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Molecular and elemental effects underlying the biochemical action of transcranial direct current stimulation (tDCS) in appetite control

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ABSTRACT

Recent studies highlight that obesity may alter the electric activity in brain areas triggering appetite and craving. Transcranial direct current brain stimulation (tDCS) has recently emerged as a safe alternative for treating food addiction via modulating cortical excitability without any high-risk surgical procedure to be utilized. As for anodal-type tDCS (atDCS), we observe increased excitability and spontaneous firing of the cortical neurons, whilst for the cathodal-type tDCS (ctDCS) a significant decrease is induced. Unfortunately, for the method to be fully used in a clinical setting, its biochemical action mechanism must be precisely defined, although it is proposed that molecular remodelling processes play in concert with brain activity changes involving the ions of: Na, Cl, K and Ca. Herein, we proposed for the first time Fourier transform infrared (FTIR) and synchrotron X-ray fluorescence (SRXRF) microprobes for a combined molecular and elemental analysis in the brain areas implicated appetite control, upon experimental treatment by either atDCS or ctDCS. The study, although preliminary, shows that by stimulating the prefrontal cortex in the rats fed high-caloric nutrients, the feeding behavior can be significantly changed, resulting in significantly inhibited appetite. Both, atDCS and ctDCS produced significant molecular changes involving qualitative and structural properties of lipids, whereas atDCS was found with a somewhat more significant effect on protein secondary structure in all the brain areas investigated. Also, tDCS was reported to reduce surface masses of Na, Cl, K, and Ca in almost all brain areas investigated, although the atDCS deemed to have a stronger neuro-modulating effect. Taken together, one can report that tDCS is an effective treatment technique, and its action mechanism in the appetite control seems to involve a variety of lipid-, protein- and metal/non-metal-ion-driven biochemical changes, regardless the current polarization.

1. Introduction

Current evidence demonstrated that the alterations affecting brain electric activity, including neuroplasticity and cortical excitability, lie behind many neuropsychiatric disorders [1]. In order to develop new treatment strategies, as long as fifty years ago, it was discovered that cortical excitability could be modified upon application of external stimuli, like those involving direct currents exerted on the brain cortex [2]. With the recent outstanding iterations of nanotechnology and electrical engineering, transcranial direct current brain stimulation (tDCS) has deservedly received great attention as a novel and safe alternative for treating a number of neuropsychiatric alterations, showing significant clinical effects either as a monotherapy or in tandem with other modalities, with pharmacology and training being selected examples [3]. By triggering the current flow, tDCS can either increase or decrease neuronal excitability by affecting membrane depolarization or hyperpolarization [4]. As for anodal-type tDCS (atDCS), we observe increased excitability and spontaneous firing of the cortical neurons, whilst for the cathodal-type tDCS (ctDCS) a significant decrease is induced [5]. Modulation of cortical excitability in major depression, chronic pain, stroke, neurodegenerative disorders including Parkinson and Alzheimer disorders covers just a few selected, most spectacular applications of it in a clinical setting [6–11]. However, over recently, translating tDCS into the field of appetite control can be particularly important due to alarmingly growing number of obese individuals in developed countries, with around 38% of the world's adult population estimated to be overweight by 2030 (even 85% in USA) [12]. Obesity is a complex and multifactorial disease that greatly increases the disease morbidity by elevating the risk for other alterations: depression, cardiovascular disease, diabetes, disability and even certain types of cancer [13,14]. Building

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upon the data from most modern obesity neuroimaging, it is often hypothesized that the imbalance in the global electric activity in the prefrontal and limbic brain circuits may be major driving forces supporting multiple aspects of obsessive eating behaviors: cognition- as well as reward-related ones [15,16]. To date, the existing research provided promising data showing a significant reduction in food craving following just one session of the dorsolateral frontal coretx (DLFC) tDCS [17,18]. There are also another candidate target brain areas integrating all fundamental aspects of eating behavior: homeostasis, reward, and cognition. One of them, the hypothalamic nuclei (HTA): the peduncular part of the hypothalamic nuclei (PLH), ventromedial nucleus of the hypothalamus (VMH) and arcuate nucleus (ARC) regulate homeostatic food intake. At the same time, the amygdala (AMY) is responsible for rewarding effects of feeding [19]. Notably, although HTA and AMY are very deep targets, possibly better reached via more invasive deep brain stimulation (DBS), the recent evidence argues that lateral prefrontal hyperactivity may be another compensatory mechanism that can be evoked by tDCS to overcome obesity using easily accessed frontal cortex targets [20,21]. Also, up to now, a number of studies on the molecular events underpinning obesity and diabetes posed the hypotheses on a putative convergence between obesity and molecular burden by oxidative stress neurotxicity in the brain [22]. Specifically, the data from several studies reported that obesity is linked to the reduced expression of several antioxidant proteins: the superoxide dismutases (SOD), glutathione peroxidase (GPx), catalase (CAT), vitamin A, vitamin C, and β-carotene [23,24]. Several studies have also documented that obesity-induced oxidative stress in the peripheria may exacerbate blood-brain barrier disruption, augmenting further neuroinflammation and levels of reactive oxygen species (ROS) in the brain [25,26]. Increased extent to glucose-driven oxidative stress via excitotoxicity was found in the brains of obese individuals owing to significantly increased levels of Ca^{2+} in HTA [27]. With this regard, the very recent study by Lu et al. provided a compelling evidence that tDCS can both ameliorate behavioral deficits and reduce oxidative stress by elevating the brain levels of GSH and SOD in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-induced (MPTP) animal model of Parkinson disease [28].

