TITLE

Prefrontal cortex neuromodulation enhances frontal asymmetry and reduces caloric intake in patients with morbid obesity.

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Running title: Neuromodulation of dlPFC reduces caloric intake

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AUTHOR CONTRIBUTIONS

RT, ASF, GR, LF and AC contributed to the conception and design of the study. AG, CS and OC were responsible for patient recruitment and OC was in charge of patient screening and medical supervision. LF and AC contributed to recruitment and data acquisition. MC carried out the interpretation and statistical analysis of the EEG data. AP conducted the endocannabinoid analyses. KP was responsible for the nutritional data collection. KL performed the statistical analysis of the clinical and cognitive data. LF, MC and AC drafted the article. RT, MA, GR, AP and AS contributed to critical revision of the manuscript for key intellectual content. All authors have read and approved the final manuscript.

DISCLOSURE

GR is a co-founder, shareholder, and employee of Neuroelectronics and Starlab which provided Starstim and Stimweaver. MC, and ASF are employees of Starlab which developed Neuroelectronics in 2011. All the other authors declare no potential conflicts of interest concerning the research, authorship, and publication of this article. Our adherence to policies on sharing data and materials remains unaltered.

BULLETPPOINTS:

- What is already known about this subject:
 - Increased activation of reward brain areas and decreased prefrontal cortex basal metabolism have been described in subjects with obesity in response to palatable food cues.
 - Neuromodulation targeted at dlPFC reduces craving and food consumption in subjects with healthy or excess weight.
- What does your study add:
 - Prefrontal tDCS (anode right-dlPFC, cathode left-dlPFC) and CT reduces kcal intake in MO.
 - Combined intervention (one week) reduces left-dlPFC dominance observed at baseline.
 - An increase of interhemispheric functional connectivity predicts BMI reduction.
- How might your results change the direction of research or the focus of clinical practice?

Our results support the use of neuromodulation interventions aiming at restoring frontal coherence and asymmetry as a new approach to regulate eating behavior.

Abstract

Objective: to test the feasibility of a combined intervention involving transcranial direct current stimulation (tDCS) on the dorsolateral prefrontal cortex (dlPFC) and cognitive training (CT). Short-term effects on food-consumption, cognition, endocannabinoid (eCB) levels, and EEG markers of future weight-loss were explored.

Methods: eighteen healthy volunteers with morbid obesity (MO) were randomized in a double-blind, placebo-controlled, parallel-trial. Participants received sham or active tDCS stimulation plus CT for 4 consecutive days. Cognitive performance, daily food intake, and eCB blood samples were collected pre- and post-intervention; EEG data were gathered before and after daily training. **Results:** active tDCS+CT group reversed left-dominant frontal asymmetry and increased frontal coherence (FC) in the γ -band (30-45 Hz) after the intervention. The strength of the latter predicted BMI reduction. Additionally, a large intervention effect on food intake was shown in the active tDCS+CT group at follow-up (-339.6 ± 639 kcal on average), and a decrease of plasma eCB concentrations.

Conclusions: dlPFC modulation through tDCS+CT is an effective tool to restore right dominance of the dlPFC and enhance FC in patients with MO. Moreover, the effect of the strength of FC on BMI suggests that the interhemispheric FC at the dlPFC is functionally relevant for the efficient regulation of food choice.

INTRODUCTION

Obesity is currently one of the most critical issues in public health (1), indeed, body mass index (BMI) has globally increased by 0.4 kg/m^2 per decade. In 2014, nearly 40% of the adult population was overweight ($\text{BMI} \geq 30 \text{ kg/m}^2$) whilst 11% and 14% presented obesity ($\text{BMI} \geq 35 \text{ kg/m}^2$) and morbid obesity (MO) ($\text{BMI} \geq 40 \text{ kg/m}^2$), respectively.

Feeding behavior regulation results from an interaction between homeostatic and hedonic processes which are controlled by differing neural pathways. The former modulates eating behavior in response to nutritional needs by matching energy intake with expenditure, and is mainly controlled by the hypothalamus and the brainstem. The latter fosters eating behavior based on the rewarding and pleasant characteristics of food, especially that which is highly palatable and energy-dense. It depends on the brain's food reward system whose principal component is the dopamine mesocorticolimbic pathway which includes various interconnected nuclei, such as the ventral tegmental area, the nucleus accumbens, and the prefrontal cortex (PFC) (2, 3).

