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Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats

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ABSTRACT

Obesity is a multifactorial disease associated with metabolic dysfunction and the prevention and treatment of obesity are often unsatisfactory. Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation technique that has proven promising in the treatment of eating disorders such as obesity. We investigate the effects of tDCS on locomotor and exploratory activities, anxiety-like and feeding behavior, and levels of brain-derived neurotrophic factor (BDNF), IL (interleukin)-10, IL-1β, and tumor necrosis factor-alpha (TNF-α) in the cerebral cortex of obese rats. A total of 40 adult male Wistar rats were used in our study. Animals were divided into groups of three or four animals per cage and allocated to four treatment groups: standard diet plus sham tDCS treatment (SDS), standard diet plus tDCS treatment (SDT), hypercaloric diet plus sham tDCS treatment (HDS), hypercaloric diet plus tDCS treatment (HDT). After 40 days on a hypercaloric diet and/or standard diet were to assessed the locomotor and exploratory activity and anxiety-like behavior to by the open field (OF) and elevated plus maze (EPM) tests respectively before and after exposure to tDCS treatment. The experimental groups were submitted to active or sham treatment tDCS during eight days. Palatable food consumption test (PFT) was performed 24 h after the last tDCS session under fasting and feeding conditions. Obese animals submitted to tDCS treatment showed a reduction in the Lee index, visceral adipose tissue weight, and food craving. In addition, bicephalic tDCS decreased the cerebral cortex levels of IL-1β and TNF-α in these animals. Exposure to a hypercaloric diet produced an anxiolytic effect, which was reversed by bicephalic tDCS treatment. These results suggest that, in accordance with studies in humans, bicephalic tDCS could modulate biometric and inflammatory parameters, as well as anxiety-like and feeding behavior, of rats subjected to the consumption of a hypercaloric diet.

1. Introduction

Obesity has become a global public health problem, and millions of people worldwide currently die from the consequences of overweight and obesity, and its prevention and treatment are unsatisfactory (Yang et al., 2018; *World Health Organization Fact sheet: obesity and overweight*, n.d.; Bastien et al., 2014). Metabolic changes resulting from overweight or obesity contribute to the development of comorbidities such as heart disease, hypertension, and liver and pancreas disease (Bluher, 2014; Lalanza et al., 2014). These comorbidities can be linked to low-grade inflammation found in obesity, with a significant expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6), and to a reduction in anti-inflammatory cytokines, such as interleukin 10 (IL-10) (Wang et al., 2013). In addition, obesity

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has also been associated with emotional disorders in humans and rodents (Kittel et al., 2015; Macedo et al., 2015). Studies show that rising obesity rates are closely related to a dramatic increase in the consumption of highly palatable and high caloric foods (Gumbs et al., 2016; Macedo et al., 2016).

The cafeteria diet has been widely used to induce obesity in rodents. This diet has a high content of sugar and fat is rich in highly palatable foods; it induces hyperphagia leading to increased body weight and alters metabolic parameters (Macedo et al., 2015; Macedo et al., 2012; Sampey et al., 2011; Cigarroa et al., 2016). Furthermore, studies indicated that animals exposed to stressful situations increased anxiety-like behaviors and the consumption of highly obesogenic, palatable food reduced these anxiogenic effects (Zeeni et al., 2015; de Oliveira et al., 2014), and can therefore trigger a search behavior similar to that seen in drug addiction (Macedo et al., 2016; Pivarunas and Conner, 2015). In rodents, the cafeteria diet contributes directly to adipose tissue and liver inflammation (Sampey et al., 2011). The release of inflammatory cytokines by adipose tissue is enhanced in obesity, and it can change the permeability of the blood-brain barrier by modifying brain homeostasis and triggering neurodegenerative diseases and neuroinflammation. While these inflammatory mediators expressed in the central nervous system (CNS) may modulate energetic metabolism and food intake (Parimisetty et al., 2016).

Another important mediator related to energy metabolism and the control of food intake is the brain-derived neurotrophic factor (BDNF), a neurotrophin involved in synaptic plasticity (Macedo et al., 2015; Woo et al., 2013; Leffa et al., 2015). Mutations in genes encoding the BDNF tyrosine kinase receptor (TrkB) lead to hyperphagia and obesity, while the central infusion of BDNF in rodents leads to increased satiety and reduction of weight gain (Hinderberger et al., 2016). Low levels of BDNF are associated with hypothalamic α2δ thrombospondin-1 receptor dysfunction; this receptor is required for normal functioning of BDNF in rats. The dysfunction of this receptor appears to contribute to increased food intake and weight gain, suggesting a central mechanism mediating the inhibitory effects of BDNF (Cordeira et al., 2014).

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulator technique, which has been investigated regarding the control of substance abuse (Conti et al., 2014; Conti and Nakamura-Palacios, 2014; Trojak et al., 2016; Pedron et al., 2017) and food intake (Macedo et al., 2016; Fregni et al., 2008). Effects of tDCS can be directly related to cortical areas linked to decision-making (Montenegro et al., 2012). The prefrontal dorsolateral cortex (DLPFC) is responsible for mediating the decision-making behavior involved in mechanisms of food reward (Fregni et al., 2008). Binge eating triggers neuroendocrine changes that are very similar to drug addiction, and obese patients who underwent bariatric surgery may therefore risk behavioral relapse leading to weight recovery, similar to the behavior observed in addicted individuals (Goldman et al., 2011). The tDCS application uses a low and continuous current that alters the excitability cortical. Positive stimulation (anodal tDCS) causes depolarization of the resting membrane potential depolarization (anodal) or negative stimulation (cathodal tDCS) induces hyperpolarization, increasing or decreasing neuron excitability, respectively (Polania et al., 2011; Nitsche et al., 2003). Human studies have shown that applying tDCS (direct anode/cathode left) in the DLPFC reduces food craving in healthy individuals (Fregni et al., 2008; Lapenta et al., 2014). Confirming data from studies in humans, previous study of our research group also showed a decrease in food craving in rats (Macedo et al., 2016).

Thus, considering that information about the brain mechanisms involved in the effects of tDCS on food consumption is scarce, the aim of this study was to evaluate the effects of tDCS on obesity as manifest in biometric, behavioral, and neurochemical parameters in rats. The behavioral parameters we evaluated were exploratory and locomotor activities, anxiety-like behavior, and preference for sweet food. The neurochemical parameters we evaluated were cerebral cortex levels of BDNF, IL-10, IL-1β and TNF-α.

