

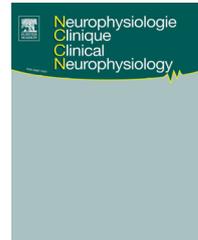


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ORIGINAL ARTICLE

# Modulation of sensory nerve fiber excitability by transcutaneous cathodal direct current stimulation



André Caetano<sup>a,b,\*</sup>, Pedro Pereira<sup>c</sup>, Mariana Pereira<sup>a</sup>,  
Mamede de Carvalho<sup>a,d</sup>

<sup>a</sup> Instituto de Fisiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

<sup>b</sup> Department of Neurology, Hospital de Egas Moniz, Centro Hospitalar de Lisboa Ocidental, Lisbon, Portugal

<sup>c</sup> Department of Neurology, Hospital Garcia de Orta, Almada, Portugal

<sup>d</sup> Department of Neurosciences, Hospital de Santa Maria, Centro Hospitalar de Lisboa Norte, Lisbon, Portugal

Received 21 September 2019; accepted 8 October 2019

Available online 15 November 2019

## KEYWORDS

Cathodal current;  
Direct current  
stimulation;  
Nerve excitability;  
Threshold tracking;  
Sensory nerves

## Summary

**Objective.** – To assess the lasting effects on sensory nerve membrane excitability of transcutaneous peripheral nerve stimulation with cathodal direct currents (pDCS).

**Methods.** – We performed pDCS in 10 healthy subjects with the active electrode placed over the distal right forearm and the reference electrode on the back of the right hand. We used 5 × 5 cm rubber electrodes and the current applied was 2.5 mA during 15 min. Three pDCS sessions were performed on the same day: first, a baseline stimulation was performed, followed by a sham stimulation and lastly a cathodal stimulation. Median sensory nerve excitability measurements were performed at baseline and immediately after each pDCS session using the TRONDNF nerve excitability protocol of the QTRAC program (measurement on the second finger).

**Results.** – The protocol was completed and well tolerated in all subjects. RRP (relative refractory period) and refractoriness at 2.5ms were significantly different across the three study conditions, with a significant increase of RRP immediately following cathodal stimulation compared with baseline assessment (mean 4.2 versus 5.3,  $P=0.002$ ). Other measurements were not modulated by the intervention. Sham-stimulation did not change axonal excitability.

**Conclusions.** – Cathodal pDCS stimulation increased RRP of sensory fibers, but no other consistent long-lasting effect was observed. This finding might suggest a reduction of sensory fiber excitability induced by cathodal pDCS.

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\* Corresponding author. Institute of Physiology, Faculty of Medicine, University of Lisbon, Av. Professor Egas Moniz, 1649-028 Lisboa, Portugal.

E-mail address: [mamedemg@mail.telepac.pt](mailto:mamedemg@mail.telepac.pt) (A. Caetano).

## Introduction

Neuromodulation using transcranial and spinal direct current stimulation (tDCS) using weak polarizing currents seems capable of inducing changes in the resting membrane potential of underlying neurons [8,13]. Despite several studies regarding the modulatory effect of transcranial DCS, cerebellar DCS and transcutaneous spinal DCS, few studies have assessed the possible after-effects of weak long-lasting DC currents on the axonal membrane of peripheral nerves. A recent study [5] explored immediate after-effects of pDCS on the excitability of peripheral motor axons; the observation of negative results supported the concept that the effect of tDCS on central neurons is probably independent from polarization effect on underlying axons [9]. Nonetheless, the properties of sensory axons are different and it would be relevant to test our protocol in these axons [2].

The main objective of this study was to assess the immediate after-effects of transcutaneous peripheral nerve stimulation with direct currents (pDCS) on sensory nerve axonal membrane excitability.