Individuals with obesity/MO exhibit a specific cognitive profile characterized by impairment in executive functions (EF) such as inhibitory control, working memory, decision-making, and risk-taking behaviors, as well as food-related attentional bias (4, 5). The PFC is common to all these cognitive functions and crucially involved in high-level cognition. Neuroimaging studies indicate that appetite decreases activation of the dorsolateral PFC (dlPFC) (6) which has been associated with higher body weight (7) and overeating conditions (8). Moreover, lateralization in dlPFC activation has led to the proposal of the right-brain hypothesis for obesity (9) which states that reduced right dlPFC (r-dlPFC) activity may lead to overeating, and poses a possible involvement in inhibitory control. The exact role of r-dlPFC activation, however, remains controversial as obese subjects who successfully resist cravings display a higher activation in the left dlPFC (l-dlPFC) (10). Such findings suggest that there is a functional asymmetry within the dlPFC that may be associated with the regulation of eating behavior. A theory that is supported by prior studies that relate enhanced right-dominant frontal asymmetry (FA) with dietary restriction (11), and left-dominant FA with reward approaches such as overeating (12, 13).

Taken together, such behavioral and neurophysiological alterations can bias food choice and hinder healthy lifestyle compliance. Adhering to a diet involves a wide range of abilities such as planning, decision-making, and the capacity to self-regulate oneself. That is to say, the ability to direct one's behavior to manage immediate impulses so as to meet long-term goals. Therefore, strategies that tap into the above-mentioned brain mechanisms aiming at strengthening cognitive control might offer a promising therapeutic approach for weight reduction (14). Following this hypothesis, we have targeted body-weight reduction through the use of a combined intervention focusing on neurophysiological and behavioral aspects employing transcranial direct current stimulation (tDCS) and cognitive training (CT). The former is a non-invasive technique that permits the controlled modulation of brain activity and behavior through the generation of electric fields that modulate cortical excitability (15). Through the scalp tDCS delivers mild currents which result in weak electric fields in the brain (16). Such electric fields influence neuronal response (although do not produce action potential per se), enhancing cortical excitability under the anode and decreasing it under the cathode (15). Previous work has demonstrated that enhancing r-dlPFC and inhibiting l-dlPFC reduces food craving in both healthy and obese patients. Nevertheless, other studies involving excess-weight participants have reported that anodal l-dlPFC decreases food craving scores and caloric intake (14). Moreover, Fregni and colleagues (2008) (17) showed that anodal tDCS stimulation at both left and right dlPFC reduced caloric intake, suggesting that dlPFC plays a crucial role in the control of appetite and food intake.

Long-lasting effects of tDCS are likely to be mediated through the neuromodulation of endogenous pathways (18). One candidate is the endocannabinoid system (ECS) whose chemical messengers (endocannabinoids, eCBs) are produced by neural depolarization (19). Their synthesis depends on the release of extracellular calcium which is facilitated

by the relaying of electrical current through synapses. The ECS regulates synaptic transmission (through CB1 receptors) in the hypothalamus and mesolimbic regions. It is thought to stimulate appetite by acting on both the homeostatic and hedonic aspects of food intake. It is noteworthy that the ECS plays a central role in memory and learning processes (20), necessary for the cognitive control of food intake, as well as the shift from habitual to goal-directed actions (21) crucial in transforming unhealthy eating habits. We hypothesize that eCB concentrations may decrease following tDCS intervention thus leading to a reduction in caloric intake.

Regarding CT, it consists of repetitive exercises designed to maintain/improve a number of cognitive skills, including attention, inhibition, and flexibility, based on the ability of the brain to form or modify its connections and pathways (i.e. neuroplasticity) (22). Previous research has demonstrated that some CT, such as response inhibition and attentional bias modification,) are useful in reducing weight, food consumption and/or food cravings in both healthy-weight and individuals with obesity. Growing evidence suggests that the combined administration of tDCS and CT may lead to better outcomes such as improved performance and longer-lasting effects on neuroplasticity (23). We therefore decided to adopt a joint tDCS and CT approach.

