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Brief Communication

Effects of transcranial direct current stimulation coupled with repetitive electrical stimulation on cortical spreading depression

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Abstract

We have recently shown that two techniques of brain stimulation – repetitive electrical stimulation (ES) (that mimics transcranial magnetic stimulation) and transcranial direct current stimulation (tDCS) – modify the velocity of cortical spreading depression (CSD) significantly. Herein we aimed to study the effects of these two techniques combined on CSD. Thirty-two Wistar rats were divided into four groups according to the treatment: sham tDCS/sham ES, sham tDCS/1 Hz ES, anodal tDCS/1 Hz ES, cathodal tDCS/1 Hz ES. Our findings show that 1 Hz ES reduced CSD velocity, and this effect was modified by either anodal or cathodal tDCS. Anodal tDCS induced larger effects than cathodal tDCS. Hereby CSD velocity was actually increased significantly after anodal tDCS/1 Hz ES. Our results show that combining two techniques of brain stimulation can modify significantly the effects of ES alone on cortical excitability as measured by the neurophysiological parameter of cortical spreading depression and therefore provide important insights into the effects of this new approach of brain stimulation on cortical activity.

Keywords: Cortical spreading depression; Cortical electrical stimulation; Transcranial direct current stimulation; Transcranial magnetic stimulation; Wistar rats; Cortical excitability

We have recently shown that cortical stimulation with repetitive electrical stimulation (ES) (a technique that mimics the effects of transcranial magnetic stimulation) and with transcranial direct current stimulation (tDCS) results in a significant change in the velocity of the phenomenon of cortical spreading depression (Fregni et al., 2005a; Liebetanz et al., 2006). Because the interest in the clinical application of brain stimulation has been increasing and recent studies investigating the use of both techniques in combination in humans subjects (Siebner et al., 2004; Lang et al., 2004) showed that priming rTMS treatment with tDCS modifies the effects of rTMS on motor cortex excitability, the further exploration of the neurophysiological effects of this strategy of brain stimulation is warranted. Therefore we aimed to investigate the effects of preconditioning of cortical electrical stimulation with tDCS on an animal model of cortical spreading depression (CSD), comparing the effects of 1 Hz ES (preconditioned with sham tDCS) vs. 1 Hz ES preconditioned with either anodal or cathodal tDCS on the phenomenon of CSD.

In this study, we used CSD as an index of brain function. This phenomenon was first described by Leão (Leao, 1944; Teive et al., 2005) and it is characterized by massive alterations in cerebrocortical ion homeostasis in response to the stimulation of a point of the brain tissue (Somjen, 2005). These alterations result in a wave of neuronal depolarization that propagates at a rate of 2–5 mm min^{-1} across the cortical surface, accompanied by reversible electroencephalogram (EEG) suppression and a negative deflection of the direct current potential (Guedes and Cavalheiro, 1997). CSD has been extensively studied in several conditions of brain function alteration, providing important information about brain activity, therefore being a useful index to study brain function experimentally (Guedes, 1984; Guedes, 2005). Finally, we used repetitive electrical stimulation, because the technical limitations of rTMS in rats result in a diffuse

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stimulation throughout the cortex and is thus not suitable for this study.

Thirty-two male adult Wistar rats (365.5±7.8 g, mean± S.E.M.) were used in this experiment. They were housed individually under controlled temperature and on a 12 h light/dark cycle (lights on at 6 h A.M.) with free access to rat chow pellets and tap water. All rats were cared for and used in strict accordance to the PHS Guide for the Care and Use of Laboratory Animals. Importantly, all efforts were made to minimize the number of animals used and their suffering. All procedures were approved by the Institutional Animal Care and Use Committee of Federal University of Pernambuco State, Brazil, where the experiments were carried out.

