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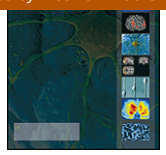

Transcranial direct current stimulation influences the cardiac autonomic nervous control

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ABSTRACT

To investigate whether the manipulation of brain excitability by transcranial direct current stimulation (tDCS) modulates the heart rate variability (HRV), the effect of tDCS applied at rest on the left temporal lobe in athletes (AG) and non-athletes (NAG) was evaluated. The HRV parameters (natural logarithms of LF, HF, and LF/HF) was assessed in 20 healthy men before, and immediately after tDCS and sham stimulation. After anodal tDCS in AG the parasympathetic activity (HF_{log}) increased ($P < 0.01$) and the sympathetic activity (LF_{log}) and sympatho-vagal balance (LF/HF_{log}) decreased ($P < 0.01$), whereas no significant effects were detected in NAG ($P > 0.05$). No significant changes in HRV indexes were provoked by sham stimulation in both AG and NAG ($P > 0.05$). In conclusion, tDCS applied on the left temporal lobe significantly increased the overall HRV in AG, enhancing the parasympathetic and decreasing the sympathetic modulation of heart rate. Consequently the sympatho-vagal balance decreased at rest in AG but not in NAG. Releasing a weak electric current to stimulate selected brain areas may induce favorable effects on the autonomic control to the heart in highly fit subjects.

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Transcranial Direct Current Stimulation (tDCS) has been used to modulate brain activity in order to optimize treatments of various neurological or psychiatric disorders, as depression and chronic pain [6,16]. In addition, previous studies have also shown the clinical effectiveness of this technique to enhance physical performance [10]. Basically, tDCS applies a weak electrical direct current to the brain cortex through two electrodes. One electrode stimulates the cortex, whereas the other is usually positioned on a contralateral area as a reference electrode [26,29]. Anodal tDCS has been shown to induce neurological changes in the cell membrane resting potential, favoring depolarization and increasing spontaneous neuronal firing rate. On the other hand, opposite effects seem to be generated by cathodal current polarity [25]. However, the underlying effect of stimulation in different cortical areas warrants future investigation.

Green et al. [17] showed that deep brain stimulation of the periventricular/periaqueductal grey area for the relief of chronic neuropathy modulated the blood pressure up or down, depending on the electrode placement. Therefore Cogiamanian et al. [9] speculated that tDCS might induce favorable blood pressure changes in humans with hypertension. Previous studies suggested that electrical stimulation applied over the insular cortex (IC) could influence the autonomic cardiovascular control [27,28]. The right anterior insular stimulation during surgery increased the sympathetic cardiovascular responses, whereas the left insular stimulation resulted in parasympathetic activity increase [27]. Although it has been shown that direct current applied on the scalp can stimulate surrounding sub-cortical areas such as IC [23], which are related to the autonomic nervous system and blood pressure control, the under-

Abbreviations: AG, athletic group; NAG, non-athletic group; HFn, normalized high frequency; LFn, normalized low frequency; LF/HF, low frequency and high frequency ratio; HRV, heart rate variability; LIC, left insular cortex; RIC, right insular cortex; tDCS, transcranial direct current stimulation.

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lying effect on the autonomic nervous system due to tDCS targeting the IC remains unclear.

The heart rate variability (HRV) has been used as a strategy to assess the autonomic control to the heart [8,36]. Previous studies showed that regular exercise practice may induce changes in the autonomic nervous system control [15]. Therefore, athletes would have lower sympathetic autonomic and higher vagal autonomic activity compared to sedentary subjects [12,30,32]. Therefore the influence of endurance training on the heart rate control would be in part due to neurocardiac mechanisms [15].

Since there are evidences indicating that the application of tDCS over the scalp can stimulate surrounding sub-cortical areas such as IC [27], the present study investigated whether tDCS applied on the left temporal lobe (T3) would affect the autonomic nervous activity as reflected by HRV indexes. Additionally, it has been hypothesized that the influence of tDCS on the HRV would be higher in subjects with better fitness levels.

