Aus der Klinik und Poliklinik für Psychiatrie und Psychotherapie des LMU Klinikums und der
International Max-Planck Research School for Translational Psychiatry (IMPRS-TP)

Dissertation
zum Erwerb des Doctor of Philosophy (Ph.D.) an der
Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

Wirkungen der transkraniellen Gleichstromstimulation bei psychiatrischen Erkrankungen

Understanding of transcranial direct current stimulation effects in psychiatric disorders

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Jahr
2023

Mit Genehmigung der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

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Date of oral defense:	11.01.2023

1. Table of content

2. List of abbreviations

ACC	anterior cingulate cortex
DLPFC	dorsolateral prefrontal cortex
ELECT-TDCS	Trial of Electrical Direct-Current Therapy versus Escitalopram for Depression
fMRI	functional magnetic resonance imaging
LTP/LTD	long-term potentiation and long-term depression
NTBS	noninvasive transcranial brain stimulation
PANSS	positive and negative syndrome scale
PFC	prefrontal cortex
STARTS	Schizophrenia Treatment With Electric Transcranial Stimulation
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation

3. List of publications

Subject of this PhD thesis

Bulubas, L.*, Padberg, F.*, Bueno, P.V., Duran, F., Busatto, G., Amaro, E., Jr., Bensenor, I.M., Lotufo, P.A., Goerigk, S., Gattaz, W., Keeser, D.*, Brunoni, A.R.*, 2019. Antidepressant effects of tDCS are associated with prefrontal gray matter volumes at baseline: Evidence from the ELECT-TDCS trial. Brain Stimul 12, 1197-1204. DOi: 10.1016/j.brs.2019.05.006

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*these authors contributed equally

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4. Candidate's contribution to the publications

4.1 Paper I - Antidepressant effects of tDCS are associated with prefrontal gray matter volumes at baseline: Evidence from the ELECT-TDCS trial.

This publication is based on the randomized, controlled "Trial of Electrical Direct-Current Therapy versus Escitalopram for Depression" that was carried out by ARB and colleagues (PVB, FD, GB, EA, IMB, PAL, WG) in Sao Paulo in Brazil from 2013 – 2016. The original results were published in New England Journal of Medicine in 2017; in summary, this non-inferiority trial included 245 subjects treated in a three-armed design by a combination of sham and verums pharmacotherapy and transcranial direct current stimulation and failed at showing non-inferiority of the brain stimulation treatment to pharmacotherapy, while – in secondary analyses – showing that brain stimulation is superior to placebo treatment.

Prof. Brunoni was a visiting scholar at our Department 2018-2019 and the candidate collaborated with Prof. Brunoni on the analysis of the neuroimaging data sets collected in the ELECT-TDCS trial; Prof. Brunoni is also candidate's fourth supervisor.

For this publication, the candidate worked with ARB on the final collection of the structural data sets, in particular by performing a quality check of available data, retrieval of missing data sets or information regarding quality issues with data sets (such as to decide whether subjects with abnormal brain morphology should be excluded due to possibly medical conditions). Furthermore, in collaboration with DK, the candidate prepared the data sets so that the final analysis could be run using DK's pre-established automated pipelines for structural analysis; this included eventual transformation of DICOM file format into nifty format and overall data cleaning. The candidate has also performed own, manual structural analyses of the datasets using different software packages (FSL for region-of-interest-based analysis, and freesurfer for analysis of cortical thickness and preparation of electric field simulations for SIMNIBS), however, for the final publication, the volumetric results from DK's pipelines were used as these pipelines are published and have been used by the group for MRI analyses in the past. The candidate created, however, Figure 1 in the manuscript that shows electric field simulations on a group template of patients receiving tDCS. The candidate and ARB, DK, and FP participated in weekly discussions regarding conception and design of this subsequent MRI analysis of the ELECT-TDCS trial.

The candidate cleaned the data sets that included further characteristics of participants, such as age, gender, years of schooling, descriptions of depressive disorder and several other characteristics and examined the dataset using descriptive statistics to receive an overview of the sample (see also Table 1 of the manuscript). In collaboration with SG, who provided expert guidance, the candidate performed the statistical analyses in this publication. Together with ARB, FP, SG, and DK, the candidate interpreted and discussed findings of the statistical analyses in weekly meetings.

Finally, following this groundwork, the candidate has written the manuscript and created all tables and figures and was major lead in the submission process (several submissions were attempted prior to Brain Stimulation). All authors revised the manuscript and provided important intellectual content and approved of the final version. In addition, the candidate has presented results of this publication at several conferences.

