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NEUROPHYSIOLOGICAL BASIS OF A NEW ELECTRODE CONFIGURATION TO POTENTIATE THE tDCS: PROTOCOLS FOR UPPER LIMBS

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ABSTRACT | Transcranial Direct Current Stimulation (tDCS) uses a direct electrical current to modulate the activity of cortical neurons. Anodal tDCS (positive pole) increases the excitability of cortical neurons, while cathodic tDCS (negative pole) reduces it. However, when applied in the peripheral nervous system the effects are the opposite of cranial application. Furthermore, when central and peripheral stimuli are used concomitantly, their effects can be summed up. This has been demonstrated by combining tDCS with other forms of sensory peripheral stimulation. We propose a new electrode configuration to potentiate the excitatory and inhibitory effects of tDCS on neuronal excitability and increase upper limb motor function. Our hypothesis is that placement of the electrodes in the primary motor cortex (M1) and the contralateral brachial plexus (BP) would promote this potentiation by central and peripheral synaptic summation. We will test our hypothesis in two proof-of-concept studies. Study 1) Secondary trial, in which we will evaluate the effects of these configurations on the neuronal excitability of healthy individuals; Study 2) A double-blind, randomized and crossover clinical trial in which we will test the stimulation with the anode in M1 and the cathode in the contralateral BP on the motor function and electrophysiological markers of individuals with cerebral palsy. The effects of the new configurations will be compared with the conventional configuration (M1/ contralateral supraorbital region). We expect that our investigations will identify a more efficient way to apply tDCS and consequently a better clinical use of this technique.

Key words: tDCS, TMS, M1, Brachial plexus, Cerebral palsy

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INTRODUCTION

Transcranial direct current stimulation (tDCS) is a low cost and easy to use therapeutic tool that promotes short and long term changes in the behavior of neurons[1,2]. Interestingly, some of these changes in neuronal behavior are correlated with improvement of clinical symptoms in sensorimotor, emotional, and cognitive disorders[3–6]. On the other hand, a substantial portion of individuals are refractory and/or resistant to tDCS[3,7].

Strategies to enhance the therapeutic effects of tDCS have been proposed mainly by combination with other interventions[8,9]. In fact, the association of tDCS with peripheral nerve electrical stimulation, aerobic exercise or conventional physiotherapy improves pain and motor function in a more pronounced way than these interventions *per se*[8–13]. These results have been interpreted on the basis of the meta plasticity phenomenon[14]. Accordingly, the direction of the modulatory effect of the electrical stimulation is influenced by the previous neuronal activity[14,15].

Association of excitatory and inhibitory stimulation (central and peripheral) potentiates the tDCS effects, probably by making the state of neuronal activity more susceptible to the modulatory effect of stimulation. Despite this, the therapeutic potential of tDCS has not been fully explored and requires further research to establish more efficient protocols and therapeutic combinations[16,17].

The direction of the effects of tDCS is dependent on polarity[18–20]. The population of neurons below the positive electrode (anode) usually increases their excitability/activity[21–24]. These effects are similar in magnitude and inverted with respect to the negative electrode (cathode). On the other hand, the effects of direct current stimulation (DCS) on peripheral neuronal excitability are reversed (cathode: excitatory and anode: inhibitory)[25,26].

These differences occur mainly due to morphological and functional characteristics between cortical and peripheral neuronal circuits. The basic biological effects induced by DCS clearly influence neuronal behavior depending on the neuronal compartment directly affected by the electric field[27,28]. In fact, in cortical circuits the anodal tDCS increases the excitatory neurotransmission and hyperpolarizes the apical dendrites of pyramidal neurons[17,18]. The resultant of this balance, more often makes these neuronal circuits more excited and/or excitable. On the other hand, in the periphery the electric field acts directly on axons[25,26]. Possibly, in peripheral neurons the balance of the attraction of ânions (-) by anodal DCS (+) makes the neuronal membrane more hyperpolarized. The rationale for the effects of transcranial and peripheral cathodal stimulation are similar but in opposite directions (excitation).