2. Materials and methods

2.1. Animals

Forty naïve adult male Wistar rats (60 days old; weighing 200–250 g) were used in this study. Rats were randomized by weight and length measurements and housed in polypropylene home cages ($49 \times 34 \times 16\ cm^3$). The animals were maintained on a standard 12-h light/dark cycle (lights on at 7:00 a.m., lights-off at 7:00 p.m.), in a temperature-controlled environment (22 ± 2 °C), with access to water and chow ad libitum (hypercaloric diet and/or standard chow diet). All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol No. 110455). The procedures for scientific animals use were realized in accordance with the Guide for the Care and Use of Laboratory Animals (8th edn. 2011, law no. 11.794, Brazil). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines (Kilkenny et al., 2010). The number of animals used in these experiments was the necessary number to produce reliable scientific data.

2.2. Experimental design

The rats were divided into groups of three or four animals per cage and habituated to the maintenance room for one week before the start of the experiment. After the habituation period, the animals were randomly selected for weight and length measurements and subsequently allocated into four treatment groups ($n = 10$/group): standard diet plus sham tDCS treatment (SDS), standard diet plus tDCS treatment (SDT), hypercaloric diet plus sham tDCS treatment (HDS), or hypercaloric diet plus tDCS treatment (HDT). The animals were weighed weekly. After 40 days on a hypercaloric diet and/or standard diet and before bicephalic tDCS treatment, the rats were exposed to the open-field apparatus (OF) and the elevated plus-maze (EPM), for evaluations of locomotor and exploratory activities and anxiety-like behavior.

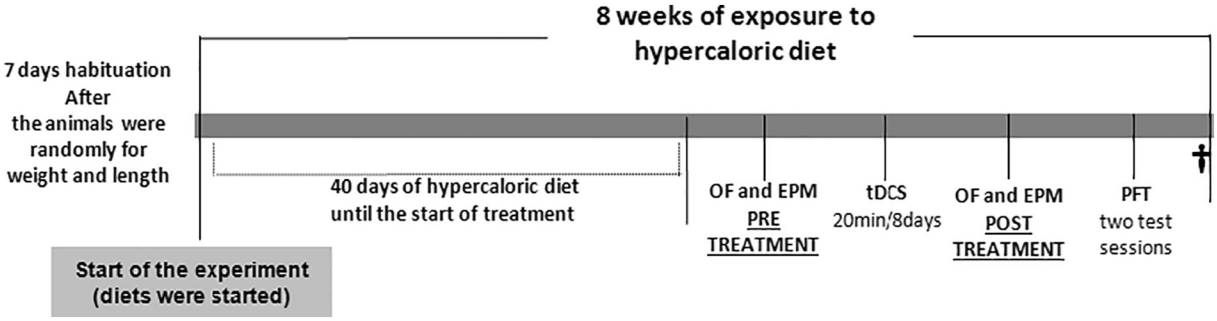

Fig. 1. Experimental Design. OF: open field test. EPM: elevated plus maze test. PFT: palatable food test. tDCS: transcranial direct current stimulation.†: euthanasia.

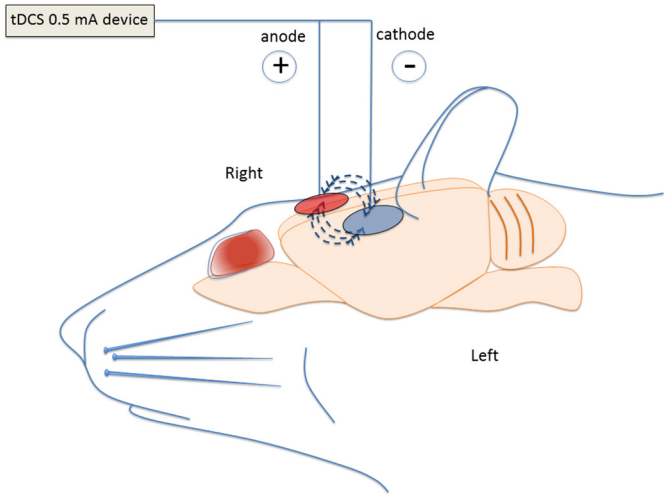

Fig. 2. tDCS model. The center of the anodal electrode was placed on the right prefrontal dorsolateral cortex (DLPFC) and the cathode on the left side DLPFC.

Subsequently, the experimental groups received active or sham tDCS treatment for 20 min each day for eight consecutive days. OF and EPM tests were repeated 24 h after the last tDCS session. The next day, the animals were exposed to two palatable food test (PFT) sessions. The rats were killed by decapitation 24 h after the PFT (Fig. 1).

2.3. Blinding

A number of steps were taken to control for possible measurement bias. The animals' hair was shaved in the area of electrode placement. In addition, the researchers responsible for the behavioral ratings were blind to the numbers assigned to the test boxes; a third researcher did number assignment. It was therefore impossible for the investigators to distinguish the groups receiving active tDCS treatment from the sham groups. Two researchers conducted the behavioral tests. Three researchers analyzed the results; importantly, these evaluators were unaware of the experimental protocol. We thus believe that any potential influence on the results of the behavioral evaluations was eliminated.

2.4. Experimental diets

The pelletized standard rat chow Nuvilab CR-1 (NUVITAL®, Curitiba, PR, Brazil) provides an energy content of 2.93 kcal/g (information provided by the manufacturer), and is composed of 55% carbohydrates, 22% protein, 4.5% lipids, and other constituents (fiber and vitamins) (Supplementary Diagram S1). The hypercaloric diet (cafeteria diet) was chosen according to our previous study (Macedo et al., 2015; Macedo et al., 2012), and this diet model mimics modern patterns of human food consumption and has been used successfully in other experimental studies to induce obesity in lean animals (Estadella et al., 2004; Kumar et al., 2011). This particular diet was adapted from the hypercaloric diet or Western diet previously described by Estadella et al. (Estadella et al., 2004). Foods included in the hypercaloric diet were crackers, sausages, snack foods, wafers, condensed milk, and soda. These are highly palatable and highly flavored, which are features of comfort food (Kumar et al., 2011). The nutritional composition of this diet was approximately 60.0% carbohydrates, 20.0% lipids, 15.0% protein and 5.0% other constituents (sodium, calcium, vitamins, preservatives and minerals) providing 4.186 kcal/g (solids) and 0.42 kcal/mL (soda drink; calculations based on information provided by the manufacturer on the package label) (Supplementary Diagram S2). The standard diet and the hypercaloric diet were replaced daily by fresh food. Animals exposed to the hypercaloric diet also had access to standard food and water. The amount of food consumed in each cage was evaluated by weighing each day the food remaining in the feeders on a digital scale, and by calculating the food consumption per cage. To evaluate the weekly caloric ingestion the amount of cafeteria diet (g) and of soda drink (mL) were converted into kcal and summed. The intake of kcal per cage of rats was calculated, and this value was divided by the number of rats in the cage to estimate the intake in kcal per rat per cage.

