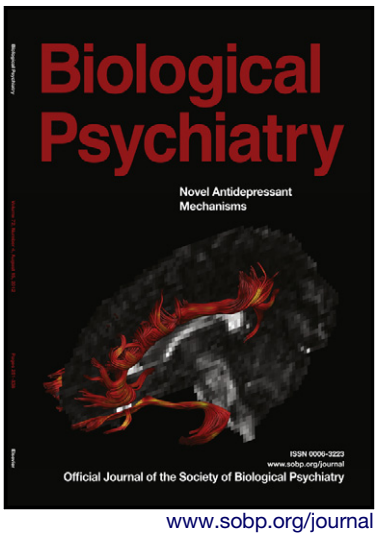

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Online effects of transcranial direct current stimulation in real time on human prefrontal and striatal metabolites

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Abstract

Background Studies have reported that transcranial direct current stimulation (tDCS) can modulate human behaviours, symptoms and neural activity, however the neural effects during stimulation remain unknown. Indeed, most studies compared the effects of tDCS before and after stimulation. The objective of our study was to measure the neurobiological effect of a single tDCS dose during stimulation.

Methods and Materials We conducted an online and offline protocol combining tDCS and magnetic resonance spectroscopy (MRS) in seventeen healthy participants. We applied anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC) and cathodal over the right DLPFC for 30 minutes, one of the most common montages used with tDCS. We collected MRS measurements in the left DLPFC and left striatum during tDCS and an additional MRS measurement in the left DLPFC immediately after the end of stimulation.

Results During stimulation, active as compared to sham tDCS elevated prefrontal N-acetyl Aspartate (NAA) and striatal Glutamate + Glutamine (Glx), but did not induce significant differences in prefrontal or striatal gamma-aminobutyric acid (GABA) level. Immediately after stimulation, active as compared to sham tDCS did not significantly induce differences in Glx, NAA or GABA levels in the left DLPFC.

Conclusions These observations indicate that tDCS over the DLPFC has fast excitatory effects, acting on prefrontal and striatal transmissions, and these effects were short living. Ones may postulate that repeated sessions of tDCS might induce similar longer lasting effects of elevated prefrontal NAA and striatal Glx levels which may contribute to its behavioural and clinical effects.

Introduction

Studies have reported that tDCS can noninvasively modulate human behaviours *in vivo.* tDCS applied over the DLPFC can influence mood, emotional perception and various cognitive processes including decision-making (1), problem solving (2) and working memory (3). Recent meta-analyses however questioned some of these effects of tDCS (4, 5). Some of these questions raised the issue that most studies have tested the effects of tDCS on behavioural and clinical outcomes. Indeed, the neurophysiological mechanisms of action of tDCS remain relatively unknown. The few studies that tested the effects of tDCS on neural outcomes used offline design, thus measuring and comparing neural substrates before and after stimulation (e.g., 6). Although these studies greatly contribute to elucidating the neural effects of tDCS, they are limited to report neural differences subsequent to tDCS delivery. There is still a need to demonstrate whether tDCS instantaneously changes neural substrates during stimulation. Such demonstration would provide evidence that tDCS directly modulates the brain.

Characterization of the neural effects of tDCS is also important to further investigate the clinical potentials of tDCS (7; 8). Indeed, the possibility of modulating the brain, and consequently inducing behavioural and cognitive changes, confers tDCS therapeutic promises. Most studies modulating behaviours and cognition in healthy volunteers have reported such effects with a single tDCS session, and therapeutic promises have mainly been reported when repetitive sessions of tDCS are applied over the DLPFC. Studies reported reduction of depressive symptoms in patients with major depressive disorders (9; 10), positive symptoms in schizophrenia (11), and craving in substance use disorders (12). Again, how the brain is modulated when tDCS induces such clinical benefits remains largely unknown. Better characterization of the neural effects of tDCS over the DLPFC will likely contribute at identifying optimal parameters to enhance clinical outcomes.