tDCS in the primary motor cortex (M1) has been explored for the treatment of pain and to increase motor function in several populations[3]. A large population of M1 neurons forms the lateral cortical-spinal tract, which controls voluntary movements of the appendicular skeleton[29,30]. The activity of these neurons is regulated by an excitation/inhibition balance from various cortical and subcortical structures that integrate sensory information and motor planning[31]. The end-point of the activity of these neurons is to control α-motoneuron activity in the anterior horn of the spinal cord. We propose a protocol that enhances the therapeutic effects of tDCS and its electrophysiological markers by the summation of excitatory and inhibitory, peripheral and central effects (without requiring two separate stimulators). Therefore, our hypothesis is that the placement of the anodal electrode on the M1 of the dominant hemisphere and the cathodic in the contralateral brachial plexus (BP) promote a synergism of the central and peripheral excitatory effects. The inverted configuration may produce similar inhibitory effects. The clinical use of these protocols will depend on the results of basic and clinical investigations, as well as whether the desired effect will be inhibitory or excitatory.

MATERIALS AND METHODS

Study design, allocation description and blinding

The effectiveness of these tDCS configurations will be tested in two proof-of-concept studies. Study

1) A secondary trial, we will compare the effects of proposed new configurations with conventional (M1/contralateral supraorbital region) on motor-cortical excitability, spinal reflexes and peripheral excitability of healthy individuals. Study 2) A double-blind, randomized and crossover clinical trial. In study 2 we will investigate the effect of the anodal tDCS on the M1 and cathodic current on the contralateral BP on the motor function of individuals with hemiplegic cerebral palsy.

The randomization will be performed with a virtual online tool (www.randomization.com). An assistant researcher who will not participate in the study will generate an allocation sheet in 7 groups in study 1 and 5 groups in study 2. Allocation of subjects will be concealed with sealed envelopes, listed in ascending order and kept hidden from the evaluator and volunteers until the end of data collection. The allocation envelope will be opened on the day of data collection according to the order of inclusion of the participant in the study.

Eligibility criteria

Study 1:
Inclusion - Male individuals aged between 18 and 49 years.
Non-inclusion - Non-literate, individuals with metal implants who have systemic arterial hypertension, diabetes mellitus or any neurological and/or psychiatric disorder. Individuals on medication with a central nervous system effect (including medication for sleep disorders).

Study 2:
Inclusion - Individuals aged between 6 and 16 years and confirmed diagnosis of hemiplegic spastic cerebral palsy.
Non-Inclusion - Individuals who have experienced epileptic seizures at some point in their lives or who have other neurological conditions. In use of medications with known action on central nervous system (including medication for sleep disorders) that have received botulinum toxin application within the past three months or are unable to understand the commands for performing the functional tests.

Discontinuity criterion

There will be discontinuity if:

A) The individual does not want to continue data collection due to discomfort or intolerances to the intervention and/or evaluation tools. Still, if the evaluator perceives any adverse effects.

B) If the participant withdraws consent at any stage of the study.

C) Occurrence of events that may interfere in the results: epileptic seizures, cranioencephalic trauma, orthopedic trauma or nervous system infections, and modification in the approach of neurorehabilitation in progress.

Strategies to improve adherence to the intervention protocol:
Study participants will receive transportation assistance to and from the research laboratory as well as a meal voucher. At the end of study 2, all participants will receive treatment with the setting that showed the best clinical effect.

Study configuration and participant schedule

Participants with CP enrolled in the basic health units of the Neuroscience Ambulatory of the Complex HUPES - Salvador - Bahia. All pre and post evaluation procedures will be performed on the same day and the data collection will be performed at the Health and Functional Studies Center (NESF) of the Health Sciences Institute of the Federal University of Bahia.