2.5. Transcranial direct current stimulation (tDCS)

After five weeks on a hypercaloric diet, the animals in the active treatment groups underwent 20 min of bicephalic tDCS every afternoon for 8 days, as described by Macedo et al. (Macedo et al., 2016). The electrodes were positioned, fixed to the head with surgical tape (Micropore™), and covered with a protective mesh to prevent removal. A constant direct current of 0.5 mA was delivered from a battery-powered stimulator using electrocardiogram electrodes with conductive adhesive hydrogel. The rats' heads were shaved for firmer adherence and the electrodes were trimmed to 1.5 cm^2 for better fit. The electrodes were placed on the scalp to emulate the tDCS method used in human food craving studies (Fregni et al., 2008). The treatment target was the DLPFC, because modulation of this area has been shown to reduce both smoking and food craving (Johann et al., 2003; Uher et al., 2005; Alonso-Alonso and Pascual-Leone, 2007); moreover, other studies suggest that DLPFC activity is associated with food craving (Alonso-Alonso and Pascual-Leone, 2007). To locate the DLPFC, a cerebral atlas was used for rats (Paxinos and Watson's, 1982) and the figure published by Alonso-Alonso and Pascual-Leone (Alonso-Alonso and Pascual-Leone, 2007) which it illustrates brain areas involved in the regulation of food intake and schematic representation of their interactions. The center of the anodal electrode was placed on the right DLPFC and the cathode on the left side DLPFC (Fig. 2), to mimic the anodal placement in human studies in which stimulating the DLPFC modifies food craving (Macedo et al., 2016). According to an earlier animal safety study (Liebetanz et al., 2009), a current density higher than 142.9 A/m^2 is associated with brain lesions. Considering this threshold, we used stimulation parameters that resulted in a current density of 33.4 A/m^2. Moreover, constant current of 1 mA intensity causes skin lesions, as current density is comparatively much higher than the traditional 1 mA tDCS using large pads in humans. We therefore chose to use 0.5 mA, an intensity that has also been used in other animal studies. For sham stimulation, the electrodes were placed and fixed in the same position as for active stimulation; however, the stimulator remained in the "off" position throughout the procedure (Macedo et al., 2016). In order to deliver the current, animals had to be immobilized with a cloth for the total duration of stimulation. This protocol, including parameters such as the intensity and period of stimulation, is in accordance with the methods our group used in other studies investigating behavior and neurochemical effects in rats (Laste et al., 2012; Spezia Adachi et al., 2012; Leffa et al., 2016; Cioato et al., 2016).

2.6. Weight parameters and food intake

The animals were weighed weekly, and the weight delta was defined as the difference between final and baseline weight. At the end of the experiment, the naso-anal length (cm) of the animals was measured to determine the Lee index. This index, which was adapted from Bernardis and Cols, corresponds to the ratio between the cube root of the body weight (g) and the naso-anal length (cm) of the animals multiplied by 1000 (Bernardis and Patterson, 1968).

According to de Oliveira et al. (de Oliveira et al., 2015) food was weighed daily to assess the amount of food consumed per cage. In order to evaluate the amount of food consumed in each cage the caloric intake was evaluated during the 8 weeks of the experiment and the daily dietary intake was calculated as the difference between the diet offered

and the leftovers collected from the cage. Food was weighed daily to assess the amount of food consumed per cage. The weight in grams was then converted to Kcal (standard rat diet 2.93 kcal/g and the cafeteria diet 4.18 kcal/g). Using a digital scale, the daily food consumption (in grams) was calculated for each cage (amount of food placed in the feeders minus the remaining food) and averaged for a week (total food consumption). To estimate the consumption for each animal, the total food consumption was multiplied by the animal weight (in grams) and divided by the total sum of weights for all animals in its cage, converting to an approximate value of individual consumption. The analyses of the consumption were calculated according to the type of diet offered, the standard chow (standard rat diet 2.93 kcal/g) or hypercaloric diet (cafeteria diet 4.18 kcal/g and included the 0.42 kcal/mL of soda drink) (de Oliveira et al., 2015). Animals were weighed weekly, and the change in body weight was calculated by the difference between the final and baseline weights (de Oliveira et al., 2015).

2.7. Open-field test (OF)

To assess locomotor and exploratory activities, an OF test was carried out; the apparatus was a varnished wooden cage ($60 \times 40 \times 50\,cm^3$) with a front glass wall. The linoleum floor was divided by black lines into twelve $13 \times 13\,cm^2$ squares. Each trial began immediately after the animals were placed in the left rear corner and allowed to explore their surroundings for 5 min (Bianchin et al., 1993; Medeiros et al., 2012). After the end of each trial, the box was cleaned with 70% alcohol, and then water, to remove any animal scent. Three measures were evaluated during the test: (1) the total number of crossings, (2) the number of occurrences of rearing behavior (i.e., vertical activity) and (3) the latency until the animal left the first quadrant (in seconds). The number of line crossings (all paws crossing the boundary of a marked-out adjacent area) was taken as a measure of locomotor activity (Roesler et al., 1999). The latency to leave the first quadrant was considered an indicator of anxiety-like behavior (Britton and Britton, 1981). The period of time that the animals were standing on hind legs (Wells et al., 2013) was used to evaluate exploratory activity (Medeiros et al., 2012; Marques Filho et al., 2016).

2.8. Elevated plus-maze test (EPM)

The EPM test was used mainly to assess anxiety-like behaviors. The EPM apparatus consisted of two closed arms ($50 \times 40 \times 10$ cm), which extended from a common central platform (10×10 cm) and elevated 50 cm above the floor. The animal was placed in the EPM central area facing one of the open arms. During a 5 min session, the following behavioral measures were recorded: (1) number of non-protected head-dipping movements (NPHD), (2) total time spent in the open arms (TOA), (3) total time spent in the closed arms (TCA), and (4) number of open arm entries (EOA). Non-protected head-dipping movements were considered to occur when the animal dipped its head over the sides of the maze while in an open arm. In the EPM, an entry into a new area was counted when all four paws crossed into the new arm or the central area (Marques Filho et al., 2016). After each test, the apparatus was cleaned to remove any scent from the previously tested rat.

