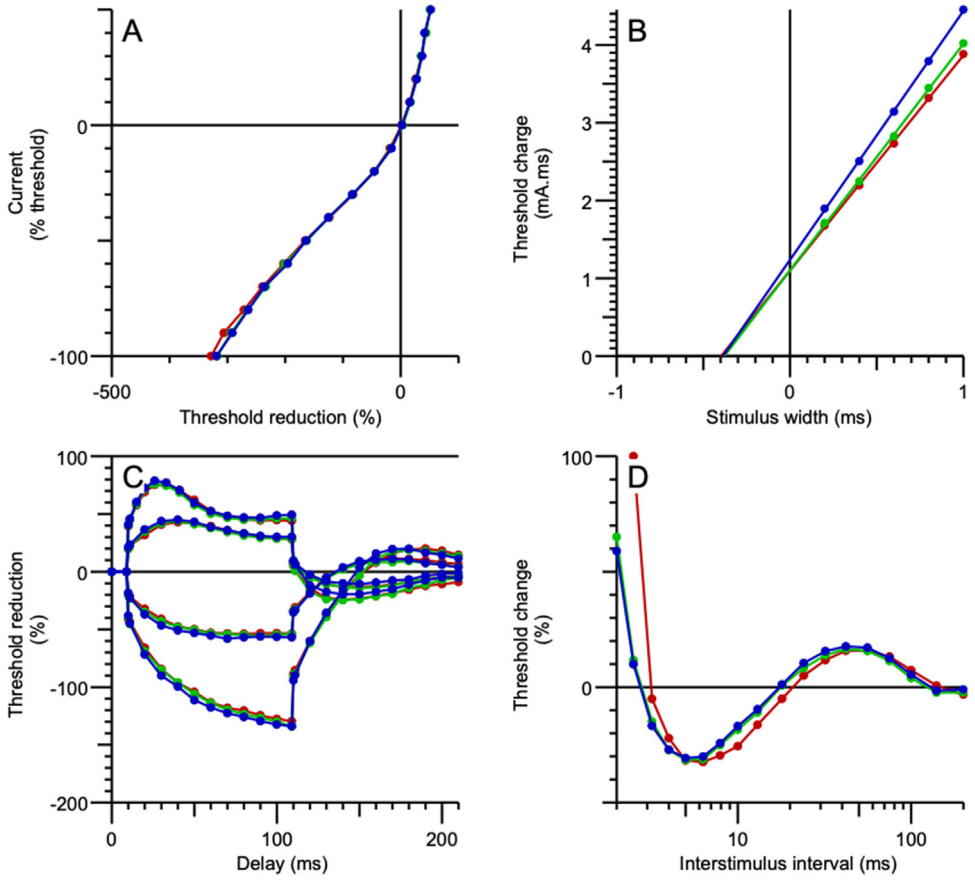

Figure 2 Example of excitability changes across the three trial conditions in one subject (blue = sham; green = anodal; red = cathodal current).

opposed to anodal stimulation which increases it [1]. The protocol we presented is not only feasible but safe, as only minor skin changes were reported by the participants. Our initial findings suggested significantly lower thresholds (stimulus for 50% CMAP) after active pDCS, which could represent an overall increase in excitability. On the other hand, we found that the relative refractory period (RRP) was significantly longer using anodal pDCS (2.97 ms versus 3.02 ms). The RRP represents a transient period of refractoriness of the axon during which transient sodium channels gradually recover from inactivation, meaning a stronger stimulus is needed to generate an action potential. It is sensitive to changes in membrane potential, as sodium channels are inactivated by depolarization and recover with hyperpolarization, and higher RRP values reflect longer membrane depolarization [12]. It seems tempting to suggest that the use of an anodal current depolarized the membrane enough to increase the refractory period, and thus decrease the excitability of the axon. However, the magnitude of change, albeit statistically significant, amounts to 0.05 ms which is probably negligible. On the other hand, this finding seems to contradict the established effects of anodal tDCS on cortical excitability (which increases with anodal stimulation). The absence of other excitability changes also argues against a true effect on membrane potential. When the protocol was repeated several weeks later in the same subjects, those findings were not confirmed, which argues against a real effect on membrane potential or ion channel expression.

Several limitations of our study should be pointed out. Firstly, we included a small sample of subjects. Secondly, we hypothesized that the applied setup was effective in modulating axonal membrane excitability. We assumed that the duration of stimulation and current intensity was sufficient to generate excitability changes, although we cannot be certain of this. It would be important to generate a computational current density model and adjust the setup accordingly [9]. The authors believe that further studies should investigate the effect of different current settings on motor and sensory nerve fibers, both in healthy controls and patients with peripheral nerve disorders. In addition, it would certainly be of interest to assess the effect on axons with different thresholds, as well as expanding the excitability assessment to slower acting K+ rectifying channels.

The negative results of this study suggest that the long-lasting neuromodulatory effect of tDCS on central neurons is not replicated when DC currents are applied on the peripheral nerve. Consequently, it seems plausible that for explaining lasting brain or spinal modulatory effects, synaptic changes (such as polarity-dependent modification of NMDA receptor functions) are involved, because inducing local persistent changes of axonal polarization seems infective [1].

In conclusion, although pDCS is a safe technique to stimulate the peripheral nerve, using the protocol applied in this study the authors were unable to demonstrate any significant and lasting effect on peripheral motor nerve excitability. Modulation of peripheral nerve excitability could be a promising approach to treat neuropathic pain and other clinical manifestations of nerve disease. Different current intensities and/or duration as well as sensory nerves should be explored in future studies.

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Disclosure of interest

The authors declare that they have no competing interest.

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