The rats were randomized into four different groups according to the stimulation protocols: sham tDCS followed by sham ES (represented by sham tDCS/sham ES), sham tDCS followed by 1 Hz ES (represented by sham tDCS/1 Hz ES), anodal tDCS followed by 1 Hz ES (represented by anodal tDCS/1 Hz ES) and cathodal tDCS followed by 1 Hz ES (represented by cathodal tDCS/1 Hz ES). Initially, tDCS electrodes implantation and baseline CSD recording session were performed. To perform stimulation and CSD recording, rats were anesthetized with a mixture of urethane and chloralose (1.0 g/kg plus 0.04 g/kg IP, respectively; one-third of the initial dose as supplement, if necessary) and placed in a stereotactic frame in a flat-skull position. For CSD recording, two holes (3–4 mm diameter) were drilled manually over the right hemisphere (Fig. 1). They were aligned in the parieto-frontal direction, located either 1 mm posterior to the coronal suture or 1 mm anterior to the lambdoid suture, and parallel to the midline (2 mm lateral to the sagital suture). A small plastic jacket was positioned over the right parietal cortex just between the two boreholes and fixed with a coating of a glass ionomer cement (Ketac Cem, ESPE Dental AG, Seefeld, Germany). The jacket was filled with saline solution (0.9% NaCl) to establish a defined contact area (7 mm^2) and, thus used as one of the electrodes for tDCS. After the tDCS electrode implant, CSD recording, tDCS and ES were performed.

To elicit CSD, we used KCl as this substance is effective in causing a massive neuronal depolarization. Recording was made with two Ag–AgCl agar-Ringer type electrodes—gently placed on the intact dura mater, in each one of the holes situated anterior and posterior to the DC-electrode, respectively. CSD induction was started after 30 min of baseline recording. It was elicited at 20 min intervals by 1 min application of a cotton ball (1–2 mm diameter), soaked in 2% KCl solution, to the anterior hole. The cortical spontaneous electrical activity (electrocorticogram) and the slow potential change accompanying CSD were continuously recorded for 80 min against a common reference electrode of the same type, placed on the nasal bones (Fig. 1). CSD velocity of propagation was indexed by the velocity of propagation of the slow potential change and calculated from the time required for a CSD wave to pass the distance between the two cortical electrodes.