Twenty male subjects were assigned in two groups: athletes (AG) ($n = 10$; 33 ± 9 yrs; 171.5 ± 5.8 cm; 72.8 ± 9.5 kg; 24.8 ± 3.2 kg/m^2) and non-athletes (NAG) ($n = 10$; 27 ± 4 yrs; 174.6 ± 6.8 cm; 90.9 ± 24.0 kg; 29.6 ± 6.3 kg/m^2). A non-exercise model developed for healthy population was used to estimate the maximal oxygen uptake (VO_{2max}) and therefore classify the physical fitness level in both groups [24]. The AG was composed by national road race cyclist competitors, with 10 ± 2 years of practice, 3×/week training frequency, and 2.5 ± 0.5 training hours/session [mean ± SD, VO_{2max}: 51.2 ± 2.2 mL kg^{-1} min^{-1}]. The NAG was composed by healthy subjects not regularly engaged in physical activity programs [mean ± SD, VO_{2max}: 35.6 ± 7.3 mL kg^{-1} min^{-1}]. Exclusion criteria included clinical diagnosis of cardiovascular disease and the use of any medication with potential cardiovascular influence. All participants signed an informed consent and the study was approved by institutional ethics committee.

The experimental protocol was completed within a month. Subjects were asked to maintain their daily routine and to avoid strenuous physical activity, as well as alcohol and caffeinated drinks in the 24 h preceding data assessment. Data were assessed in 2 non-consecutive days separated by 48- to 120 h intervals. In both sessions the participants initially remained seated at rest during 15 min in a quiet room with low lights, controlled temperature (21 °C) and humidity (65%).

Afterwards an anodal or sham tDCS was applied in a counterbalanced random order over T3 [2 mA during 20 min]. Immediately after the end of the stimulus the participants remained seated for another 15 min. The breathing cycle was controlled [15 breaths per minute] throughout the testing sessions. Adequate hydration was provided during the experimental protocol. The continuous recording of HRV was made along the experiment, however, only the last 5 min of each registration (before, during and post stimulation) was used for HRV analysis. The HRV data was analyzed in a single blind fashion. The evaluators did not know whether the subjects were assigned to AG or NAG, or whether data corresponded to anodal tDCS or sham condition.

The electric current was applied using a pair of sponges soaked in saline solution (140 mM of NaCl dissolved in Milli-Q water) involving both electrodes (35 cm^2) [26]. The electrodes (anodal and cathodal) were connected to a constant current stimulation device with three power batteries (9 V) presenting a maximal output of 10 mA. The batteries were regulated by a digital multimeter (EZA EZ 984, USA) with a standard error of ±1.5%.

For the anodic stimulation targeting left IC, the anode was placed over T3 area according to the international EEG 10–20 system. The cathode was placed over the supraorbital contralateral area (Fp2) and fixed by elastic bands. The electrodes were placed in the same position of the anodal stimulation to perform the sham condition. However, the stimulator was turned off after 30 s [33]. Thus, the subjects reported to feel a tingling or itching sensation coming from the initial electrical stimulation, but they did not receive any further current. This procedure allowed the subjects to remain "blind" in respect to the type of polarity stimulation received during the test and to assure a sham control effect [5].

The HR and R–R intervals were registered using an R–R heart rate recorder (Polar®, model S810i, Polar Electro Oy, Finland). For data analyses R–R intervals were processed in a Matlab routine (MATLAB 6.0, Mathworks Inc, Natick, MA) as described elsewhere [34]. The artifacts were removed by filtering or manually by visual inspection whenever necessary. In the frequency domain, spectral power were calculated to obtain: (a) low frequency band (LF, 0.04–0.15 Hz), which has been associated to both sympathetic and parasympathetic activity; (b) high frequency band (HF, 0.15–0.40 Hz), which reflects the parasympathetic activity; (c) LF-HF ratio (LF/HF) as an index of sympathetic-vagal balance. The LF and HF components were presented also in normalized units (LFn and HFn). Just frequency domains were used, since it has been shown that these components would be more accurate to evaluate HRV at rest in short-term measurements [18].