Measurement of results

Electrophysiological measures

Motor-cortical excitability

The cortico-motor excitability will be assessed by means of motor evoked potentials (MEPs) using a BiStim transcranial magnetic stimulation (TMS) device (Magstim Co. Dyfed, UK).

To identify the hot-spot, the coil will be positioned at $\pm$ 45 $^{\circ}$ (relative to the median sagittal plane) and pulses applied in steps of 1 cm until the largest amplitude

of MEP is achieved. Before starting MEP recordings, the motor threshold at rest will be determined using a software with a motor threshold assessment tool (MTAT 2.0; Clinical Researcher, Knoxville, TN, USA). MEP recordings will be performed with stimuli at an intensity of 120% of the resting motor threshold. Inhibition of short latency (ISL) and intracortical facilitation (ICF) will be evaluated by the application of paired pulses paradigm. Inter-pulse intervals of 2 milliseconds preferentially recruit GABAergic inhibitory neurotransmission, via GABAA receptors. Inter pulse intervals of 15 milliseconds preferentially recruit glutamatergic excitatory neurotransmission via AMPA/kainate receptors. The first conditioning pulse will have an intensity of 80% of the resting motor threshold and the second test pulse of 120% of the resting motor threshold (32). We will position two electromyographic (EMG) bipolar electrodes in the muscle belly of the first dorsal interosseous of the right hand. Electromyographic signals will be amplified and filtered with a low-pass filter of 2 kHz and high-pass of 1 Hz (Cambridge electronic design - CED 1902). The signals will be collected at a frequency rate of 4 kHz and transferred to the laboratory computer for posterior offline analyses.

Evaluation of spinal reflexes and peripheral excitability

Measures of the compound muscle action potentials (CMAP), M-wave and H-reflex evoked by brachial plexus stimulation will be used to evaluate spinal reflexes and peripheral excitability. Initially a H-reflex/M-wave recruitment curve will be evaluated with a constant current stimulator, DS7A (NL703, Digitimer, Welwyn Garden City, UK). Squared current pulses lasting 1 ms will be applied to the cutaneous nerve and recorded on the biceps brachii muscle. The peak-to-peak amplitude of the events evoked by peripheral stimulation will be measured. To record the CMAP the stimuli will be delivered at 125%, 150%, 175% and 200% of the motor neuron threshold[25].

Muscle cutaneous reflexes will be evoked by a train of short stimuli (15 ms) applied to the ulnar fossa (five pulses, 200 μs pulse width and 300 Hz, with inter-stimulus intervals of 3 seconds.) The stimulus intensity will be immediately below the nociceptive threshold to evoke a clear EMG response with few events (<10) .The electromyographic signal will be amplified and filtered (bandpass 1 Hz to 2 kHz) (Power1401, 1902 amplifier, CED)[33].

Motor function

In study 2 the motor function of the upper limb of individuals with hemiplegic cerebral palsy will be evaluated.

Jebsen Taylor Manual Function Test

The test is used to evaluate the function of the upper limb from single and bimanual tasks involved in activities of daily living. It consists of seven subtests performed with the dominant and non-dominant hand (stack checkers, simulate feeding, grab small objects, lift light and heavy objects, turn cards). The measurement is based on the time and success of the performed tasks. To enable better analysis of the results, subjects will be filmed during the test.

Determination of the movement amplitude

The subject will be filmed while performing standardized movements of the upper limbs (elbow flexion and extension, wrist flexion and extension), neutralizing the action of gravity with the limb positioned on a table adapted to the subject height. The recording will be performed with the camera positioned above the head.

Measurement of manual grip muscle strength

A measurement of the maximum grip strength of the hands will be performed using a dynamometer.

Experimental procedures

Study 1
Groups
- anodal tDCS in the M1 and cathodic in the contralateral supraorbital region;
- anodal tDCS in the M1 and cathodic in the contralateral brachial plexus;
- sham tDCS in the M1 and in the contralateral brachial plexus.