The goal of this study was to develop an online tDCS/MRS design in order to characterize the simultaneous and subsequent neurometabolism differences induced by tDCS using ^{1}H MRS. Our hypotheses were that tDCS will (1) elevate Glx levels in the left DLPFC (under the anode electrode) and left ventral striatum, (2) elevate NAA levels in the left DLPFC, and (3) decrease GABA levels in the left DLPFC. Furthermore, these neurometabolic differences in the left DLPFC will be observed simultaneously and subsequently to the tDCS delivery. In order to test these hypotheses, we simultaneously delivered anodal and cathodal tDCS over the left and right DLPFC, respectively, and measured total Glx, GABA and NAA concentrations in the left DLPFC and left striatum with MRS. We selected this electrode montage (anodal tDCS over the left DLPFC and cathodal tDCS over the right DLPFC) because these regions are the most targeted areas to modulate behaviours and cognition as well as to alleviate neuropsychiatric symptoms. We studied metabolites in the left DLPFC and left striatum because of the importance of corticostriatal fibers as connections within the forebrain, and to probe potential subcortical effects of tDCS. We studied Glx and GABA because the effects of tDCS are primarily thought to be ascribable to local differences in cortical excitability, thus implicating glutamate (13) and GABA transmissions (14). We also measured NAA, a metabolite implicated in neuronal regulatory processes such as protein synthesis and lipid production (15), as well as an indicator of neuronal viability and metabolism activity (16). Finally, we also focused on these neurotransmitters because they have been shown in numerous papers to be affected in the aforementioned pathological conditions (17) in which tDCS have shown some clinical potential.

Materials and Methods

This study was a randomized, crossover, sham-controlled, blind at three levels experiment (participant, MRS experimenter, data analysis conductor). Each participant took part to the two experimental sessions: one with active tDCS and one with sham tDCS. Order of the tDCS sessions was randomized

with a Latin square (eight participants received active tDCS first and sham tDCS second). Sessions were separated by seven days to minimize potential carry-over effects of tDCS.

Participants

We recruited seventeen healthy participants through the electronic mail distribution service of Université Laval. The local Institutional Review Board committee (*Institut de Réadaptation en Déficience Physique de Québec*) approved the study (2013-349). We obtained informed written consent from all participants and screened them for neurological, medical and psychiatric conditions. Of note, two participants presented moving artifacts during scanning and were omitted from further analysis. The remaining fifteen participants (eight men) had an average age of 27 years (range of 21-41 years) and were right-handed as assessed by the Edinburgh Handedness Inventory. Please refer to Table 1 for participant characteristics.

Transcranial direct current stimulation parameters

We delivered stimulation using an MR-compatible DC-Stimulator (neuroConn, GER). We disposed the anode electrode over the left DLPFC (F3) and the cathode electrode over the right DLPFC (F4) using the EEG 10-20 system. We have used electrodes of 35 mm^2 and electrode positioning was verified on the individuals' T_1-weighted scan. Active stimulation was delivered for 30 minutes at a current intensity of 1 mA. Sham stimulation was delivered for 30 minutes following standard procedure with a ramp up and a ramp down of 30 seconds with the remaining time with no active current (18). Participants and the MRS experimenter filled a questionnaire on the stimulation conditions for each session to test the integrity of blinding at the end of the study. Eleven out of fifteen participants guessed which tDCS session (active or sham) were conducted with a confidence level of 55% determined on a visual analog scale. The MR experimenter (A. H-B) had minimum interaction with the participants and

remained fully blind of the tDCS conditions (active, sham; which was delivered by S. F.), with a confidence level of 100%, until the interpretation of results.

Transcranial direct current stimulation and magnetic resonance spectroscopy design

We delivered tDCS during MRS scanning (please refer to Figure 1 for the study design). Specifically, we started tDCS five minutes before acquiring the first spectroscopy scan. To our best knowledge, no studies have reported online neural effects of tDCS when targeting the DLPFC. There are however results reporting that tDCS over the primary motor cortex had to be delivered for five minutes in order to induce significant differences in the amplitude of motor evoked potentials as captured by electromyography (e.g., 19). We are aware that the effects of tDCS over the DLPFC or the primary motor cortex may have a different timeline, but we also made this choice of starting the MRS scan after five minutes of stimulation because the most likely side effect of tDCS is an itching sensation during ramp periods (the first and last 30 seconds of tDCS delivery), which might cause head movement and impact data quality. Each scanning period lasted a total time of 50 minutes at our facility. Following the acquisition of the 7-minute anatomical MRI and the 30-minute tDCS/MRS session, we only had time for one post-stimulation measurement. As the main goal of our study was to capture the effect of tDCS applied over the DLPFC, we chose to focus on the region under the anode, the left DLPFC.