All data were expressed as mean ± SD. Because absolute spectral indexes are skewed, LF (LF_{log}) and HF (HF_{log}) power indexes were transformed using the natural logarithms to allow parametric statistical comparisons. A 3-way repeated measures ANOVA with time (pre and post), physical fitness (athletes and non- athletes), and stimulation (anodic or sham) as factors was used for between and within group comparisons. In the case of significant F ratios, Tukey post hoc tests were performed. The effect-size was calculated by dividing the difference between mean values associated with either anodic or sham stimulations by the pooled SD. The significance level was set at $P \leq 0.05$. All calculations were performed using SPSS 17 software (SPSS Inc., IL, USA).

No adverse effects were described by the participants during the application of tDCS. All subjects reported to feel an itching sensation in the sham condition. Fig. 1 shows the natural logarithms of LF, HF, and LF/HF during anodic and sham condition in AG and NAG. Non significant main effects were found for LF_{log} due to isolated physical fitness ($F_{(1,19)} = 0.76$; $P = 0.40$), stimulation ($F_{(1,19)} = 1.13$; $P = 0.30$), and repeated measures ($F_{(1,19)} = 0.67$; $P = 0.42$), but a significant interaction was detected for the combination of these factors ($F_{(1,19)} = 4.40$; $P = 0.04$). In which concerns HF_{log} there were no significant main effect of isolated physical fitness ($F_{(1,19)} = 0.10$; $P = 0.76$) or stimulation ($F_{(1,19)} = 2.43$; $P = 0.14$), whereas the repeated measures effect (time factor) was significant ($F_{(1,19)} = 5.42$; $P = 0.03$), as well the physical fitness × stimulation × time interaction ($F_{(1,19)} = 10.74$; $P < 0.01$). No isolate main effect on the LF/HF_{log} was detected for the physical fitness ($F_{(1,19)} = 0.0.3$; $P = 0.88$). Significant main effects were found for the stimulation ($F_{(1,19)} = 4.91$; $P = 0.04$), time ($F_{(1,19)} = 7.81$; $P < 0.01$), and physical fitness × stimulation × time interaction ($F_{(1,19)} = 22.99$; $P < 0.01$).

Post hoc analyses revealed that anodic tDCS elicited significant increase in HF_{log} ($P < 0.01$) and decreases in both LF_{log} ($P < 0.01$) and LF/HF_{log} ($P < 0.01$) in AG, whereas no significant alterations were observed in NAG ($P = 1.00$). The sham condition did not alter LF_{log}, HF_{log}, and LF/HF_{log} in both AG ($P = 0.97$; $P = 1.00$; $P = 0.93$, respectively) and NAG ($P = 0.33$; $P = 1.00$; $P = 1.00$, respectively).

The effect-sizes of the differences related to sham and anodal conditions in the different groups are presented in Table 1. The effect-size was higher in athletes stimulated by anodic current in all HRV indexes. The lower effect-sizes were obtained for the athletes in the sham condition and non-athletes in both anodic and sham conditions.

The present study is probably the first to verify the effects of tDCS applied over T3 on the autonomic nervous activity reflected by HRV in athletes and non-athletes. Our findings indicated that the