This study examined the effect of an intervention combining tDCS on the dlPFC with CT as a novel approach to modulate food intake in participants with MO. The main aims were to: (1) evaluate the short-term effects of the intervention on food intake (as well as other secondary measures related to eating behaviors such as anthropometric, cognitive, and eCB measures); 2) explore whether we could quantify the neurophysiological impact of the intervention through the measurement of dlPFC functional characteristics (e.g. FA and intra/interhemispheric frontal coherence, FC); and 3) predict further weight-loss. We conducted a double-blind, randomized, placebo-controlled, parallel trial. In addition to

recording pre- and post- sessions EEG, we carried out an executive function assessment, a blood test (eCBs), and a nutritional evaluation of all patients before and after intervention (including anthropometric measurements) (Fig. 1A).

MATERIALS AND METHODS

Participants

21 volunteers with MO from the Unit of Endocrinology and Nutrition at the Parc de Salut Mar, Barcelona, were recruited (January 2016-August 2017). Participants were following the standard 1-year program prior to bariatric surgery (i.e diet advice) and naïve to tDCS. Inclusion criteria were the following: 1) BMI $\geq$ 40 or BMI $\geq$ 35 together with diabetes mellitus, dyslipidemia, or high blood pressure; 2) aged 18-60 years; 3) right-handed; 4) enrollment in the program <2 months. Exclusion criteria: 1) past or current neurological conditions or learning disorders that affect cognitive function; 3) mental disorders based on DSM-5 (24); 4) psychoactive drug consumption; 5) past or current disorder treated with lithium; and 6) implanted metal devices. Following the screening interview (conducted by a physician), 18 participants (12 females) met all the inclusion/exclusion criteria. The demographic characteristics, eCB levels, and EEG parameters at baseline are summarized in Tables 1, 4, and 5, respectively. The study was approved by the Institutional Review Board at the Parc de Salut Mar (IMIMFTCL/EC-TDCS OM) according to the Declaration of Helsinki. Participants provided written informed consent.

Study protocol and procedure

Participants in the randomized, double-blind, placebo-controlled, clinical trial received sham tDCS+CT or active tDCS+CT over four consecutive days. The study lasted three weeks and was organized as follows (Fig. 1A). (1) Basal evaluation (day -1 to 5, 2h): basal clinical, dietary, and neuropsychological measures were collected; (2) Intervention

(day 8-11): participants received sham or active tDCS+CT at the same time of the day over 4 consecutive days. Each stimulation session was preceded by and ended with a 3-minute resting-state EEG recording during which patients were instructed to “fix your eyes on the cross located in the middle of the screen and think of nothing”. Participants were asked to eat 2 to 4h before the intervention and fast for 2h after it; (3) Post-intervention evaluation (day 12): the same as the basal evaluation; and (4) Day 18: a 4-day follow-up dietary assessment was collected the week after finishing the training. See supplementary Table 1 for detailed information of all the variables included in the analyses.

Clinical, neuropsychological and neurophysiological measures

Anthropometric measures

Height and weight were measured by a registered dietitian and used to calculate BMI (subjects wore light clothing, and a digital scale adjusted to the first decimal was employed).

Food intake

Food consumption was self-reported. The participants were provided with a 4-day dietary record to register their meals and beverages during (1) the week prior to the intervention; (2) the week of the intervention; and (3) one week after it. They were asked to describe and weigh the ingredients of their meals (including beverages) and to specify the brands of the products they chose. A nutritionist checked the records with the patients to clarify doubts and validate information. Data were then processed with the food analyzer program PCN Pro 1.0© (Universitat de Barcelona) to obtain energy and specific nutrient information.

Cognitive Assessment Battery

Participants were evaluated with several tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) (25): Stockings of Cambridge-SOC (*Spatial Planning and problem-solving*), Intra-extra dimensional set-shift-IED (*Flexibility*), Spatial span-SSP, forward and backward variants (*Attention/Working Memory*), Simple Reaction Time-SRT (*Attention*); as well as other standard tests: Stroop Colors and Words Test-SCWT (26) (*Inhibition*), Iowa Gambling Task-IGT (27) (*decision-making*), Symbol digit modalities test- SDMT (28) (*Attention/ Processing speed*), Conner's Continuous Performance Test-CPT (29) (*Attention/impulsivity*). (See supplementary Table 2 for detailed information).