2.9. Palatable food consumption test

All animals had access to palatable food for five days for 3 min to adapt them to the new diet. For this purpose, the rats were placed in an illuminated rectangular box ($40 \times 15 \times 20\,cm^3$) with floors and wooden walls and a roof glass, and 10 Froot Loops (Kellogg's®-pellets consisting of wheat and corn starch and sucrose) were placed at one end of the box. Forty-eight hours after the learning period all animals were subjected to two test sessions of 5 min in the same rectangular box, while the number of ingested pellets was recorded. In one test session, the rats were fed ad libitum (post-feeding test), while in the other test session they fasted for 22 h prior to the behavioral task (post-fasting test). A protocol was established for counting when results were partially consumed (eg 1/3 or ¼) (Macedo et al., 2016; Gamaro et al., 2003).

2.10. Tissue collection

The animals were killed by decapitation 24 h after the completion of the palatable food test. The cortex was removed and frozen at −80 °C for subsequent analysis. The liver and visceral adipose tissue were dissected manually and weighed using a semi-analytical balance. The relative weight were calculed (weight tissue/body weight × 100) and expressed as grams of tissue per 100 g of body weight.

2.11. Biochemical assays

Levels of BDNF of IL-10, IL-1β and TNF-α were determined by Enzyme-Linked Immunosorbent Assay test (ELISA) sandwich using monoclonal antibodies specific for BDNF, IL-10, IL-1β and TNF-α, (R & D Systems, Minneapolis, USA). Total protein was measured by Bradford method using bovine serum albumin as standard.

2.12. Statistical analysis

Normality was verified for all variables using the Shapiro-Wilk test, and parametric and nonparametric tests were used to analyze data with or without normal distribution. The weight delta, Lee index, palatable food consumption, tissue weight, cytokines, and BDNF concentration were analyzed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls tests as a post hoc correction for multiple comparisons. A generalized estimating equation (GEE) followed by Bonferroni corrections was performed to analyze the results of food intake. Data from OF and EPM tests did not show normal distribution and were analyzed using the Kruskal-Wallis test followed by Dunn's multiple comparison test for the analysis pre-treatment and post-treatment individually. Data are expressed as mean ± standard error of the mean (S.E.M.) (parametric data) or median ± interquartile range (nonparametric data). Statistical significance was set at $p < .05$. The data were analyzed statistically using SPSS software version 20 (SPSS Inc., Chicago, IL).

3. Results

3.1. Assessment of weight parameters and caloric intake

The animals that consumed the hypercaloric diet showed an increase in the weight delta (HDS and HDT groups) and Lee index (HDS group), confirming the obesity induction (one-way ANOVA, $F_{(3,36)} = 7.48$, $p < .05$; Fig. 3 panel A; $F_{(3,35)} = 4.59$, $p < .05$; Fig. 3 panel B, respectively). These results demonstrate that bicephalic tDCS treatment reversed the increase in the Lee index, so that the HDT group became more similar to the groups that received a standard diet. The caloric intake was evaluated during the 8 weeks of the experiment and we found an interaction between group and time (GEE test, Wald $\chi^2 = 1134.731$, $p < .05$, $n = 80$; Fig. 3 panel C). As expected, the caloric intake of rats fed a high calorie diet (HDS and HDT) was higher than that of rats fed only standard chow (SDS and SDT), over the course of eight weeks. However, in the fifth week the HDS group showed increased caloric intake compared to the HDT group.

3.2. Assessment of relative tissues weights: liver and visceral adipose tissue

An increase in relative liver weight was observed in both hypercaloric diet groups (one-way ANOVA, $F_{(3,36)} = 6.49$, $p < .05$; Fig. 4 panel A). The hypercaloric diet lead to an increase in visceral adipose tissue weight in sham animals (HDS) (one-way ANOVA, $F_{(3,23)} = 12.36$,

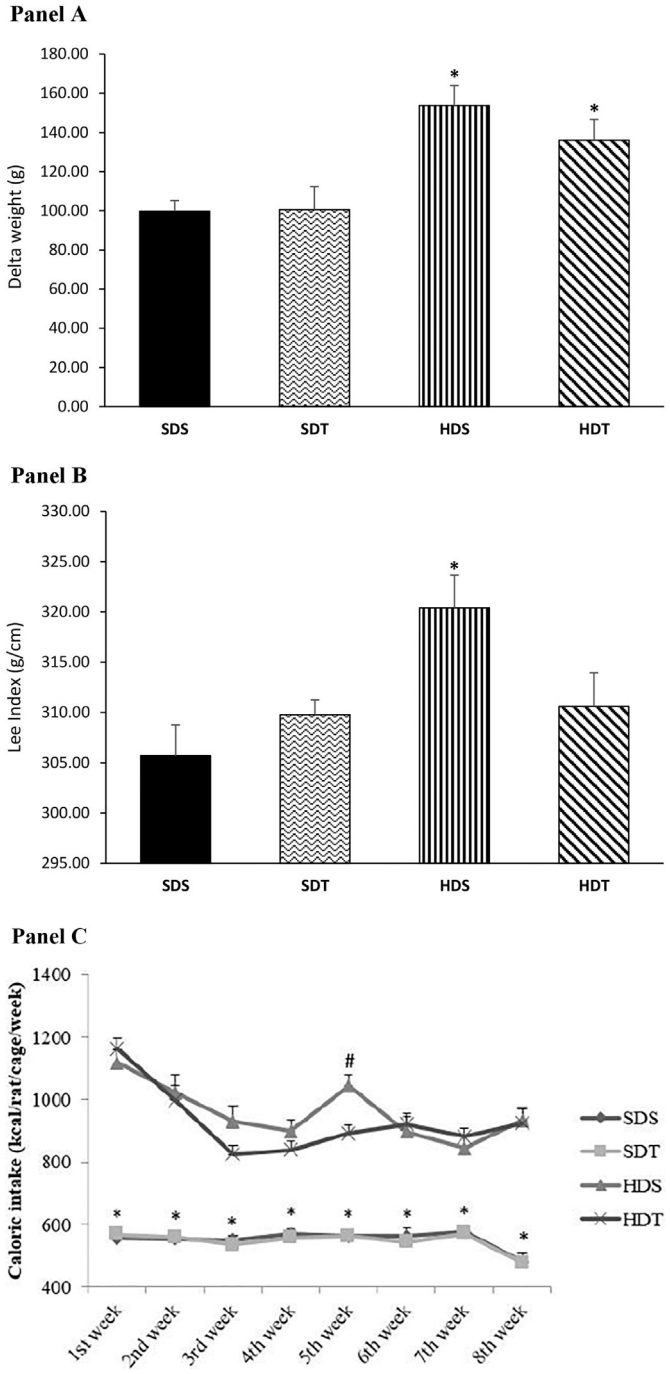

Fig. 3. Assessment of weight parameters and caloric intake. Data expressed as mean ± SEM. (SDS) standard diet plus sham tDCS treatment; (SDT) standard diet plus tDCS treatment; (HDS) hypercaloric diet plus sham tDCS treatment; (HDT) hypercaloric diet plus tDCS treatment. Panel A. Delta weight (n = 10 per group). * significant difference from SDS and SDT groups (one-way ANOVA/ SNK, $p < .05$). Panel B. Lee Index (n = 10 per group). * significant difference from other groups (one-way ANOVA/ SNK, $p < .05$).