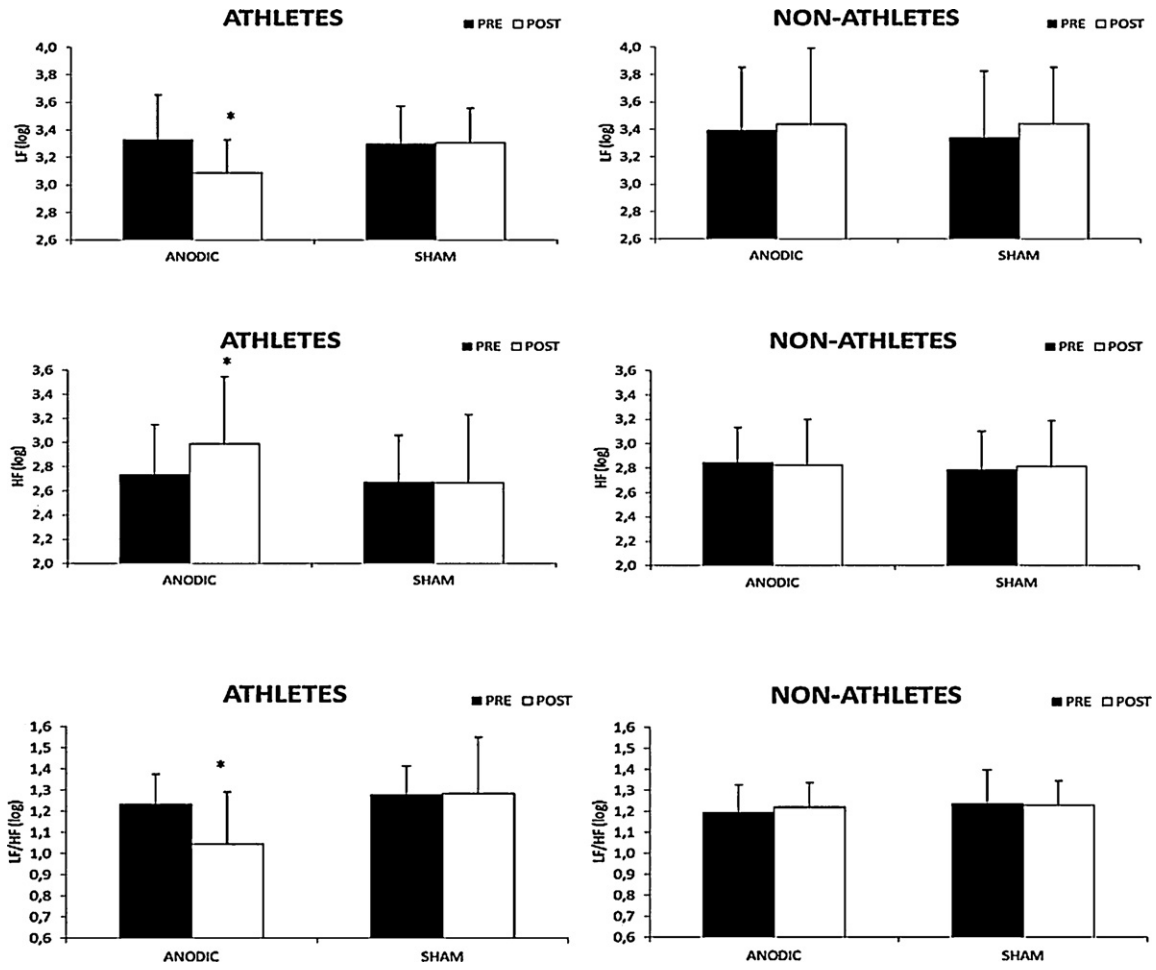

Fig. 1. Mean (SD) for HRV components of low frequency (LF_{log}), high frequency (HF_{log}), and sympathetic-vagal balance (LF/HF_{log}) during anodic and sham condition in athletes and non-athletes. *Significant difference from PRE ($P < 0.001$).

parasympathetic activity increased whereas the sympathetic activity decreased with anodal tDCS in well trained athletes. However, no changes were found in the untrained group. These data ratified the hypothesis that tDCS might be able to reach deep cerebral areas related to autonomic nervous control (e.g., IC). Moreover, it is feasible to think that such effect also relies on the individual fitness level.

Although the effects of physical activity on cardiac autonomic control have been extensively described [2] its possible influence on cerebral responses are not clear [1]. It has been demonstrated that exercise can induce plasticity in some cerebral areas according to its specific demand [1,11,14,19,21]. Endurance exercise (e.g., long distance cycling) has been shown to increase the metabolic demand of cortical neurons, inducing angiogenesis and increasing blood flow [35]. Upregulation of neurotrophic factors promoting neuronal survival and differentiation has been observed in endurance athletes [22]. Furthermore, Williamson et al. [39] showed that during mild exercise the insular region is evoked by the central command and that LIC may be a site for the parasympathetic control of HR [39]. Thus, endurance athletes may have specific adaptations on cardiovascular brain-related areas such as IC, which is possibly due to a more supportive neural environment. These adaptations might improve the responsiveness of an endurance athlete's brain to tDCS and explain the differences found in the present study, since only AG showed differences on the autonomic responses.