Quantification of circulating endocannabinoid concentration (eCBs)

Blood samples were obtained in the morning, after 12h fasting, the day of the basal evaluation, and the day of the post-intervention evaluation, and stored at -80°C until analysis. The eCBs anandamide (AEA) and 2-AG were quantified by LC/MS-MS (see ref 26 for a complete description of the method of extraction and processing) (30).

tDCS protocol

tDCS was delivered through multichannel tDCS (Starstim, Neuroelectronics) that aimed to activate r-dlPFC (within BA 9 and 46) and inhibit the contralateral lobe (l-dlPFC). The positioning of the multichannel tDCS (electrode location and currents) was determined using the Stimweaver optimization service by Neuroelectronics (16), ensuring that the current maximally affected the target areas of interest with higher focality than other approaches (31, 32). The resulting montage employed 8 gelled Ag/AgCl electrodes of π cm² size (Pistim- Neuroelectronics) placed at AF3(-1093uA), AF4(1178uA), F3(-1161uA), F4(1104uA), F7(-414uA), F8(530uA), FC5(1189uA), and FC6(-1332uA). Stimulation

duration was 20 minutes. The indicated currents were used for the active condition (peak to baseline), while the sham condition was defined as a 3-second ramp-up and ramp-down stimulation at the start of the intervention. The component of the electric field distribution in the active condition is shown in Fig.1B (standard head model). tDCS was delivered concurrent to CT so that both protocols were launched after the pre-EEG recording.

Cognitive Training (CT) protocol

The CT involved 5 different exercises to train EF and attention, based on the cognitive impairments that patients with obesity present. Information about the tasks and the trained domains is summarized in Supplementary Table 1. The difficulty level was automatically adjusted on a trial-by-trial basis. Tasks are available at the Guttman NeuroPersonalTrainer® platform, a computerized CT tool certified by the Spanish Agency for Medicines and Health Products as a Class I Health Product(RPS/430/2014). All exercises were completed every intervention day (week 2) and lasted about 20-30 minutes depending on the subject's execution.

EEG recording

Resting EEG (eyes open) was recorded daily before and after tDCS/sham at AF3, AF4, F3, F4, F7, F8, FC5, and FC6 (Starstim, Neuroelectronics), with a sampling frequency of 500 Hz. Recording time for pre-EEG was fixed for all participants at 3min, while post-EEG had a variable length (11.48min on average) depending on the CT protocol. Only the last 3 min of the post-EEG which contained resting-EEG (eyes open) were analyzed. Electrode impedance was kept below 10k Ω , and the electrical reference and ground placed at the right earlobe. Two different EEG-metrics were extracted: FA and frontal coherence (FC) for daily pre- and post-EEG intervals at the bands θ =[4,8 Hz], α =[8,13 Hz] β =[13,25 Hz], γ =[30, 45 Hz], and broadband=[4-45Hz]. EEG data were analyzed

offline by customized Matlab code (MathWorks Inc. Natick, MA, USA). See supplementary material for a detailed description. *Statistical analysis*

Differences between pre- and post-measures were assessed within and between groups. Cohen's d was used to quantify the effect size of the interventions (small: $d \leq 0.20$; medium: $d \geq 0.50$; large $d \geq 0.80$; and very large $d \geq 1.30$) It was defined as the standardized difference between two means, indicated as d throughout the text. Cohen's d is a standardized score, analogous to a Z-score, Following Cohen's effect-size conventions, only differences over medium effect size ($d \geq 0.50$) were considered of relevance (33, 34). Multivariate analysis of EEG metrics (FA and FC) pre- and post-intervention (and differences between groups) was conducted by means of Ranksum U-test per frequency bands (θ , α , β , γ , and broadband) with a Bonferroni correction of $N=5$. The correlation coefficient between EEG-metrics (FA and FC) and food intake metrics was computed by means of Pearson's correlation coefficient, indicated as r . Statistical significance of the correlation was obtained through a permutation test, with a Bonferroni correction of $N=6$. P-values < 0.05 after Bonferroni correction were considered statistically significant.