The authorships were shared between the candidate and FP and ARB and DK, respectively, as these authors carried the largest part of work needed to analyze the imaging data sets of ELECT-TDCS.

4.2 Paper II - Prefrontal resting-state connectivity and antidepressant response: no associations in the ELECT-TDCS trial

This publication is also a subsequent analysis of the ELECT-TDCS MRI data sets as explained above. The above-mentioned contributions apply here as well, as this analysis of the resting-state functional networks builds upon the first publication.

In particular, the candidate worked on completion of the MRI data sets and patient characteristic data sets, as described above. The candidate prepared the MRI data sets so that DK's automated data analysis pipelines could be run on the Department's servers (as otherwise the analysis of resting-state functional MRI data sets is very time and memory consuming). With SG as expert guide, the candidate performed the statistical analyses in this publication. The candidate and ARB, DK, SG, and FP participated in weekly discussions regarding conception and design of this analysis of resting-state networks in ELECT-TDCS, as well as interpretation and discussion of the results. The candidate has written the manuscript and created all tables and figures and was the major lead in the submission process. All authors revised the manuscript and provided important intellectual content and approved of the final version. In addition, the candidate has presented results of this publication at several conferences.

4.3 Paper III (Apendix A) - Cognitive outcomes after tDCS in schizophrenia patients with prominent negative symptoms: Results from the placebo-controlled STARTS trial

This publication is a result of the candidate's participation at the Workshop "Multimodal approaches in experimental psychology, clinical neuroscience and psychiatry" – a bilateral workshop between researches in Bavaria and Sao Paulo, Brazil. It is based on the double-blind Schizophrenia Treatment With Electric Transcranial Stimulation (STARTS) randomized clinical trial conducted 2014 – 2018 in Sao Paulo, Brazil, by LV, ARB and colleagues (JBC, BSP, HE, WFG). In 100 patients, this trial succeeded at showing efficacy and safety of transcranial direct current stimulation for treatment of schizophrenia patients with predominantly negative symptoms.

At the workshop, the candidate and SG, LV, ARB, FP, JSG, and AKB discussed extensively a subsequent analysis of the cognitive data sets from the STARTS trial; these authors concepted and designed the subsequent analysis which was ground for this publication. LV provided data sets which included patient characteristics and cognitive performance from the STARTS trial. After the workshop, the candidate and SG worked together on the statistical analysis – the analysis itself was performed by SG while the candidate provided intellectual content and further help in regular weekly meetings – and both, the candidate and SG, interpreted the results. Several times, SG and the candidate have updated the other workshop participants who then also provided intellectual content for the analysis. The manuscript itself was written by the candidate and SG together (SG has written methods and results and created figures and tables, the candidate has written the introduction and discussion and took over the submission process incl. reviews, however, both authors contributed to each other's sections). All authors revised the manuscript and provided important intellectual content and approved of the final version.

As the major work for this publication was carried out by the candidate and SG, the first authorship was shared between these two authors.

5. Introductory summary

5.1 Project's background

5.1.1 Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is a noninvasive transcranial brain stimulation (NTBS) technique that has been newly reinvented as a promising novel treatment for psychiatric disorders. Anecdotal evidence of effects of electric stimulation goes back as far as to the ancient Romans who used torpedo fish to treat pain (Tsoucalas et al., 2014). First evidence closest to what we now consider psychiatry is attributed to the 18th century, when electric stimulation of the head was used to treat patients with personality disorders (Parent, 2014). In the 20th century, the effects of electric stimulation were extensively investigated in animals and humans and another NTBS modality was invented, one that utilized the principle of Faraday's electromagnetic induction - the transcranial magnetic stimulation (TMS) (Barker et al., 1985). Electromagnetic stimulation had the advantages of evoking action potentials (for example muscle twitches when stimulated over motor cortex) at a much lesser intensity of electric currents and, hence, lower pain levels, as the magnetic field is less influenced by passing different types of tissues compared to the electric field.