Magnetic resonance spectroscopy measurements

Scanning was performed with a Philips 3T Achieva scanner. T_1-weighted structural MR images were acquired with an MPRAGE sequence (TR= 8.2 ms, TE= 3.7 ms, FoV= 250 mm, flip angle = 8◦, 256×256 matrix, 180 slices/volume, slice thickness = 1 mm, no gap). Spectroscopy measurements were acquired during and after stimulation. We obtained each spectroscopy scan lasting 10 minutes 33 seconds in the same order for all participants: left DLPFC during stimulation; left striatum during stimulation; left DLPFC immediately after the end of stimulation. We obtained spectroscopy

measurements using the MEGA PRESS acquisition sequence (20), an efficient and reliable sequence for detecting endogenous GABA (21, 22) and other brain metabolites. MEGA PRESS spectra were acquired from 3x3x3 cm^3 voxels (see Figure 2 for sample MEGA PRESS spectra). We positioned the left DLPFC voxel over the Brodmann's Area 46 and 9, located in the middle frontal gyrus, with one face parallel to the skull. Of note, the voxel might have encompassed more than this gyrus because of the large voxel volume. We positioned the striatum voxel over the head and tail parts of the caudate nucleus, encompassing the internal capsule and ventral striatum on the coronal plane. On the sagittal plane, the voxel was positioned under the lateral ventricle with one face of the voxel parallel to the ventricle (see Figure 1). We used the following spectroscopy parameters: TR/TE 2000/68 ms; spectral bandwidth= 2000 Hz; 2048 samples with 320 averages; 14 ms Gaussian editing pulses applied either to the GABA spins at 1.9 ppm or at 7.46 ppm in an interleaved manner. This typical acquisition protocol (22) results in a GABA signal that is contaminated by macromolecular signal, and often referred to as GABA+.

We analyzed Glx and NAA measurements with Tarquin 4.3.4 (23) and GABA measurements with Gannet 2.0 (24). Tarquin has comparable reliability in metabolite levels quantification to other softwares, such as LCModel (22). Metabolite levels were calculated relative to the unsuppressed water signal from the same voxel and the Tarquin fitted amplitudes were summed to give a total estimate of Glx. No data were excluded from the study due to poor quality, else than the two subjects who presented movement artifacts. We assessed quantification reliability of Tarquin results with Cramer-Rao Lower Bounds (CRLBs), with CRLB value over 20% considered unreliable. The normalized residuals of the Gannet model of GABA data all fell within the range 10-15%. We compiled and entered data for all participants in SPSS 22.0 (SPSS Statistics, IBM) to compare mean differences between active and sham conditions for each metabolite (Glx, NAA and GABA) and measures (left DLPFC during stimulation, left striatum during stimulation, left DLPFC after the end of stimulation)

within each participant. We used a multiple-related samples Wilcoxon test (Wilcoxon related samples t-test), which enables to test the difference between matched pairs when the population cannot be assumed to be distributed normally.

Results

During stimulation, tDCS induced a significant change in NAA level (active vs. sham, Wilcoxon related samples t-test; $P = 0.041$; see Figure 3A), but no change in Glx ($P = 0.906$) or GABA levels ($P = 0.850$) in the left DLPFC. tDCS also provoked a significant change in Glx level (active vs. sham, Wilcoxon related samples t-test; $P = 0.027$; see Figure 3B), but no differences in NAA ($P = 0.246$) and GABA levels ($P = 0.342$) in the left striatum. tDCS did not induce significant differences in NAA ($P = 0.820$), Glx ($P = 0.619$) or GABA levels ($P = 0.243$) when measured in the left DLPFC immediately after the end of stimulation.