RESULTS

Food intake and anthropometric outcomes

Table 2 summarizes the effect of the intervention on food intake and anthropometric measures at basal, post-intervention, and follow-up evaluations. All participants completed all training and assessment sessions. At follow-up, however, 5 subjects (3 active and 2 from the sham group) did not return the dietary diaries. Briefly, compared to the sham tDCS+CT, the active tDCS+CT intervention showed a small positive effect on total Kcals consumed at post-intervention (basal-post differences: sham tDCS+CT, -78.4 ± 489 ; active tDCS+CT, -255.2 ± 383), and a further increase of this effect over the

following week (follow-up evaluation) up to $d=0.85$ (basal-follow-up differences: sham tDCS+CT, 55.1 ± 235 ; active tDCS+CT, -339.6 ± 639). This decrease in total Kcal consumed was mainly related to a reduction in lipid intake, as shown by the large effect size of the active group both at post-treatment (basal-follow-up differences: sham tDCS+CT, -34.6 ± 226 ; active tDCS+CT, -252 ± 312), and at follow-up evaluations (basal-follow-up differences: sham tDCS+CT, 43.2 ± 145 ; active tDCS+CT, -253.5 ± 441). There was an unexpected effect of the active intervention at post-intervention on the Kcal of sugar consumed (basal-post differences: sham tDCS+CT, -28.2 ± 84.2 ; active tDCS+CT, 30.3 ± 75.8), favoring the sham condition, that was not maintained at the follow-up assessment (basal-follow-up differences: sham tDCS+CT, 41.8 ± 72.8 ; active tDCS+CT, 19.8 ± 31.8). No anthropometric changes were observed at post-intervention or follow-up evaluation.

Executive function outcomes

Table 3 summarizes the effect of the intervention on the cognitive assessments from basal to post-treatment. With respect to the inter-group pre- and post-difference comparisons, results showed significant differences in: 1) SRT omission errors (basal-post differences: sham tDCS+CT, 0.3 ± 0.7 ; active tDCS+CT, 0.0 ± 0.0), the performance of the sham group was significantly worse at post-treatment (i.e. were less accurate in their responses) compared to the active one; 2) SOC Mean initial thinking time subscale (basal-post differences: sham tDCS+CT, -653 ± 919 ; active tDCS+CT, 381 ± 1295) where the sham group significantly decreased its latency of response after the intervention. Such a finding may indicate more impulsive or less reflective responses; 3) SDMT (basal-post differences: sham tDCS+CT, 3.3 ± 4.5 ; active tDCS+CT, -1.6 ± 8.4), showing an attention and processing speed improvement in the sham group at post-treatment; 4) SCWT interference index (basal-post differences: sham tDCS+CT, 6.8 ± 6.5 ; active tDCS+CT, -

1.6±8.4) with the sham group presenting less interference resistance at post-treatment; and 5) CPT omission error subscale (basal-post differences sham tDCS+CT, 4.4±11.5; active tDCS+CT, 0.3±1.2) the active group was more accurate in giving responses after treatment.

Endocannabinoid outcomes

Table 4 summarizes the effect of the intervention on the eCB plasma concentrations. Compared to sham tDCS+CT, the active tDCS+CT group had a moderate to high effect on the plasma concentrations of AEA (pre- and post-difference: sham tDCS+CT, -78.4±489; active tDCS+CT, -255.2±383), with a decrease in the active condition after the intervention (Fig. 2C). No effect was found on 2-AG plasma concentrations.

EEG outcomes

To understand the neurophysiological changes associated with the intervention, we examined the FA and FC in the different bands of interest (Table 5 and Table 6).

FA reflects differences across hemispheres so that an FA<0 indicates left-hemisphere dominance. Within-group analyses showed that in the sham tDCS+CT and active tDCS+CT group participants displayed a negative FA at baseline (i.e. greater left-sided brain activation). Additionally, FA tended to be closer to zero in post-EEG as compared to pre-EEG in active tDCS+CT, so that the left-side dominant activation was reduced, especially in the γ -band ($d=0.77$; $p=0.01$). In fact, results comparing between-groups showed that, compared to sham, the active tDCS+CT intervention tended to display a greater increase in FA in β and γ -power after the intervention ($d=0.75/d=0.78$; basal-post differences: sham tDCS+CT, -0.06 ± 0.19 ; active tDCS+CT, 0.1 ± 0.21 , $p=0.06/p=0.03$, respectively), suggesting that tDCS+CT may reverse the dominance of l-dlPFC (Fig. 2A).