Despite increased workforce towards explaining the biochemical action of tDCS, there are many unexplored fundamental aspects that may render its pre-clinical application successful before it is attempted in clinical trials on human individuals: putative relation with energy metabolism and potential metabolic interferences [20]. To do so, the fundamentals aspects thereof, including the relation with lipid/protein composition along with any changes involving brain currents including: Na^{+}, K^{+}, Cl^{-}, Ca^{2+} levels must be known [29]. To our greatest knowledge, there is not at all any in-situ study showing any association between molecular and electrical activity changes directly in the brain areas regulating food craving in relation to the current polarity: atDCS vs ctDCS. Herein, we propose for the first time the use of both benchtop Fourier transform infrared (FTIR) and synchrotron radiation induced X-ray fluorescence (SRXRF) micro-spectroscopies for a combined molecular and elemental brain tissue imaging, so that these complimentary analytical tools can be robustly used to study fundamental aspects behind the biochemical response to atDCS and ctDCS in the brains of rats fed with high caloric nutrients. FTIR spectroscopy has recently experienced tremendous progress in terms of acquisition time and spectral resolution, and is now commonly used for a nonperturbative, label-free analysis of biochemical information enabling in-situ imaging of neural functionality and metabolism [30,31]. Interestingly, the method has been successfully implemented for molecular lipid/protein profiling of tissues taken from animal models of obesity [32,33]. In turn, burgeoning interest on the significance of alkali and alkaline metals in active cell transport and cell signaling made it viable to perform their direct imaging at regional, cellular and even sub-cellular spatial resolution using SRXRF [34]. Despite the low energy K_{α} lines of metal ions, in particular: Na, strongly absorbed in the air, Gianocelli et al. demonstrated the feasibility of synchrotron-based soft X-ray imaging modalities for their fast imaging in complex biological systems using highly brilliant synchrotron facilities [35,36]. Szczerbowska-Boruchowska et al. proposed for the first time the use and tested the utility of external standard SRXRF for major and trace metals' quantitative elemental mapping in brain tissue taken from the rats subjected to UVNS and the baseline sham stimulated ones. Of all the results, the authors concluded statistically significant aberrations in Cl/Ca metabolism in the corpus striatum and substantia nigra [37].

Having highlighted the analytical advantages of SRXRF and FTIR, one can infer that the methods have sufficient scientific capacity for boosting our knowledge on the interplay between the molecular and electric activity remodelling changes in the brain upon experimental treatment by tDCS. Therefore, in this study, we aimed at showing whether atDCS/ctDCS have any significant impact on lipid/protein composition and brain current ions of Na, Cl, K, Ca levels directly in the brain areas triggering feeding behavior. The research like this is critical for precisely defining the biochemical action of tDCS, which is a major prerequisite for building an adequate knowledge base to guide the global development of this emerging treatment method in a clinical arena.

2. Materials and Methods

2.1. tDCS Protocol

The procedure for tDCS was applied using a constant current stimulator (BrainStim, EMS, Bologna, Italy) for a continuous application of low currents. The currents were applied transcranially through an epicranial electrode fixed to the right or left frontal part of the scalp four days before the stimulation had been started. The procedure for the electrode implantation was based on the surgical technique described by Liebetanz et al. [38]. Under general anesthesia (10% ketamine and xylocaine; 10 and 3 mg/kg intramusculary), the 3.5 mm diameter circular electrode (contact area of 9.6 mm^2), composed of a tubular plastic jacket, was placed over the frontal cortex: 3 mm anterior to the coronal fissure and 3 mm right or left to the sagittal fissure, using a glass ionomer cement (Ketac Cem, ESPE Dental AG, Seefeld, Germany). The treatment target was the dorsolateral prefrontal cortex (DLPFC). The cranial electrode remained fixed over the entire experiment. The counter electrode, composed of a conventional rubber plate electrode (10.5 cm^2, Vermed, Graphic Controls, Poland), was placed on the animal's back using a corset. An unipolar setting of the epicranial electrode was chosen to prevent the bypassing brain currents that would occur when two head electrodes are close to each other. Additionally, asymmetric electrode sizing was used to achieve the highest current density directly beneath the active electrode. A jacket electrode was filled with 0.9% NaCl before stimulation. Both the epicranial electrode and larger back electrode were connected to the DC stimulator controlled by a dedicated software. In atDCS, the current flew from epicranial to back electrode while in cathodal stimulation the direction of electric fields flow was opposite. The procedure for tDCS was applied for conscious rats. Two 10 min sessions daily of sham (SH - 0 μA) or active (200 μA), atDCS or ctDCS was applied throughout eight consecutive days. The current intensity was automatically ramped for 10 s to avoid the abrupt on/off switching. For the stimulation, the rats were placed in separate plastic cages and carefully observed for any behavioral abnormalities. In Fig. 1, the details of the experiments are shown. From this data, one can see that the rats were fed high caloric diet through the whole experiment. The stimulation started from the 47th day of the experiment. The appetite of the animals was assessed by calculation the difference between the amount of chow placed in the feeders (in grams) and the remaining food in each cage using a digital scale (cf. Fig. 2). Histological examination of the rat brain tissue was performed in our previous study with the same tDCS protocol (not published data). The brain tissue was examined to detect possible injury following active tDCS application. Macroscopically, no abnormalities were observed. No signs of neurotrauma, edema, hematoma of other