## Methods

### Subject selection

We included 10 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

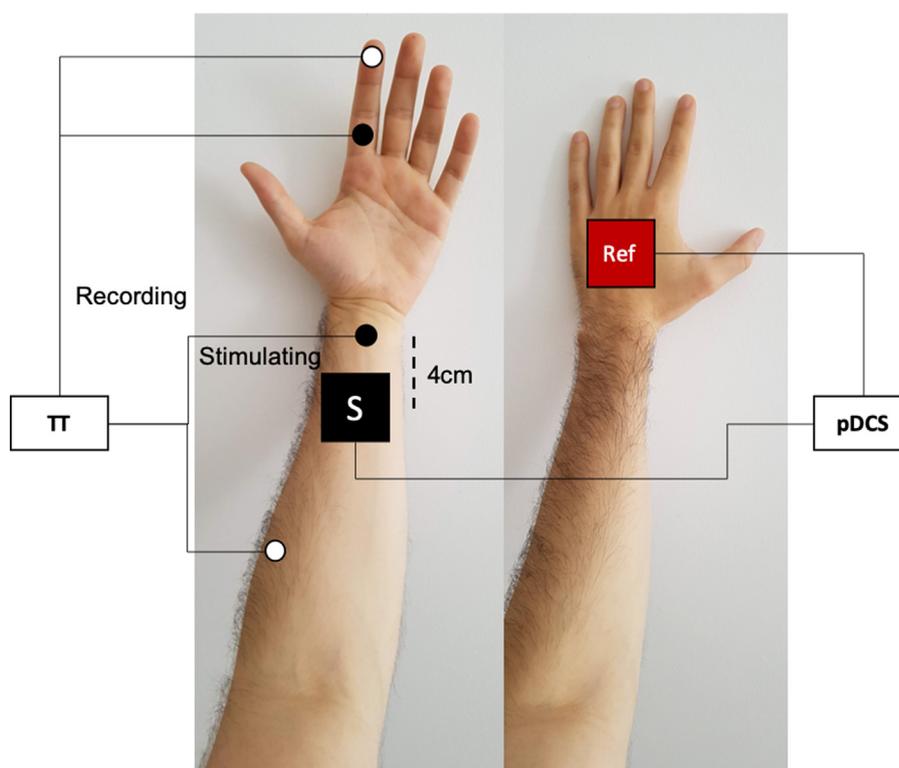
the study. Both pDCS and threshold tracking studies were performed on the right arm of every subject.

### Peripheral nerve direct current stimulation (pDCS)

The authors used a previously published setup for peripheral nerve transcutaneous direct current stimulation - see Fig. 1 of Caetano et al. [5]. The authors used a current setting of 2.5 mA during 15 min which was chosen arbitrarily, considering safety parameters described elsewhere [14]. Three different settings were used, in the same order: baseline assessment, sham pDCS and cathodal current stimulation.

### Peripheral nerve excitability assessment - Threshold tracking

Excitability studies were performed according to previously published standard protocols [3,4,6,7]. Every subject was seated in a relaxed position, with the right arm resting on a pillow, and when needed, heated either by a heater device or a hot water bag placed underneath the right 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 [6]. 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



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

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 proximal phalanx of the second finger and the reference on the distal phalanx of the second 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 lateral region of the forearm. The amplitude of the sensory nerve action potential (SNAP) was measured from baseline to negative peak. For all tracking studies, the target SNAP was set to 40% of the peak response [3,4,6,7,10]. The overall excitability variables used are described below.

### Test sequence

Two different pDCS sessions were tested on each subject. The first stimulation was always the sham stimulation, and the second was always cathodal current stimulation. Threshold tracking studies were performed at the beginning of the protocol (baseline assessment) and immediately after each pDCS session, for a total of three (baseline, after sham, after cathodal) studies for each subject. Each threshold tracking study had a duration of 13 to 14 minutes.

### Variables and statistical analysis

Using previously published protocols [3–7,10], 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 200ms), 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 (90–100ms),
  - TE h (90–100ms),
  - TE d (undershoot).
- Recovery-cycle parameters:
  - relative refractory period (RRP) (ms),
  - refractoriness at 2.5ms (%),
  - supraexcitability (%),
  - subexcitability (%).

Changes in excitability measures across the three stimulation conditions (sham and cathodal) were searched using the Friedman test (given non-normal distribution). For post-hoc testing to find differences between groups, when relevant, Dunn's multiple comparison test was used, according to non-normal distribution. Significance was set at  $P < 0.003$  (Bonferroni Correction). The analysis was performed using IBM SPSS version 23.0 (IBM, Armonk, NY, USA).

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

### 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 4 (40%) males and median age was 31 years (ranging from 21 to 43 years). Excitability indices and plots are represented in Table 1 and Fig. 2, respectively.

All excitability measures were tested for normality; given the small sample, 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 with regards to TE d (peak), RRP (relative refractory period) and refractoriness at 2.5ms. Post-hoc tests revealed that only RRP and refractoriness at 2.5ms (%) were significantly different across the three study conditions (with Bonferroni correction). In particular, RRP following cathodal stimulation was significantly higher when compared with baseline assessment (mean 4.2 versus 5.3,