$p < .05$; Fig. 4 panel B), and this effect was reverted by exposure to bicephalic tDCS.

3.3. Effect of tDCS on locomotor and exploratory activities and anxiety-like behavioral parameters

The OF test showed no significant difference between the groups before and after applying tDCS regarding the total number of crossings,

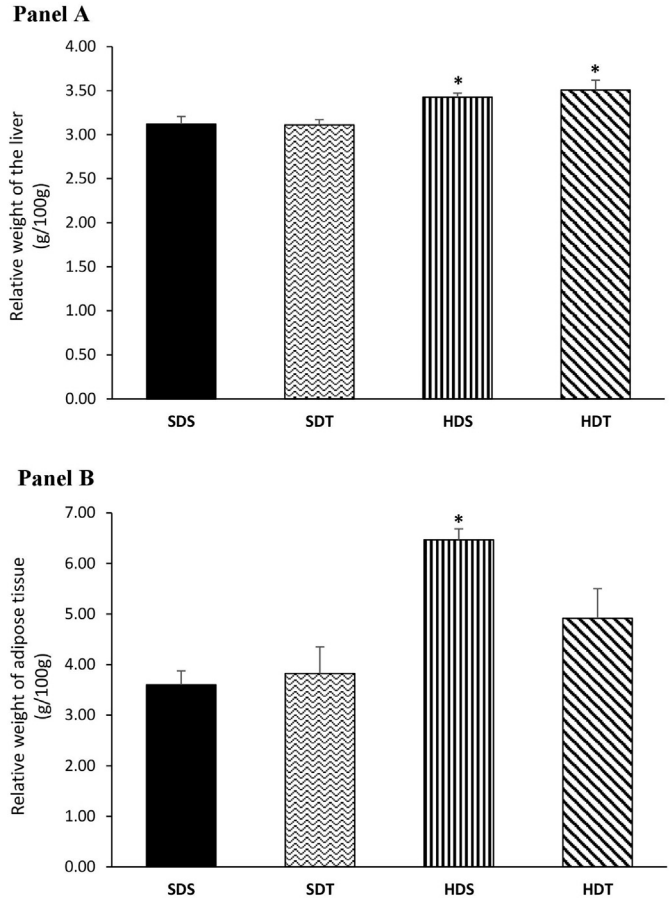

Fig. 4. Assessment of the relative tissue weights. Data expressed as mean ± SEM. (SDS) standard diet plus sham tDCS treatment; (SDT) standard diet plus tDCS treatment; (HDS) hypercaloric diet plus sham tDCS treatment; (HDT) hypercaloric diet plus tDCS treatment. Panel A. Relative weight liver (n = 10 per group). * significant difference from SDS and SDT groups (one-way ANOVA/ SNK, $p < .05$). Panel B. Relative weight adipose tissue (n = 6–9 per group). * significant difference from other groups (one-way ANOVA/ SNK, $p < .05$).

rearing behavior, and latency to leave the first quadrant (Kruskal–Wallis test, pre-treatment: $\chi^2 = 2.27$, $\chi^2 = 5.57$ and $\chi^2 = 5.13$, respectively, $p > .05$ and post-treatment: $\chi^2 = 2.91$, $\chi^2 = 6.31$ and $\chi^2 = 1.65$, respectively, $p > .05$). When comparing intra group pre and post tDCS treatment, there is significant reduction in the number of rearing behavior in the animals fed a standard diet (SDS and SDT) (Wilcoxon test, $Z = -2.80$ and $Z = -2.39$, respectively, $p < .05$; Table 1). The active tDCS group (SDT) showed a decrease in the latency to leave the first quadrant (Wilcoxon test, $Z = -2.69$, $p < .05$; Table 1). The latter effect also was observed in the HD groups (HDS and HDT) (Wilcoxon test, $Z = -2.61$ and $Z = -2.81$, respectively, $p < .05$; Table 1). The EPM test revealed no significant difference between the groups before treatment with tDCS in the parameters for NPHD, TOA, TCA, and EOA (Kruskal–Wallis test, pre-treatment: $\chi^2 = 2.98$, $\chi^2 = 4.45$, $\chi^2 = 2.77$ and $\chi^2 = 3.41$, respectively, $p > .05$; Table 2). However, in the post-treatment analysis the HDS group showed increased NPHD and decreased TCA compared to the SDS group (Kruskal–Wallis test, post-treatment: $\chi^2 = 11.04$, $\chi^2 = 8.76$, respectively, $p < .05$; Table 2), suggesting an anxiolytic effect of the hypercaloric diet. There were no significant differences for TOA and EOA between groups (Kruskal–Wallis test, $\chi^2 = 5.55$, $\chi^2 = 3.77$, respectively, $p > .05$; Table 2). However, comparing pre and post tDCS treatment, the HDS group showed increased NPHD, TOA, EOA, and decreased TCA (Wilcoxon test, $Z = -2.11$, $Z = -2.80$,

Table 1
Open field test. Data expressed as median ± interquartile range (n = 10 per group).

	Pre treatment			Post treatment		
	Crossing	Rearing	Latency	Crossing	Rearing	Latency
SDS	70.50 ± 36.25	23.50 ± 13.25	6.00 ± 11.00	59.00 ± 64.75	17.00 ± 70.75*	2.50 ± 4.50
SDT	78.50 ± 43.00	24.50 ± 11.25	7.50 ± 4.00	79.00 ± 34.25	13.50 ± 13.25*	2.50 ± 1.00*
HDS	85.50 ± 49.75	19.00 ± 6.50	4.50 ± 5.25	65.50 ± 64.25	15.50 ± 13.50	2.50 ± 3.25*
HDT	88.50 ± 22.00	24.00 ± 9.25	10.50 ± 12.50	89.50 ± 55.00	25.50 ± 24.25	3.00 ± 6.75*

Note: (SDS) standard diet plus sham tDCS treatment; (SDT) standard diet plus tDCS treatment; (HDS) hypercaloric diet plus sham tDCS treatment; (HDT) hypercaloric diet plus tDCS treatment; Crossing and rearing presented in absolute numbers, and latency, in seconds.
* Significant difference from pre treatment (Wilcoxon test, $p < .05$).

Z = −2.56 and Z = −2.19, respectively, $p < .05$; Table 2); however, this effect was not observed in the active tDCS group (HDT) (Wilcoxon test, $p > .05$ for all, Table 2).