When comparing online and offline metabolite concentrations within the DLPFC during active and sham tDCS, there was a difference in NAA levels (Wilcoxon related samples t-test; active, $P = 0.002$; sham, $P = 0.056$), but not in GABA (active, $P = 0.344$; sham $P = 0.943$) or Glx levels (active, $P = 0.407$; sham, $P = 0.136$). More specifically, NAA concentration within the left DLPFC was significantly elevated during tDCS as compared to after tDCS in the active condition.

We co-registered the active and sham MRS voxels for the DLPFC and striatum using the header information of native T1 images in Gannet 2.0 (25). Subsequently, we used SPM 12 to segment tissues within the voxel into gray matter, white matter and cerebrospinal fluid. Results from these supplementary analyses suggest there was no bias in voxel placement in relative gray matter proportion between sessions in the left DLPFC (active vs. sham, Wilcoxon related samples t-test; $P = 0.959$) and striatum ($P = 0.878$). Additionally, using the same analysis, there was no change in cerebrospinal fluid

between the two sessions DLPFC ($P = 0.163$) and striatum voxel ($P = 0.233$), nor was there change in the white matter voxel compositions, DLPFC ($P = 0.332$) and striatum voxel ($P = 0.502$). This suggests there was no significant bias in voxel placement between sessions. Furthermore, this indicates water tissue content was not different between acquisitions.

We administered a standardized side effect form and a 16-item visual analog scale questionnaire on mood before and after each experimental session. All side effects reported are presented in Table 2. There were no significant differences in the number of reported side effects (paired samples t-test, $P = 0.332$) nor in mood (paired samples t-test, $P = 0.438$) between active and sham tDCS conditions.

Discussion

In this study, we have found that a mild (1 mA) dose of anodal tDCS over the left DLPFC coupled with cathodal tDCS over the right DLPFC induced a significant and rapid elevation in prefrontal NAA (within 15 minutes) and striatal Glx (within 30 minutes). This elevation was normalized directly after the end of stimulation. Overall, these results may suggest that tDCS has an excitatory effect on the prefrontal cortex and that this effect is primarily represented in the ipsilateral striatum, down the corticostriatal pathways, within the basal ganglia. Although they are not direct elevation of prefrontal glutamate, they support work that had proposed excitatory effects of anodal stimulation promoting synaptic plasticity, mediated through NMDA receptor activity. For instance, anodal stimulation can enhance motor cortical excitability and NMDA receptor partial agonist D-cycloserine can prolong the length of this augmented motor cortical plasticity (26).

The present findings demonstrated an elevation of NAA levels in the DLPFC during active compared to sham tDCS. This effect was no longer significant immediately after the end of stimulation delivery, suggesting that this effect is normalized directly after the end of stimulation. NAA is critical to cellular

regulatory processes, protein synthesis, lipid production and is a marker of mitochondrial function (27). Decreases in NAA levels, reflecting cellular dysfunction or impaired functioning, have been correlated with neurological lesions and psychopathology. Reduced NAA levels have been observed in major depressive disorders (28) and schizophrenia (29, 30). Importantly, NAA levels are known to be elevated following psychostimulant medication (31, 32), antipsychotic medication (33), and repetitive TMS administration (34). NAA is also metabolically close to glutamate, with aspartate the intermediate in a two-step conversion, and NAA and glutamate may track in MRS studies, as both metabolites are found in neurons. Previous results have demonstrated that tDCS applied to the parietal cortices, with the anode over P4 and cathode over the contralateral arm, elevated NAA and Glutamatergic metabolites under the anode after stimulation (35). Our results concur with the results of Clark *et al.* (35) with a similar effect over NAA levels in the prefrontal cortex. However, we did not measure a significant elevation of Glx in the prefrontal cortex.