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

	Baseline (mean $\pm$ sd)	Sham (mean $\pm$ sd)	Cathodal (mean $\pm$ sd)	<i>P</i> <sup>a</sup>
<b>Excitability parameter</b>				
Peak CMAP (mV)	7.4	8.0	7.7	n.s.
Stimulus for 50% CMAP (mA)	5.4	5.2	4.7	n.s.
Stimulus response/slope	2.6	2.9	2.6	n.s.
Rheobase (mA)	2.5	2.4	1.9	n.s.
Strength duration time constant	0.6	0.6	0.6	n.s.
<b>I/V parameters</b>				
Resting I/V slope	0.7	0.5	0.5	n.s.
Minimum I/V slope	0.2	0.2	0.2	n.s.
Hyperpolarizing I/V slope	0.4	0.4	0.3	n.s.
<b>Threshold electrotonus parameters</b>				
TE d (peak)	64.2	60.1	59.8	0.034 <sup>b</sup>
TE d20 (peak)	44.2	43.6	45.2	n.s.
Accommodation half time (ms)	36	35.5	36.2	n.s.
TE d (90–100ms)	51.6	46.4	46.2	n.s.
TE h (90–100ms)	–131.6	–122.4	–126.3	n.s.
TE d (undershoot)	–25.1	–26.6	–27.3	n.s.
<b>Recovery cycle parameters</b>				
RRP (ms)	4.2	4.6	5.3	<b>0.002<sup>c</sup></b>
Refractoriness at 2.5ms (%)	51.4	62.2	72.2	<b>0.004<sup>c</sup></b>
Supraexcitability (%)	–18.3	–18.8	–17.6	n.s.
Subexcitability (%)	12	11.9	11.9	n.s.

<sup>a</sup> Although the mean values  $\pm$  standard deviation are represented, the *P* values refer to the Friedman test. Numbers in bold means significant *P*-values (corrected for multiple comparisons)

<sup>b</sup> Statistical trends.

<sup>c</sup> Significant difference (Bonferroni correction).

$P = 0.002$ ). Sham stimulation did not change the measured excitability parameters.

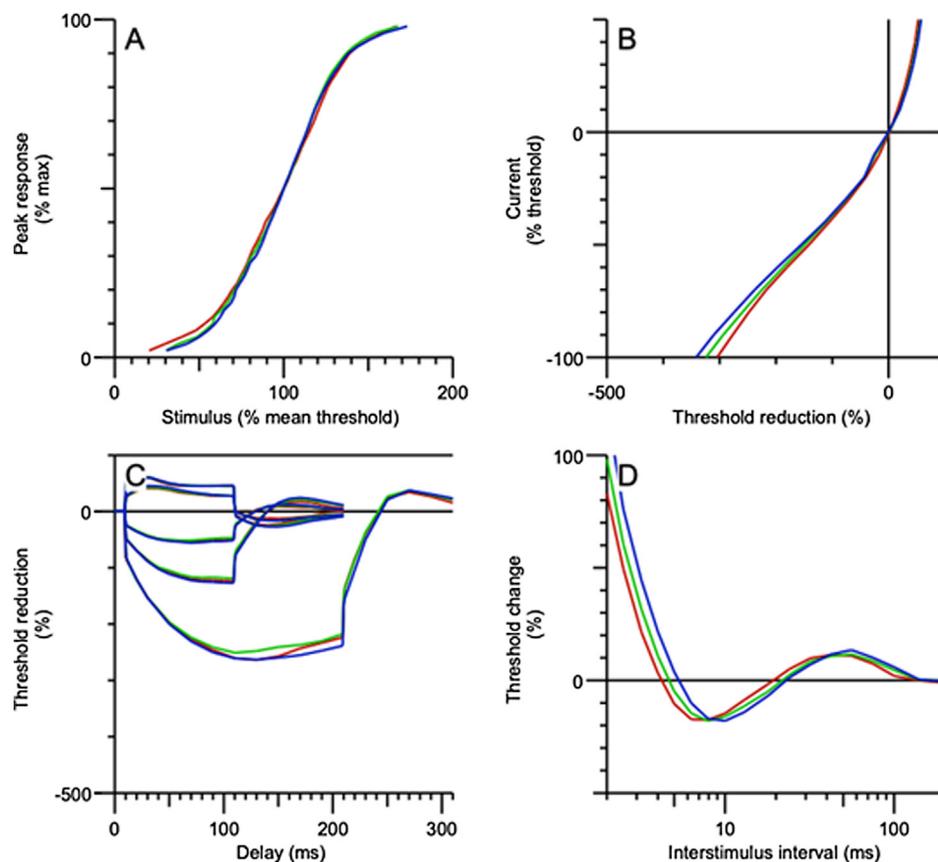
## Discussion

As previously reported [5], the present peripheral nerve stimulation protocol is feasible and safe. Our findings suggest that the relative refractory period (RRP) was significantly longer using cathodal pDCS. The RRP represents a transient period of refractoriness of the axon during which transient sodium channels are gradually recovering from inactivation, meaning that a stronger stimulus is needed to generate an action potential. It is sensitive to changes in membrane potential, since sodium channels are inactivated by depolarization and recover with hyperpolarization, and higher RRP values reflect longer membrane depolarization [10]. It seems tempting to suggest that the use of a cathodal current depolarized the membrane enough to increase the refractory period, and thus decrease the excitability of the axon. Still, the absence of other excitability changes also argues against a true effect on membrane potential. A similar peripheral DCS setup used by Bolzoni et al. [1] albeit using the tibial nerve as the study nerve and the variation of maximal peak-to-peak amplitude of the H-wave and M-wave as surrogate measures of excitability, found that cathodal polarization increased their amplitude while anodal polarization reduced it, an effect that carried over for a few minutes (after-effect). They concluded that DCS changed