3.4. Effect of tDCS on palatable food test

In the PFT test, bicephalic tDCS treatment did not alter food consumption in the first phase of the test (post-feed) (one-way ANOVA/ SNK, $F_{(3,38)} = 2.14$; $p > .05$; Fig. 5), but we found a decrease in consumption in the second phase of the test (post-fasting) for the HD group (HDT) (one-way ANOVA/SNK, $F_{(3,38)} = 3.82$; $p < .05$; Fig. 5) compared to the sham treatment group (HDS).

3.5. Effect of tDCS on central levels of BDNF, IL-10, IL-1β, and TNF-α

We found a decrease in BDNF levels in the HD groups (HDS and HDT) compared to the standard diet groups (SDS and SDT) (one-way ANOVA, $F_{(3,36)} = 10.96$, $p < .05$; Fig. 6 panel A). The IL-10 levels also decreased in the HD groups (HDS and HDT) compared to the standard diet groups (SDS and SDT) (one-way ANOVA, $F_{(3,34)} = 14.21$, $p < .05$; Fig. 6 panel B). However, pro-inflammatory cytokine levels (IL-1β and TNF-α) decreased in the HDT group (one-way ANOVA, $F_{(3,36)} = 5.69$ and $F_{(3,35)} = 7.19$, respectively, $p < .05$; Fig. 6 panel C and D) compared to the other groups, suggesting a state-dependent effect of tDCS, since the reduction occurred only in animals that were fed a hypercaloric diet and, as a consequence, became obese.

4. Discussion

In this study, we show that bicephalic tDCS treatment reverses the increase in the Lee Index as well as in relative visceral adipose tissue weight, and reduces the post-fasting intake of palatable foods of animals that were fed a hypercaloric diet. As expected, we found an increase in the caloric intake of animals on a high caloric diet, and bicephalic tDCS treatment did not reverse this effect. Surprisingly, in the fifth week, animals on a hypercaloric diet that were submitted to sham tDCS (HDS) showed an increase in caloric consumption in comparison to animals submitted to the active treatment (HDT). In addition, in the OF test, the bicephalic tDCS treatment reduced the latency to leave the first quadrant in animals placed on a standard diet. Moreover, animals fed a hypercaloric diet showed a decrease in BDNF and IL-10 levels, and the association with bicephalic tDCS treatment decreased IL-1β and TNF-α levels in the cerebral cortex. We can therefore assume that bicephalic tDCS treatment has a state-specific effect on biometric parameters, behavior, and inflammatory mediators, since the observed effect is different according to the diet type of the animals.

It is important to highlight that for the tDCS technique to be applied without the use of anesthetics, it is necessary to restrain the animal. Thus, to avoid a restraint stress bias in our study, we insert in the sham group, which the animals were restrained during 20 min. During this time, the electrodes were positioned on the animal's head, while the stimulator was turned off. In this way, all groups were exposed to the apparatus and all groups were homogeneous regarding behavioral parameters.

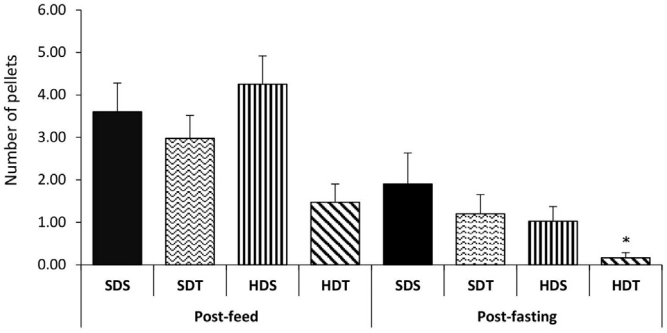

Fig. 5. Palatable food consumption test. Data expressed as median ± mean ± SEM (n = 10 per group). (SDS) standard diet plus sham tDCS treatment; (SDT) standard diet plus tDCS treatment; (HDS) hypercaloric diet plus sham tDCS treatment; (HDT) hypercaloric diet plus tDCS treatment. Post-feeding. There was no significant difference between groups (one-way ANOVA/ SNK, $p > .05$). Post-fasting. * significant difference from HDS group (one-way ANOVA/ SNK, $p < .05$).

Table 2
Elevated plus maze test. Data expressed as median ± interquartile range (n = 9–10 per group).

	Pre treatment				Post treatment			
	NPHD	EOA	TOA	TCA	NPHD	EOA	TOA	TCA
SDS	1.00 ± 4.00	1.50 ± 3.25	10.50 ± 23.00	269.00 ± 85.00	0.00 ± 1.00	0.50 ± 4.00	3.00 ± 44.50	267.50 ± 35.00
SDT	3.00 ± 6.50	2.00 ± 3.00	36.00 ± 53.00	222.00 ± 46.50	2.00 ± 4.50	2.00 ± 1.00	32.00 ± 40.50	255.00 ± 77.50
HDS	0.00 ± 3.50	0.00 ± 2.00	0.00 ± 34.75	274.00 ± 65.75	3.00 ± 3.25[a,b]	2.00 ± 2.00[a]	40.00 ± 48.50[a]	232.50 ± 60.25[a,b]
HDT	2.50 ± 6.75	1.00 ± 3.00	14.00 ± 48.75	258.00 ± 63.25	2.00 ± 4.25	1.50 ± 1.25	22.50 ± 25.00	259.50 ± 45.75

Note: (SDS) standard diet plus sham tDCS treatment; (SDT) standard diet plus tDCS treatment; (HDS) hypercaloric diet plus sham tDCS treatment; (HDT) hypercaloric diet plus tDCS treatment; (NPHD) non-protected head-dipping movements; (EOA) entries into open arms; (TOA) time on open arms; (TCA) time on closed arms. NPHD and EOA presented in absolute numbers, and TOA and TCA, in seconds.
[a] Significant difference from pre treatment (Wilcoxon test, $p < .05$).
[b] Significant difference from SDS group in post treatment (Kruskal-Wallis test, $p < .05$).