The present results also show a transient and rapid elevation of Glx levels in the striatal region. This indicates that excitatory stimulation of the DLPFC may provoke an ipsilaterally distal effect over the circuitry of the basal ganglia. We believe such elevation of Glx in the ipsilateral striatum may result of increased Glutamatergic transmission as a results of tDCS aplied to the prefrontal cortex. Stimulation of neuronal metabolism in the prefrontal cortex could lead to Glu release in downstream structures. This may partly explain some behavioural effects of tDCS. Indeed, the corticotriatal glutamate pathways play a crucial neuromodulatory part within the striatum (36, 17). This is of particular interest, as corticostriatal and mesocorticolimbic circuitries are often found impaired in several neuropsychiatric conditions (37), including substance use disorders (38) and schizophrenia (39, 40). Although our results did not show a significant modulation of Glx within the prefrontal cortex, they suggest that tDCS may act through Glutamatergic transmission. Glu transmission is impaired in depression as seen with lower prefrontal Glx levels (41) and decreased Glu metabolism of individuals with depression (42). Repeated

sessions of tDCS applied to the prefrontal cortex can reduce depressive symptoms in patients with major depressive disorder (8, 10). These therapeutic effects may thus be imputable to stimulation of Glutamatergic transmission, supporting tDCS as a non-pharmacologic alternative treatment in major depression. In sum, a systemic explanation for possible therapeutic potential of tDCS would be that direct stimulation of cortical Glutamatergic neurons entails important modulation on a wide array of cerebral structures, as the prefrontal cortex has crucial Glutamatergic outputs to the nucleus accumbens and striatum.

We did not find significant differences in Glx under the anode during or after stimulation, whereas Clark *et al*. (35) observed differences in Glx levels under the anode after stimulation. This may be explained by methodological differences between these studies, such as the current intensity (1 vs. 2mA) and electrode montage (uni-, vs bi-lateral stimulation paradigm). Specifically, they delivered 2mA with the anode over P4 and cathode on the contralateral arm; we delivered 1mA with the anode over F3 and cathode over F4. They found differences by comparing Glx levels before and after tDCS at P4; we found no significant differences in Glx levels by comparing active and sham tDCS at F3 or during and after active tDCS. Our results also differ from those of Stagg *et al*. (6). They delivered 1mA for 10 minutes with the anode over the primary motor cortex (M1) and cathode over the supraorbital area, alternatively. They compared Glx levels before and after tDCS. They report that tDCS with the cathode applied to M1 caused a local decrease in Glx compared to sham, whereas tDCS with the anode over M1 did not change Glx compared to sham. Again, methodological choices differ greatly between the work of Stagg *et al*. (6) and our study, which makes it difficult to compare results.

Findings from this work also show that the observed neurometabolism differences, elevated levels of prefrontal NAA and striatal Glx, during tDCS delivery were no longer significant immediately after the end of stimulation. This may partially explain some discrepancies on the effects of tDCS in the

literature, especially when comparing online and offline designs with single tDCS sessions. One might find behavioural and cognitive differences when tested during stimulation but these differences might disappear immediately after stimulation delivery. Longer lasting effects of tDCS might be observed with repeated tDCS sessions.

Finally, results from this work also show that anodal stimulation applied over the left DLPFC coupled with cathodal stimulation over the right DLPFC did not induce significant differences in prefrontal and striatal GABA levels. It has been shown that anodal tDCS-related excitatory effects over M1 are silenced by administration of NMDA antagonist Dextromethorphan (13) and reduced by GABA receptors agonist Lorazepam (14), thus suggesting a paramount role for Glutamatergic and GABAergic transmission in tDCS effects. Stagg *et al*. (6) reported differences in GABA levels when comparing before and after 1mA for 10 minutes with the anode over M1 and cathode over the supraorbital area, alternatively. They report that following tDCS with the anode over M1, GABA levels decreased compared to sham. The lack of significant differences in GABA levels in the present work may result from important technical discrepancies in current intensity, tDCS duration and MRS parameters.