While FA reflects hemispheric dominance in terms of oscillatory power, FC aims to differentiate between the inter and intra-hemispheres (Table 6 Fig. 2B). Results of the within-group analysis show that no changes in FC appeared in the sham tDCS+CT group. However, in the active tDCS+CT group the γ -band FC across hemispheres tended to increase after the intervention ($d=0.99$; pre-EEG, 0.17 ± 0.03 ; post-EEG, 0.20 ± 0.03 ; $p=0.02$). Interestingly, results comparing FC between groups showed that, compared to the sham condition, the active tDCS+CT intervention increased FC within the right-hemisphere at the β and γ -bands (β -bands: active tDCS+CT, 6.69 ± 6.67 ; sham tDCS+CT, -3.94 ± 2.55 , $p=0.02$; γ -bands: active tDCS+CT, 10.59 ± 6.59 ; sham tDCS+CT, -7.16 ± 2.61 , $p=0.002$), as well as inter-hemispheric coherence at the γ -band (active tDCS+CT, 12.09 ± 7.51 ; sham tDCS+CT, -4.72 ± 3.68 ; $p<0.01$). However, no statistically significant change in l-dIPFC coherence was observed in the active intervention as compared to sham in either β or γ -band ($p=0.240$ and $p=0.558$, respectively). *Predicting clinical outcomes from neural correlates*

We then attempted to better establish whether the neurophysiological modulations associated with training could be used as a predictor of changes in feeding behavior (35).

Results demonstrated that, within the active group, larger changes in the FA within the β band tended to correlate with a reduction in BMI after treatment ($r=-0.42$), although this was not statistically significant ($p=0.30$). Regarding FC, however, an increase within the γ -band intra- and inter l-dIPFC was observed to predict a larger reduction in BMI ($r=-0.89$; $p=0.003$ / $r=-0.80$; $p=0.02$, respectively, see Fig. 2D). This correlation tended to be maintained in the β -band between BMI and interhemispheric/l-dIPFC coherence ($r=-0.59$; $p=0.18$ / $r=-0.53$; $p=0.18$, respectively). There were no significant findings for the sham group.

DISCUSSION

Patients with MO that seek bariatric surgery are requested to adhere to healthier eating habits for extended periods prior to the intervention. While current weight-loss strategies are focused on energy expenditure paradigms (36), evidence suggests that ultimately adhering to a diet involves a wide range of cognitive abilities such as planning, decision-making, and self-regulation (37). In this study, we proposed a combined intervention (tDCS plus CT) to enhance cognitive regulation of food intake for patients with MO who are at the preparatory period of the intervention (i.e. dieting). In order to better understand the immediate impact of the proposed intervention on food intake, we analyzed anthropometric measures, cognitive functions, eCBs, and neurophysiological signals (i.e. EEG) which can be employed to predict future weight-loss.

The results of this study demonstrate that patients with MO who received 20 min dlPFC tDCS (anodal r-dlPFC, cathodal l-dlPFC) in combination with CT reduced their kcal intake during the week of stimulation. It is of note that this effect was more pronounced at follow-up (1-week after finishing the intervention) in those participants who returned the dietary assessment (n=13). Despite the fact that no anthropometric changes were observed, our study suggests that a brief tDCS+CT intervention can reduce energy intake in patients with MO, an effect that may last or even increase over the short term.

Lateralization of the dlPFC activation observed in obese patients has led to the proposal of the right-brain hypothesis for obesity (9) which states that reduced r-dlPFC activity may lead to overeating. Moreover, whilst relatively left-dominant FA has been observed in overeating behaviors (12, 13), r-dlPFC hyperactivation has been associated with anorexia-like symptoms (38). In addition, r-dlPFC activation has been related to decision-making and risk-taking behaviors, and its disruption biases risk-taking responses in tasks

such as gambling (39). Activation of l-dlPFC has been reported for successful response-inhibition, for example in patients with obesity who successfully resist cravings (10). Our study aimed to quantify this dlPFC activation by analyzing EEG changes following the intervention. In agreement with the above-mentioned studies, participants involved in our trial showed an l-dlPFC dominant activation at baseline. Following the active tDCS+CT intervention and the reduction in caloric intake, we observed a decrease in the l-dlPFC dominance observed at baseline and an increase in γ -band functional connectivity across the frontal hemispheres. These results suggest that altered feeding behaviors may stem from a communication dysregulation between bilateral dlPFC, and that a combined tDCS and CT approach could be a potential intervention to decrease food intake (40).