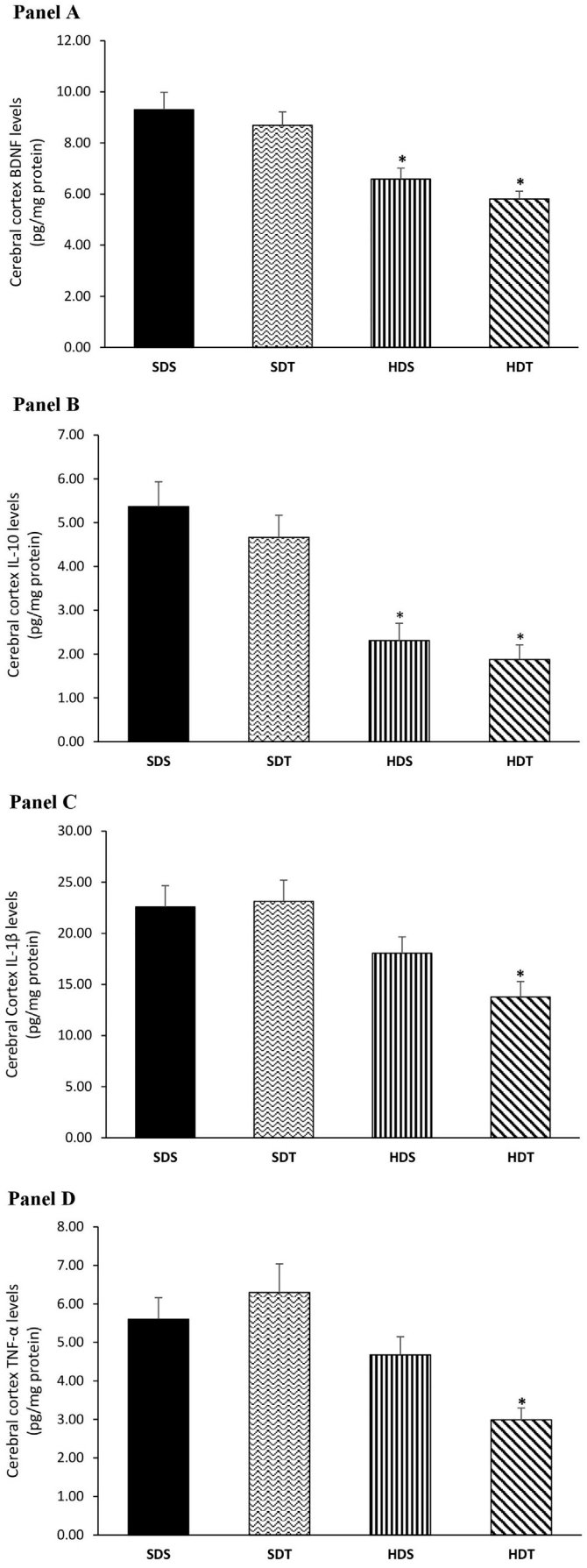

Fig. 6. BDNF, IL-10, IL-1β and TNF-α levels in cerebral cortex. Data expressed as mean ± SEM. (SDS) standard diet plus sham tDCS treatment; (SDT) standard diet plus tDCS treatment; (HDS) hypercaloric diet plus sham tDCS treatment; (HDT) hypercaloric diet plus tDCS treatment. Panel A. BDNF levels (n = 10 per group). * significant difference from SDS and SDT groups (one-way ANOVA/ SNK, $p < .05$). Panel B. IL-10 levels ($n = 9–10$ per group). * significant difference from SDS and SDT groups (one-way ANOVA/ SNK, $p < .05$). Panel C. IL-1β levels (n = 10 per group). * significant difference from SDS, SDT and HDS groups (one-way ANOVA/ SNK, $p < .05$). Panel D. TNF-α levels (n = 9–10 per group). * significant difference from SDS, SDT and HDS groups (one-way ANOVA/ SNK, $p < .05$).

Comparing pre and post bicephalic tDCS treatment (intra group), the hypercaloric diet groups presented an increase in the EOA, TOA and NPHD in the EPM. These results can indicate an anxiolytic-like effect of hypercaloric diet, which was able to reverse some characteristics of anxious behaviors. In addition, the restraint exposure decreases the time latency to leave the first quadrant in the open field test in these animals; however, the bicephalic tDCS treatment is not able to reverse this effect.

Between-group comparisons showed that after bicephalic tDCS treatment, the animal exposure to restraint and standard diet lead to a decrease in rearing in the OF test, and this effect was not reverted by active bicephalic tDCS treatment. Hypercaloric diet exposure increased NPHD and reduces TCA compared to standard diet groups, in agreement with the anxiolytic effect already observed in the intra group's analyses. These results suggest that hypercaloric diet may be attenuating the effect of restraint stress required to bicephalic tDCS technique application. It is known that acute or chronic stress can cause changes in food control (Dallman et al., 2004), and palatable foods may decrease the stress response of rats (Pecoraro et al., 2004). This could also explain a higher caloric intake of the animals that were exposed to restraint (HDS group) in the fifth week and this effect was attenuated in the following weeks. A recent study undertaken in our research group showed that exposure to chronic stress increases anxiety-like behavior and this effect is reversed by consumption of a hypercaloric diet (de Oliveira et al., 2015).

Stress, combined with overeating can lead to overweight and abdominal obesity, which is related to higher waist-to-hip-ratio and body mass index (BMI). In addition, a previous study showed a relationship between energetic supply of the brain and body weight regulation (Schmoller et al., 2010). A study in humans suggests that tDCS can modulate brain energy in the DLPFC and thus reduce body weight (Jauch-Chara et al., 2014). In this study, bicephalic tDCS treatment reversed the increase in the Lee index as well as in visceral adipose tissue weight induced by a hypercaloric diet. These findings therefore demonstrate that bicephalic tDCS is able to reduce obesity parameters, and suggest tDCS that could be a non-pharmacology tool for the treatment of metabolic diseases.

In addition, a diet high in fat and sugar can trigger an irresistible motivation for consumption known as “food craving.” A recent study from our research group showed that tDCS reduces palatable food craving in rats (Macedo et al., 2016), corroborating earlier studies in humans (Fregni et al., 2008; Goldman et al., 2011). In the present study, bicephalic tDCS decreases food intake after fasting (HD group) in relation the sham treatment (HDS). The molecular mechanisms by which repeated tDCS reduces food craving are not yet established. It is known that the consumption of highly palatable foods can trigger binge eating, similar to compulsive drug mechanisms (Gearhardt et al., 2011). These highly palatable foods high in sugars activate the reward system, modified eating behavior (Novelle and Diéguez, 2018; Dalton et al., 2013). The DLPFC, an important area of the brain responsible for motivation and decision-making, has been investigated regarding treatment for addiction and compulsion. Studies have shown that DLPFC plays an important role in taste (Brody et al., 2002; McBride et al., 2006; Wilson et al., 2004). In addition, the repeated application of tDCS

in DLPFC reduces food craving (Fregni et al., 2008; Uher et al., 2005), alcohol consumption (Boggio et al., 2009), and nicotine and smoking craving (Amiaz et al., 2009). The tDCS modulates dopaminergic pathways by cortical stimulation (Nitsche et al., 2006), and palatable food intake acts way mesolimbic dopamine system that has an important role in reward circuits (Nicola, 2016).