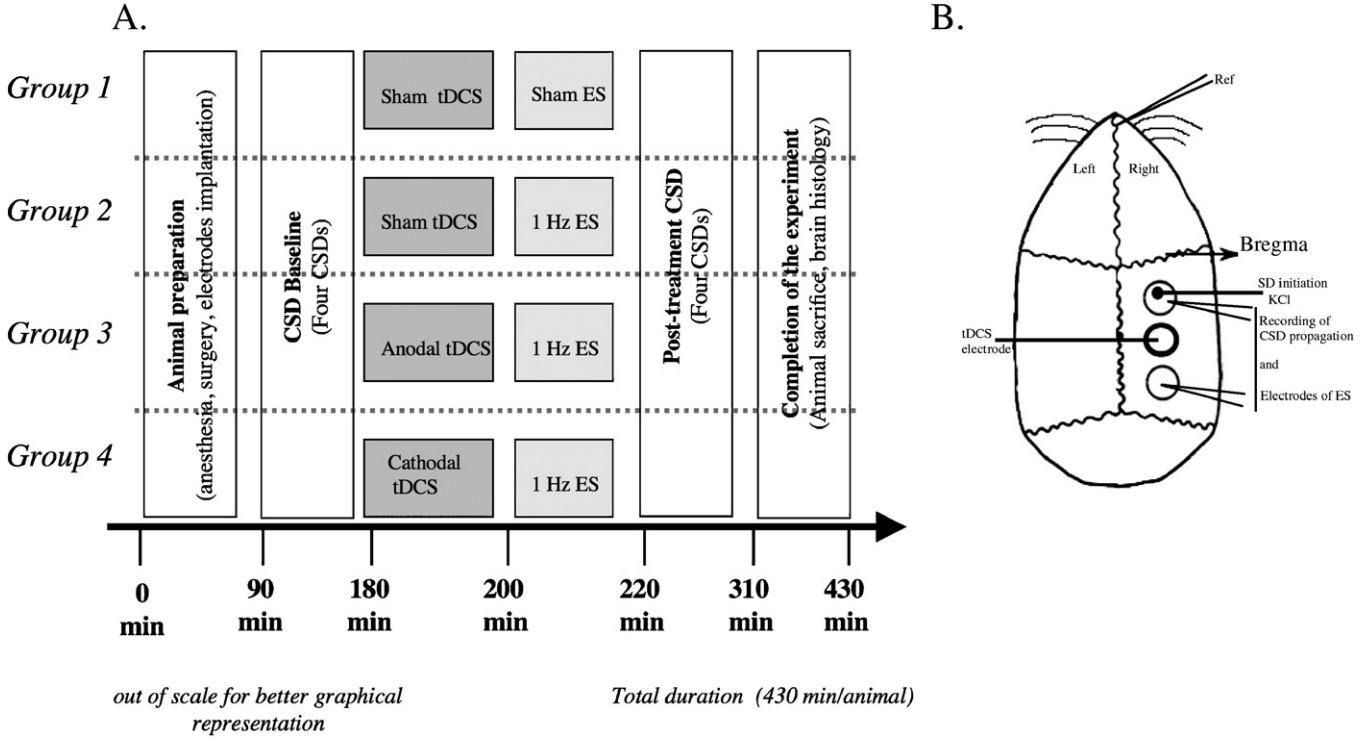

Fig. 1. A—This diagram summarizes the timeline for our experiment. Initially the animals were operated to implant electrodes for cortical spreading depression (CSD) recording and transcranial direct current stimulation (tDCS)/electrical stimulation (ES). We then record baseline CSD, applied tDCS (sham, anodal or cathodal), ES (1 Hz or sham) and recorded post-treatment CSD. At the end, the animals were sacrificed and the brains were removed for histological analysis. B—Schematic illustration showing the location of the two holes in each hemisphere for CSD recording, the location of epidural KCl application (frontal hole), the location of the two electrodes (for CSD recording and ES), the nasal reference for cortical spreading depression (CSD) recording and the location of the tDCS stimulating electrode (between two holes of CSD recording).

For transcranial direct current stimulation, we used a specially developed, battery-driven, constant current stimulator (Schneider Electronic, Gleichen, Germany) with a maximum output of 10 mA that transferred electric current to the electrode on the cranium (jacket fixed on the parietal cortex filled with saline solution—area of 7 mm^2) and to the electrode placed onto the ventral thorax that served as a counter electrode (large, conventional rubber plate electrode −10.5 cm^2 (Physiomed Elektromedizin AG, Schnaittach, Germany)). For anodal stimulation, the electrode on the cranium was the anode and the electrode on the thorax the cathode, whereas for cathodal stimulation, the connections were reversed. A constant current of 0.2 mA intensity (current density of 2.86 mA/cm^2) was applied for 20 min. For sham stimulation, the electrodes were placed in the same position; however, the stimulator was turned off after 30 s, similarly to the method used in human studies.

For repetitive electrical stimulation, we used the recording CSD electrodes to deliver repetitive electrical stimulation. This procedure assured that CSD would be recorded in the brain area that received ES. Electrical stimulation was delivered by an electrical stimulator (Insight, Equipamentos Cientificos, Ribeirão Preto, Brazil) and applied using the following parameters: 1 Hz continuous stimulation for 20 min (total of 1200 pulses using a pulse intensity of 0.6 mA and pulse duration of 5 ms). We used the same stimulation protocol as in a previous study that evaluated the effects of repetitive electrical stimulation on CSD in Wistar rats (Fregni et al., 2005a) and indeed showed that 0.6 mA corresponds to a suprathreshold stimulation (approximately 120% of the motor threshold (MT)).

At the end of the experiment, brains were removed and serial frozen sections were cut using a microtome, mounted on glass slides, and stained with cresyl violet. The slices were examined under a light-microscope to evaluate histological lesions induced by either tDCS or ES.

Statistical analyses were done with SAS statistical software (version 8.0, Cary, North Caroline, USA). The main outcome of this study was the CSD velocity change—defined as the difference between pre- and post-stimulation CSD velocity in the same animal. Because there were 4 different groups of stimulation (sham tDCS/sham ES, sham tDCS/1 Hz ES, anodal tDCS/1 Hz ES, cathodal tDCS/1 Hz ES), we used analysis of variance to detect whether there was an overall significant effect of stimulation condition on CSD velocity. When appropriate, post-hoc comparisons were carried out using Fisher LSD correction for multiple comparisons. Statistical significance refers to a two-tailed p value <0.05.