Table 1
Effect size for the comparison of heart rate variability indices following sham and anodal stimulation.

	Athletes		Non athletes	
	ANODIC	SHAM	ANODIC	SHAM
LF	−0.88	0.02	0.09	0.22
HF	0.68	−0.02	−0.07	0.06
LF/HF	−1.43	0.04	0.22	−0.05

Another possible explanation for the differences between AG and NAG is that well conditioned subjects show different cardiac autonomic control when compared to non-athletic subjects [2]. Usually, fit subjects are expected to show higher parasympathetic activity and reduced sympathetic activity at rest [7,20], as well as faster recovery from exercise [2]. In other words, athletes might have better neural efficiency on the central command for the autonomic nervous system, which could help explaining the increase of autonomic modulation via parasympathetic pathways after tDCS in AG, and therefore significant changes on HRV.

The HF component is generally considered as a marker of cardiac vagal control [31]. In AG the anodal tDCS induced significant changes in HF in comparison with the rest condition ($P < 0.01$). These results agree with previous data showing that electric stimulation applied over LIC may induce bradycardia and, consequently, a reduction of blood pressure at rest [27]. Furthermore the anodal tDCS decreased the LFn values, which are associated with cardiac sympathetic activity [4]. This finding suggests that there would be an antagonistic relationship between the function of IC in the left

and right cerebral hemispheres [27]. The LIC would be related to parasympathetic autonomic activity, whereas the right IC would mediate sympathetic autonomic activity [27]. Hence it is feasible to believe that tDCS increased the LIC relative influence on the autonomic control. The sympathetic-vagal balance decrease in AG after anodal tDCS concurs with this idea. Some previous studies corroborate these findings. It has been extensively demonstrated that LIC stimulation together with surrounding cortical sites (as the nucleus tractus solitarii or rostral ventrolateral medulla) induces significant changes in the heart rate and blood pressure [9,13]. Vernieri et al. [38] demonstrated that tDCS applied to the left motor cortex could also induce a decrease in HRV consequently to an increase in LFn.

In the present study we have chosen to place the anodic electrode over T3. Although tDCS stimulated only the cortical area directly beneath the electrode, it could also modulate subcortical areas since there is a connection within cortico-cortical neural networks [23]. The IC was found to connect with the temporal pole and superior temporal sulcus of the temporal lobe [3]. It can be therefore speculated that at least in AG the tDCS applied over T3 may have modulated subcortical areas such as IC and change the cardiac autonomic regulation.

In which concerns the electrodes distribution used in this study (bi-cephalic), the "active" electrode was placed over T3 and the "reference" electrode over the contralateral orbita [26]. It should be considered that usually one electrode is defined as the reference and the other as the stimulation electrode. However, both electrodes have similar current and both are placed on the scalp. Therefore, this is a functional definition and does not imply that the "reference" electrode is physiologically inert. Given this, the cephalic reference electrode might have also helped modulating brain regions involved in cortical cardiovascular modulation [37] such as prefrontal cortex (PFC). In addition, frontal lobe afferents to IC come from the orbital cortex [3], which may also yield some influence of the "reference" electrode on the autonomic nervous modulation.

Despite the fact that the brain related areas to the autonomic nervous system control have been well described, we have not presently verified the specific function of the activated cells due to tDCS. This significant limitation might compromise some of our interpretations for the results. Furthermore, the projection fields of such neurons and their identity as excitatory or inhibitory neurons are still unknown. Other studies are necessary to a better understanding of the underlying mechanisms associated with our findings.

In conclusion, tDCS applied over T3 targeting autonomic nervous control areas (e.g., LIC) significantly increased the parasympathetic activity and decreased the sympathetic activity in endurance trained athletes, whereas no changes were found in non-athletic subjects. Hence, releasing a weak electric current to stimulate selected brain areas may induce favorable effects on the autonomic control to the heart. Additional research should be encouraged to ratify these results and to help clarifying the physiological mechanisms underlying possible changes in the autonomic control pattern due to tDCS.

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