Due to the specific EF impairment displayed by subjects with obesity this cognitive domain was measured pre- and post-intervention. Results showed a tendency to improve the participant's response inhibition (as measured by CPT), and minor effects on planning and sustained attention capabilities (as measured by SRT and SOC, respectively) after active tDCS+CT. Such abilities may play a role in cognitive control and explain the reduction in caloric intake. An improvement in response inhibition (measured by SWCT) was also present in the sham tDCS+CT condition. However, the neuropsychological results must be taken with caution since the duration of the treatment may not have been long enough to produce measurable brain changes, as confirmed by the EEG data analysis. Although cognitive training has been observed to improve cognitive control (14), the training protocols usually last for 1-3 months, an issue that may have diminished the impact of CT alone in our population. Moreover, few studies have tested the combined effect of multiple subdomain CT approaches in eating behavior (compared to training a single cognitive aspect such as response inhibition (41)). Results, however, are promising(14). Our findings demonstrate that whilst CT resulted in the improvement of

some cognitive tests, CT alone had no impact on reducing kcal intake or blood eCB levels. Overall, our results suggest tDCS may enhance the effect of CT.

Following the active tDCS+CT intervention and reduction in caloric intake, we observed a moderate decrease in AEA plasma concentrations, consistent with the known effects of eCBs. Although the exact relationship between blood eCB levels and brain eCB levels is unknown, blood eCBs are considered a good biomarker of the ECS since the peripheral ECS is overactive in human obesity (42). This may suggest a possible mechanism underlying the effects of the tDCS+CT intervention on caloric intake.

Finally, we investigated to what extent brain changes associated with the intervention can be used to predict patients' future feeding behavior. We observed that at post-treatment an increase in interhemispheric and l-dIPFC coherence within the γ -band predicted a larger decrease in BMI at follow-up. A finding that may serve as a predictor of future BMI reduction.

Our results should be taken with caution as our sample of patients with morbid obesity seeking bariatric surgery may not be representative of the larger population. Future studies could overcome some of our limitations which include the relatively short follow-up, small sample size, inherent biases of serial neuropsychological testing (i.e. personal motivation or practice effect), and self-reported measurement of food intake (which was confirmed by a registered dietitian). Moreover, the use of food-related stimulus could have increased the behavioral specificity of the cognitive training. Nevertheless, our study shows that tDCS+CT reduces energy intake in MO patients thus enhancing the results of previous studies by proposing that anodal r-dIPFC tDCS restores right dominance of the dIPFC. In addition, our findings suggest that behavior may not only be modulated by FA, but also by functional connectivity between hemispheres, which

permits the prediction of future changes in eating behavior. Overall, tDCS+CT is a strategy that could be effective in modulating cognitive biases observed in obese patients, whilst the combined use of EEG-eCB as quantitative metrics offers a new approach to explore the mechanistic understanding of eating disorders.

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Participant data (after de-identification), study protocol, statistical analysis plan, informed consent, and analytic code are available to investigators whose proposed information use has been approved by an independent review committee. Proposals should be directed to rtorre@imim.es.

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Fig. 1:

A) Experimental design of the double-blind, randomized, placebo-controlled, parallel trial.

B) Specifications of the tDCS montage with anode over the right-dlPFC and cathode at left-dlPFC (left), and the resulting multichannel position of the tDCS (right) using the standard head model.

C) Intervention effect on kcal intake (total kcal consumed) in both Sham + CT and Active tDCS + CT conditions.

Fig. 2:

A) Frontal Asymmetry changes in beta and gamma bands (dB, post-intervention versus baseline) for both Sham and Active groups.

B) Visualization of the significant intra (r-dlPFC) and interhemispheric frontal coherence observed changes.

C) Changes in plasma concentration of AEA/LEA.

D) Correlation between the changes in BMI (post-intervention as compared to baseline) and changes in the intra-hemispheric FC due to intervention in the gamma band (post-intervention as compared to baseline).