Obesity results in a low-grade inflammatory state with increased body fat, particularly visceral fat, impairing to metabolic control and body weight. This low-grade inflammatory state also affects other regions and organs, such as the liver, pancreas, and brain (Gregor and Hotamisligil, 2011). Previous studies describe some metabolic changes induced by obesity and brain sensitivity to these changes (Lopez et al., 2007; Kanoski et al., 2007; Velloso and Schwartz, 2011). The present study shows that a hypercaloric diet is associated with a decrease in BDNF levels in the cerebral cortex, corroborating findings from previous studies (Franco-Robles and Lopez, 2016; Meireles et al., 2016). In addition, we showed that exposure to a hypercaloric diet also reduces cortical levels of IL-10. BDNF is a neurotrophin responsible for cell survival and synaptic plasticity (Leal et al., 2014; Bekinschtein et al., 2014), as well as to modulates orexigenic and anorexigenic signaling pathways (Rios, 2013). Studies have indicated that different regulators of appetite, such as leptin, insulin, neuropeptide Y (NPY), galanin-like peptide (GALP) and pancreatic polypeptide potentially exert anorexigenic effects through BDNF (Sainsbury et al., 2010; Rosas-Vargas et al., 2011; Sergeant et al., 2017; Fukasaka et al., 2018). Furthermore, in the adult central nervous system, BDNF displays a widespread distribution pattern on structures such as the hippocampus, amygdala, hypothalamus and cerebral cortex (Pruunsild et al., 2007). Likewise, there are important neurotransmitters such as serotonin, which participate in the controlling appetite as an inhibitor of calorie intake in rodents (López-Alonso et al., 2007; Lam et al., 2008), and the dopamine which plays an important role in palatable food consumption (Szczypka et al., 2001). Although, tDCS may not modify feeding behavior through BDNF in our study, it is well established that the DLPFC is also implicated in higher-order reward processing as part of its involvement in the mesocortical dopaminergic pathway (Lee et al., 2018). Indeed, dopaminergic signaling within the DLPFC has been linked to regulation of food intake. In this way, we understood as a limitation of the study not to have measured the levels of leptin, Neuropeptide Y for example, as well as brain activity of dopamine neurotransmitter, since these dosages could have contributed to the observation of behavioral modifications. Additionally, it has been demonstrated that IL-10 may play an important anti-inflammatory role in the central nervous system (Valdearcos et al., 2015), and IL-10 hypothalamic infusion inhibits inflammatory action in this brain region of obese rats, contributing to a decreased caloric intake and control of energy balance (Ropelle et al., 2010). Our findings agree with the results of previous studies that showed that the obesity process induces a decrease in anti-inflammatory activity (Arslan et al., 2010; Galic et al., 2010), and we show an increase in pro-inflammatory interleukins. Hypercaloric diet does not increase central levels of pro-inflammatory interleukins which can be explained by adaptive processes maintaining CNS homeostasis. In addition, it is possible that these effects were related to diet type and exposure time. Interestingly, it was observed that the tDCS decreased IL-1β and TNF-α levels in the HD groups, compared to SD groups. The role of TNF-α is complex; it has pleiotropic effects and may have positive and negative effects on the brain (Parimisetty et al., 2016). Highlight that bicephalic tDCS decrease pro-inflammatory interleukins levels the only in altered state, as obesity. The mechanisms by which tDCS exerts its effects are not fully understood.

It is important to highlight that this study has some limitations, including difficulty in the employment of the tDCS technique due to the small size of the rat head, that contributed to the designation of "treatment tDCS bicephalic," as well as the lack of evaluation of other structures involved in feeding behavior and the reward system. Furthermore, our result confirms the important role of the prefrontal cortex in food behavior, which can be modulated by noninvasive brain stimulation. Importantly, although our results give additional evidence to support the relationship between dorsolateral prefrontal cortex activity and its effectiveness in obese animals after fasting craving; the exact mechanisms by which dorsolateral prefrontal cortex modulation by tDCS decrease craving are still unknown. Thus, this study has some limitations. First, tDCS conventional technology has certain limitations and these include focality (area stimulated), depth of penetration, and targeting-location control (Brunoni et al., 2012; Macedo et al., 2016). Second, we did not assess the effects food craving in others brain structures such as the brainstem, and striatum due to its participation in the mesocorticolimbic system (Biederman and Faraone, 2002). Third, measure the levels of leptin, Neuropeptide Y for example; also, to evaluate central levels of serotonin and dopamine as well as receptor expression directly related to the reward system because another alternative explanation is that stimulation of the prefrontal cortex stimulated dopaminergic pathways. In this way, modeling studies are expected to play a critical role in the development of next-generation tDCS technologies and approaches.

Taken together, this study demonstrates that bicephalic tDCS treatment is effective in reducing food craving in obese animals after fasting, as demonstrated in animals (Macedo et al., 2016) and human studies (Fregni et al., 2008). In addition, tDCS treatment reverses the increased Lee index and the increase in the weight of visceral adipose tissue in these animals. Furthermore, we found that exposure to a hypercaloric diet decreases levels of BDNF and IL-10 in the cortex. On the other hand, bicephalic tDCS treatment reduces IL-1β and TNF-α levels in the cortex. Exposure to a hypercaloric diet induced an anxiolytic effect that was reversed by treatment with bicephalic tDCS. Therefore, exposure to repeated treatment with tDCS appears to act in pathways related to eating behavior that modulate neuroplastic changes in obesity features. The mechanism by which the tDCS inhibits food craving in animals and humans probably involves neurobiological processes linked to reward, motivation, and decision-making (Wang et al., 2006).

5. Conclusion

In conclusion, the present study demonstrates that bicephalic tDCS can modulate biometric and inflammatory parameters, as well as feeding and anxiety-like behaviors in rats submitted to a hypercaloric diet, a finding that corroborates human studies. This research provides a framework for the exploration of the behavioral and neurochemical effects of tDCS in the prevention or treatment of obesity and food craving. Further research to determine the mechanisms underlying the effects shown in this study could benefit both our understanding of the pathophysiology of metabolic diseases and the neurobiological substrates of tDCS. Therefore, new studies are warranted to fully elucidate the mechanisms of tDCS, thus enhancing the knowledge about the immediate and delayed effects of this technique.

Panel C. Caloric intake (n = 10 per group). * significant difference between SDS and SDT groups and HDS and HDT groups (GEE, $p < .05$). # significant difference from HDT group in the fifth week (GEE, $p < .05$).

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Conflict of interest

There was no financial interest between any of the authors or any commercial interest in the outcome of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.npep.2018.09.006.

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