A one-way ANOVA showed that the main effect of stimulation condition was significant ($F=6.37$, $df=3,21$, $p=0.003$). After sham tDCS/1 Hz ES, a decrease in the CSD velocity of 6.9% (±1.9%) was observed as compared to baseline. Post-hoc comparisons furthermore showed that CSD velocity changes after sham tDCS/1 Hz ES were significantly different when compared to sham tDCS/sham 1 Hz ($p=0.011$), anodal tDCS/1 Hz ES ($p=0.0003$) and cathodal tDCS/1 Hz ES ($p=0.045$).

The effects of anodal stimulation had a larger magnitude as compared to cathodal stimulation using sham stimulation as the reference group (i.e., anodal vs. sham compared with cathodal vs. sham), whereas anodal stimulation changed the velocity of CSD as compared with sham tDCS/1 Hz ES in 14.4% (CSD velocity (mm/min) after anodal tDCS/1 Hz ES: 3.41 (±0.39) and after sham tDCS/1 Hz ES: 2.98 (±0.28)), this difference was only 4.3% for the same comparison with cathodal stimulation (CSD velocity after cathodal tDCS/1 Hz ES: 3.11 (±0.26)). There was also a significant difference ($p=0.041$) between the effects of 1 Hz ES preceded by anodal vs. 1 Hz ES preceded by cathodal stimulation on CSD (Fig. 2). In addition anodal tDCS/1 Hz ES resulted in a significant increase in the velocity of CSD propagation as compared with baseline (see Table 1 for details).

Finally, we tested whether sham tDCS/sham ES resulted in CSD velocity change. This analysis revealed no difference between the post-stimulation CSD compared to pre-stimulation CSD (change of −0.5%±0.6%, $p=0.75$). Histological examination showed that there were no histological lesions induced by the electric current from either tDCS or ES and combination of both techniques.

The results of this study extend our previous study showing that the combination of tDCS with ES can modify the effects of ES alone. Furthermore this effect depends on the direction of the current such as that anodal stimulation had a larger effect on CSD changes modulated by ES.

We showed that preconditioning of electrical stimulation with anodal or cathodal tDCS modulates the after-effects (i.e., the modulatory effects that are observed beyond the stimulation period) of 1-Hz ES on CSD. Although these findings can be explained by different mechanisms, we hypothesize that the most likely mechanism is that different effects of anodal and cathodal tDCS on membrane polarization are responsible for the results of subsequent ES on CSD velocity. Low-frequency ES alone induced long-term-depression (LTD)-like effects in our

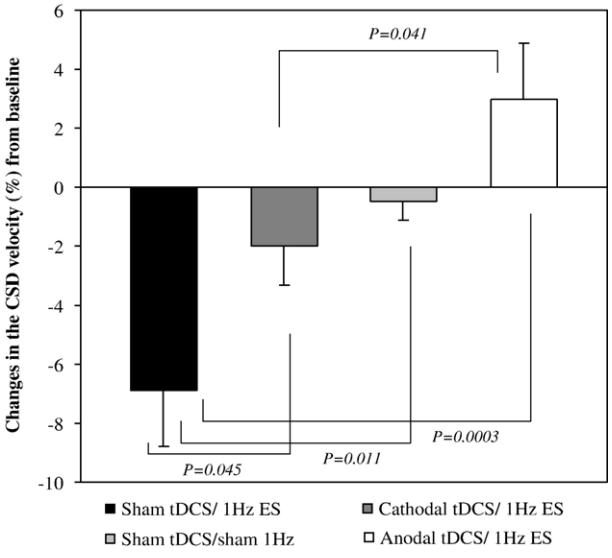

Fig. 2. Changes in the CSD velocity compared to baseline (%) can be observed after sham tDCS/1 Hz-ES (black column); cathodal tDCS/1 Hz-ES (dark grey column); sham tDCS/sham ES (light grey column) and anodal tDCS/1 Hz ES (white column). Each column represents mean velocity of CSD changes from baseline. Error bars indicate S.E.M.