the excitability of both motor and sensory fibers, and in particular that cathodal DCS increased the excitability of the peroneal nerve, which contradicts our findings. Nonetheless, since the H-reflex depends on the excitability of the motoneuronal pool in the spinal cord, it seems plausible to consider that peripheral DCS can influence spinal cord excitability and not just generate local nerve changes. In fact, not only has long-term synaptic plasticity been proposed as a possible after-effect of transcutaneous DCS [9], previous studies using transspinal DCS have also demonstrated that there are supraspinal after-effects concerning both sensory and motor networks [11,12]. Although threshold tracking studies assess local excitability changes, one cannot fully exclude possible after-effects dependent on plasticity changes at a more central level.

As previously noted using a motor nerve excitability testing protocol [5], it is reasonable to assume that the settings used are not ideal for modulating the peripheral nerve membrane potential, which reinforces the importance of generating a current density model and adjusting the setup accordingly.

Our study has a number of limitations, of which the three most critical are the small number of subjects, absence of anodal stimulation and application of a single protocol for pDCS. Since we observed a number of trends in favor of a depolarizing effect, we propose that the results are promising but require a larger number of subjects to attain a definitive conclusion.



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

In conclusion, pDCS is a safe technique for stimulating peripheral nerves. We were unable to demonstrate significant and lasting effects on peripheral sensory nerve excitability other than an increase in RRP. Modulation of peripheral nerve excitability could be a promising approach to treat neuropathic pain and other clinical manifestations of sensory nerve hyperexcitability, and as such different current intensities and/or duration should be explored in future studies.

## Funding

UID/BIM/50005/2019, project funded by Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through Fundos do Orçamento de Estado.

## Disclosure of interest

The authors declare that they have no competing interest.

## References

[1] Bolzoni F, Esposti R, Bruttini C, Zenoni G, Jankowska E, Cavallari P. 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. *Eur J Neurosci* 2017;46:2499–506.
- [2] Bostock H, Burke D, Hales JP. Differences in behaviour of sensory and motor axons following release of ischaemia. *Brain* 1994;117:225–34.
- [3] Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. *Muscle Nerve* 1998;21:137–58.
- [4] Burke D, Kiernan MC, Bostock H. Excitability of human axons. *Clin Neurophysiol* 2001;112:1575–85.
- [5] Caetano A, Pereira M, de Carvalho M. A 15-minute session of direct current stimulation does not produce lasting changes in axonal excitability. *Neurophysiol Clin* 2019;49:277–82.
- [6] Casanova I, Diaz a, Pinto S, De Carvalho M. Motor excitability measurements: the influence of gender, body mass index, age and temperature in healthy controls. *Neurophysiol Clin* 2014;44:213–8.
- [7] Kiernan MC, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: a new approach in clinical testing. *Muscle Nerve* 2000;23:399–409.
- [8] Lefaucheur JP. Methods of therapeutic cortical stimulation. *Neurophysiol Clin* 2009;39:1–14.
- [9] Lefaucheur JP, Wendling F. Mechanisms of action of tDCS: a brief and practical overview. *Neurophysiol Clin* 2019;49:269–75.
- [10] Nodera H, Kaji R. Nerve excitability testing and its clinical application to neuromuscular diseases. *Clin Neurophysiol* 2006;117:1902–16.

- [11] Schweizer L, Meyer-Frießem CH, Zahn PK, Tegenthoff M, Schmidt-Wilcke T. Transcutaneous spinal direct current stimulation alters resting-state functional connectivity. *Brain Connect* 2017;7:357–65.
- [12] Song W, Truong DQ, Bikson M, Martin JH. Transspinal direct current stimulation immediately modifies motor cortex sensorimotor maps. *J Neurophysiol* 2015;113:2801–11.
- [13] Terzuolo CA, Bullock TH. Polarization by imposed voltage gradient adequate to modulate neuronal firing. *Proc Natl Acad Sci U S A* 1956;42:687–94.
- [14] Woods AJ, Antal A, Bikson M, Boggio PS, Brunoni AR, Celnik P, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin Neurophysiol* 2016;127:1031–48.