- cathodic tDCS in the M1 and anodal in the contralateral supraorbital region;
- cathodic tDCS in the M1 and anodal in the contralateral brachial plexus;
- anodal tDCS in the brachial plexus and cathodic in the deltoid muscle;
- cathodic tDCS in the brachial plexus and anodal in the deltoid muscle.

The experimental protocol will consist of four evaluations of cortical and peripheral excitability with transcranial magnetic stimulation (TMS) or peripheral electrical stimulation. Initially, the individual's central and peripheral neuronal excitability will be assessed (baseline recording). After this recording the individual will be submitted to an electrical stimulation protocol. Neuronal excitability will then be recorded immediately, 30 minutes and 60 minutes after the stimulation.

Direct current stimulation protocols will be performed using a specific electrostimulator for this procedure (DCStim, Neurocom, Germany). All experimental groups will be stimulated for 20 minutes with an intensity of 1 mA, with electrodes of 35 cm2.

Study 2
Groups
- anodal tDCS in the M1 and cathodic in the contralateral supraorbital region;
- anodal tDCS in the M1 and cathodic contralateral brachial plexus;
- anodal tDCS in the M1 and cathodic in the contralateral supraorbital region followed by neuromuscular stimulation in elbow and wrist extensors;
- anodal tDCS in the M1 and cathodic in the contralateral brachial plexus followed by neuromuscular stimulation in elbow and wrist extensors;
- sham tDCS of neuromuscular stimulation in elbow and wrist extensors.

The experimental protocol will consist of the evaluation of the motor and electrophysiological function of the individuals for baseline recording. The functional test and the recording of the uniplanar movements with the simultaneous EMG measurements will be performed followed by electrophysiological evaluation of the cortico-motor excitability of spinal reflexes and peripheral excitability. After baseline recordings, one of the electrical stimulation protocols will be performed and motor and electrophysiological function reassessed. After 7 days (washout) the subjects will undergo another intervention according to the previous randomization. All subjects will be submitted to all intervention protocols.

tDCS will be performed using a specific electrostimulator for this procedure (DCStim, Neurocom, Germany). All experimental groups will be stimulated for 30 minutes with an intensity of 2 mA and 35 cm2 electrodes.

Potential for adverse effects and damage

tDCS can generate relatively subtle, self-limiting and short-term adverse effects that include mild tingling sensation, itching, burning and mild pain under the electrodes surface, fatigue and sleepiness. All of these potential adverse effects can be avoided through appropriate training in the management of the technique. In addition, these adverse effects will be monitored with a specific questionnaire. Evaluation with TMS may also evoke adverse reactions. These effects are rare and will be minimized following the protocol of care and the eligibility criteria (by not including individuals at risk). All individuals will be properly informed about these risks at the time of recruitment and declaration of free and informed consent. The NESF is comprised of physiotherapists and physicians and all will be accessible to assist in case of any risk of harm to the participant in this study.

Sample size

The sample calculation was performed using G-Power software using effect size of 0.3, alpha of 5% ($p < 0.05$) and study power of 80%. For the study one an estimated a sample of 133 individuals, with 19 per group was obtained. In study 2 a sample of 12 individuals was estimated. The Kolmogorov-Smirnov test will be applied to the continuous variables for analysis of normality. Continuous descriptive data will be presented in measures of central tendency and dispersion. The categorical data will be presented in relative and absolute frequencies. The comparison between groups will be performed through two-way ANOVA for repeated measures and post-hoc test

for difference detection. The data will be analyzed in SPSS software v.23.

Neurophysiological background

Changes in neuronal excitability in response to tDCS have been evidenced by several experimental approaches[20,34,35]. Accurate imaging and functional assessment techniques for measurements of cortico-motor excitability and pattern of human brain activity allowed identification of mechanisms underlying the therapeutic effect of tDCS[34,36,37].