Table 1
CSD velocity before and after stimulation

Treatment group	CSD velocity (mm min^{-1}) (±S.D.)	
	Baseline	After stimulation
Sham tDCS/Sham ES	3.26 (±0.32)	3.25 (±0.34)
Sham tDCS/1 Hz ES	3.21 (±0.29)	2.98 (±0.28)
Cathodal tDCS/1 Hz ES	3.17 (±0.25)	3.11 (±0.26)
Anodal tDCS/1 Hz ES	3.31 (±0.28)	3.41 (±0.39)

tDCS—transcranial direct current stimulation; ES—electrical stimulation; CSD—cortical spreading depression.

study, which is in accordance with previous animal studies (Malenka and Bear, 2004). On the other hand, anodal tDCS might have depolarized postsynaptic membranes relevantly and thus favored long-term-potentiation (LTP)-like effects of the same ES protocol, as it was demonstrated in animal experiments that postsynaptic depolarization combined with low-frequency electrical stimulation generates LTP (Fregnac et al., 1994). Conversely, a postsynaptic hyperpolarization generated by cathodal tDCS might have diminished LTD-like effects of our ES protocol, since a slight postsynaptic depolarization, however smaller than the amount of depolarization needed for LTP-induction, is also a pre-requisite for most LTD-inducing protocols (Malenka and Bear, 2004). For us, this hypothesis seems to be the most parsimonious one to explain the results, because it is applicable for the effects of anodal as well as cathodal tDCS on ES. Therefore the results of this study shed light on the mechanisms and effects of two techniques of brain stimulation on cortical physiology.

The results of this study are important and advance the knowledge in the field of brain stimulation for several reasons: (1) we showed, by neurophysiological data, that tDCS is a powerful method of brain stimulation that when combined with ES changes its effects; (2) we confirmed and extended the notion that the effects of tDCS are specific to the polarity of stimulation using another neurophysiologic method of assessment – cortical spreading depression – as anodal and cathodal stimulation yield different results as indexed by cortical spreading depression; and (3) anodal tDCS in combination with 1 Hz ES changes the velocity of CSD propagation by 14.4%, and thus to a greater degree than anodal tDCS alone (change of 11.1%), as shown in our previous study (Liebetanz et al., 2006) and therefore suggesting that this combined approach of brain stimulation might have a greater modulatory impact on brain activity.

Finally, our results might have a potential relevance for the clinical application of brain stimulation in migraine and epilepsy. The phenomenon of cortical spreading depression is associated with these pathologies (Leniger et al., 2003). As we have shown that anodal and cathodal tDCS change the features of CSD induced by repetitive ES, we speculate that tDCS in humans could also shape the likelihood of migraine and epilepsy attacks induced by stimulatory, trigger factors such as sleep deprivation, stress and hypoglycemia.

Although we believe that our results might have a clinical relevance for humans, parameters of DC stimulation were not the same as those used in human studies and thus the transferability of these results for application in humans are limited. We used a current density several times higher than the one usually used in humans (2.86 mA/cm^2 (in this study) vs. 0.06 mA/cm^2—given a current of 2 mA and an electrode of 35 cm^2 or 0.03 mA/cm^2 with a current of 1 mA in humans), the electrode was fixed directly on the skull instead of being applied to the skin (as performed in humans) and the duration of stimulation was 20 min – that is twice the duration used in some human studies (Fregni et al., 2005b; Nitsche and Paulus, 2001) – however, 20 min of stimulation has also been used in other clinical studies (Fregni et al., 2006; Hummel et al., 2005). Although the parameters of stimulation might change the magnitude of its effects, its direction (i.e., facilitation or inhibition) remains the same. Similarly, although electrical stimulation and TMS might be roughly comparable, these two types of stimulation might induce at least gradually different biological effects as epidural electrical stimulation might induce a more focal and deeper stimulation as compared to TMS.

In conclusion, the results of this study show that tDCS alters the subsequent effects of 1 Hz ES on the phenomenon of CSD. Further studies exploring different parameters of stimulation such as the combination of anodal tDCS with high frequency ES are necessary in order to develop this novel strategy of brain stimulation.

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