Future studies are needed to characterize the neurophysiological effects of tDCS and, to a greater extent, the effect of repeated sessions of tDCS on Glx and GABA levels. Given that the vast majority of striatal medium spiny neurons contain GABA, prefrontal Glutamatergic activation of corticostriatal fibers may facilitate GABAergic transmission and thus GABA release from the striatum to nearby subcortical structure in the basal ganglia. Therefore, it is possible that modulation of GABA transmission may only be perceptible in other structures rather than the striatal region, such as the internal and external globus pallidus. Our methodology and timeline prevented us from taking such measurements. It is also possible that repeated sessions of tDCS over the DLPFC at 2 mA, as mainly delivered in studies targeting clinical populations, may have greater and longer lasting effects on

Glutamatergic facilitation. This in turn may modulate prefrontal and striatal GABA release. It should also be noted that we used a $(3\ cm)^3$ voxel in to order to obtain three MRS measurements within the 50-minute scan period. However, the large voxel volume is difficult to position in the studied regions, the DLPFC and basal ganglia, which consists of a limitation of our work. Future studies should investigate the effects of tDCS using smaller voxel volumes to test whether similar neurochemical differences would be observed. As an additional limitation to the interpretation of our results, it is worth mentioning that if applying a post-hoc Bonferonni-type correction, the corrected alpha level of significance would prevent our data of reaching significance.

In conclusion, we have shown prefrontal NAA and striatal Glx neurochemical differences during a single session of 1mA tDCS. To our best knowledge, this is the first study combining tDCS and MRS online demonstrating the direct modulation of metabolites with tDCS in real-time. An offline design would not be sensitive to transient differences such as those we observed. Future studies are needed to address the proper mechanistic effect of tDCS in the region most likely to be targeted in future clinical practice, the prefrontal cortex. Findings from this work thus indicate the importance to further evaluate the safe dosage and optimal stimulation target of tDCS in order to delimitate its full potential, for both clinical and healthy populations.

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Table 1. Participants' characteristics

ID participant	Sex	Age	Handedness
1	M	31	80
2	F	27	50
3	M	29	100
4	M	22	80
5	F	41	100
6	F	27	100
7	M	23	90
8	M	23	100
9	F	24	50
10	F	23	100
11	F	28	60
12	F	21	88
13	M	28	78
14	M	26	100
15	M	33	100

Table 2. Side effects reported by participants at each tDCS/MRS session. Side effects were rated as absent, mild, moderate or severe

ID participant	Active tDCS	Sham tDCS
1	Headache (mild)	Trouble concentrating (mild)
4		Trouble concentrating (mild)
7	Neck pain (mild)	Neck pain (mild)
9	Light tingling (mild)	
11		Trouble concentrating (mild)
12		Headache (mild)
13	Trouble concentrating (mild)	Trouble concentrating (mild)
14	Neck pain (mild)	Neck pain (mild)

Table 3. Mean results for Glx and NAA levels for each voxel of interest during and after tDCS (standard error means)

		DLPFC during tDCS	**Striatum during tDCS**	**DLPFC after tDCS**
Active tDCS	Glx	5.19 (0.24)	6.62 (0.38)	4.45 (0.37)
	NAA	6.59 (0.28)	5.52 (0.48)	4.49 (0.39)
Sham tDCS	Glx	5.13 (0.31)	5.85 (0.33)	4.38 (0.37)
	NAA	5.82 (0.22)	4.99 (0.46)	4.35 (0.48)

Figure legends

Figure 1: Experimental timeline

Following the acquisition of a T_1-weighted anatomical image, we delivered active or sham stimulation to the DLPFC with the anode electrode over the left DLPFC and the cathode electrode over the right DLPFC. Five minutes after the start of stimulation, we acquired Glx, NAA and GABA levels in the left DLPFC (ipsilateral to the anode) and left striatum, during stimulation. Immediately after stimulation, we acquired the same metabolites in the left DLPFC.

Figure 2: Sample MEGA PRESS spectra from the prefrontal voxel illustrated in Figure 1

Figure 3: Elevation of prefrontal NAA and striatal Glx level by tDCS applied over the DLPFC

A) NAA levels during active and sham tDCS. NAA levels (N=14) in the left DLPFC during active and sham stimulation. Light grey bars represent group averages for active and sham stimulation. Results are in arbitrary units (AU). Error bars represent s.e.m. (* $P < 0.05$).

B) Glx levels during active and sham tDCS. Glx levels (N=15) in the left striatum during active and sham stimulation. Light grey bars represent group averages for active and sham stimulation. Results are in arbitrary units (AU). Error bars represent s.e.m. (* $P < 0.05$).