The magnitude of these effects is dependent upon the time, intensity of the stimulation and electrode size(38). Experimental studies *in vitro* correlated with computational modeling suggest that a part of these effects are mediated by a mechanism of cell membrane polarization[35,39,40]. Accordingly, brain slices containing the hippocampus undergoing anodal stimulation displace the negative charges from the inner face of their membrane to the apical dendrites (closest to the surface). As consequence, the soma and basal dendrites become more depolarized. The result of this balance makes the neuron more likely to trigger an action potential or more frequently spontaneous firing. On the other hand, the cathodic stimulation repels the negative charges of the inner face of the membrane in the apical dendrites, hyperpolarizes the soma and the basal dendrites making the neuron less excitable[20,35,37].

In humans, the mechanisms of changes in neuronal plasticity induced by tDCS have been investigated with pharmacological manipulation[34,41,42]. In fact, blocking voltage-dependent channels for Na+ and Ca2+ inhibited the short and long-term effects of anodal stimulation, without affecting the effects of cathodic stimulation. This suggests that a component of the effects of neuronal hyperexcitability induced by anodal stimulation is dependent on intrinsic membrane properties related to voltage-sensitive channels for Na+ and Ca2+[34,43–45]. In addition, these effects are potentiated by factors related to excitatory neurotransmission. Specifically, NMDA glutamatergic receptor agonists potentiate the effects of long-term anodal stimulation, whereas antagonists produce inverse effects[37]. On the other hand, dopaminergic and cholinergic agonists potentiate the long-term effects of cathodic stimulation[34,35,41].

Although the effects of direct current stimulation directly influence the electrical properties of the membrane, alterations in the neuronal microenvironment may also justify the changes induced in its behavior[46–50]. In fact, migration and conformational change of proteins, alteration in tissue pH and incorporation of cholinergic receptors are some of the biological effects promoted by the application of exogenous continuous currents[25]. An important phenomenon induced by the application of direct current is electrolysis, that can alter acid-base balance, generating alkalosis or acidosis, which markedly alters cellular function. Changes in intracellular Ca2+ concentration have also been associated with direct current stimulation[46–49]. These results demonstrate that although tDCS promotes direct effects on the intrinsic electrical properties and parameters of neurotransmission, it also alters the cellular microenvironment and the molecules that compose it. Accordingly, cathodal stimulation in M1 reduces the amplitude of MEPs. On the other hand, cathodal stimulation of the ulnar nerve reduces amplitudes of CMAPs[25].

α-Motoneuron of the brachial plexus are activated by descending motor pathways related to the control of voluntary[50]. In addition, they are modulated by afferences originating from several somesthetic sensory inputs. The tested DCS protocols explore the measures of excitability related to the region in which the stimulation (peripheral or central) was applied. It is plausible that the direct current stimulation of the M1 and the brachial plexus with inverted polarities can generate a summation of the cortical and peripheral effects, with an increase in the functional result. An alternative hypothesis is that a higher current density reaches the cortical neurons due to the direction of current flow between the electrodes and the greater distance between them.

Direct current stimulation in peripheral and central tissues promotes changes in the excitability of neurons with inverse effects. Interestingly, we did not find in the literature any protocol that proposed the stimulation with transcranial and transcutaneous DC current simultaneously and with inverted polarities in synaptic communication pathways. Therefore, we

hope that our future results may identify a greater therapeutic effect of tDCS for upper limb motor function. Also, identify the electrophysiological markers associated with this effect.

AUTHOR CONTRIBUTIONS

Zugaib J designed the protocol and wrote the manuscript. Gomes IO participated in all phases of the research as undergraduate junior researcher. Baptista AF and Lucena RCS did the critical review and approval of the final draft. Sá KN and Matos MA participated in the critical review.

COMPETING INTERESTS

No financial, legal or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

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