However, starting in early 2000s, tDCS was reinvented as a kind of a softer and broader (i.e. less focal) form of neuromodulation with its own scope of application. In comparison to TMS, tDCS modulates the resting membrane potential of underlying neurons towards depolarization (anodal stimulation) or hyperpolarization (cathodal stimulation), not evoking action potentials, but making the induction of an action potential, when intrinsic signals arrive, more or less likely (Nitsche and Paulus, 2000). This novel application of tDCS utilizes weaker electric current intensities in the range of 1-2 mA that make tDCS a safe and well tolerable treatment alternative. First therapeutical applications of tDCS in psychiatric cohorts aimed at improving mood and cognitive symptoms in patients with depression (Fregni et al., 2006a; Fregni et al., 2006b) and positive and negative symptoms of schizophrenia (Brunelin et al., 2012) and showed remarkable clinical efficacy in these first studies. In the last 20 years, numerous clinical trials, reviews, and meta-analyses have been published and while tDCS is not (yet) as well established (nor e.g. part of the German Guidelines) like TMS, it is still is an inherent part of alternative NTBS therapeutical modalities offered in psychiatric hospitals worldwide.

5.1.2 TDCS for treatment of affective and non-affective psychoses

From its beginnings in the 2000s, a large body of evidence for the application of tDCS as treatment for symptoms associated with depression and schizophrenia has emerged. A recent meta-analysis has counted 27 studies and 1204 patients with depression treated with tDCS and showed

beneficial effects of active over sham tDCS (Zhang et al., 2021). Several guideline papers recommended the usage of tDCS in this cohort with probable to definite (latter in newer guidelines) efficacy (Fregni et al., 2021; Lefaucheur et al., 2017). For schizophrenia, the evidence is more limited, however the newer guidelines do recommend (with probable efficacy) anodal left prefrontal/cathodal left temporoparietal tDCS montage for auditory hallucinations in schizophrenia and - to some extent - also for negative symptoms (Fregni et al., 2021)

The largest randomized, controlled trial on efficacy of tDCS for depressive symptoms to date (Trial of Electrical Direct-Current Therapy versus Escitalopram for Depression, ELECT-TDCS) included 245 patients that received, in a three-armed design, either escitalopram and sham tDCS, tDCS and placebo pills, or double-placebo (sham tDCS and placebo pills) (Brunoni et al., 2017). TDCS was delivered at 2 mA using a bi-frontal electrode montage (anodal left, cathodal right) for 30 minutes daily (workdays) over 3 weeks, followed by 7 weekly treatments. ELECT-TDCS failed at showing non-inferiority of tDCS to escitalopram in the primary analysis, however, in secondary analyses, tDCS treatment was shown to be superior to double-placebo (Brunoni et al., 2017). In terms of side effects, patients who received tDCS showed higher rates of skin redness (from the electrodes), tinnitus, nervousness, and mania (2 out of 94 patients in the tDCS group) (Brunoni et al., 2017).

The largest randomized, controlled trial on efficacy of tDCS for schizophrenia aimed at improving negative symptoms (Schizophrenia Treatment With Electric Transcranial Stimulation, STARTS) and included 100 patients in a two-arm design; tDCS was delivered using a left anodal prefrontal/left cathodal temporoparietal electrode montage at 2 mA intensity for 20 minutes, twice-daily at 5 consecutive days (which is considered state-of-the-art) (Valiengo et al., 2019). Patients receiving active tDCS showed a significant improvement (active>sham) in Positive and Negative Syndrome Scale (PANSS) negative symptom sub-scores, however, no significant differences for other outcome variables, such as total PANSS score or positive symptoms/auditory hallucinations (Valiengo et al., 2019). This is rather unexpected as this protocol was rated “probably efficient” in the previously mentioned guidelines for auditory hallucinations (Fregni et al., 2021). TDCS was well tolerated; burning sensations occurred more often in the active group (Valiengo et al., 2019).

Impairment of cognition is common in depression as well as in schizophrenia. NTBS effects on cognition have been much investigated in healthy and clinical cohorts with highly heterogeneous results per se, varying strongly between the several domains of cognition such as working memory, processing speed, attention, learning, social cognition and others. A recent large meta-analysis has pooled the effects of NTBS on cognition over several psychiatric and neurologic disorders, including depression and schizophrenia, but also dementia, Parkinson's disease, stroke, traumatic brain injury, and multiple sclerosis thus meaningfully increasing the sample size (Begemann et al., 2020). With this approach, the authors were able to identify overall effects of tDCS on working memory and attention/vigilance underlying NTBS potential for this application (Begemann et al., 2020).

One of the major limitations of tDCS lays in the sham control condition; often, when tDCS trials fail to show superiority of the active treatment, the question rises whether this is due to a lack of

improvement in the active group or due to “too much” effects excerpted by the sham condition (Loo et al., 2018). Even though the meta-analysis by Zhang et al. (2021) supports active tDCS, the difference in rates of patients who showed an improvement in the active and sham tDCS group, respectively, was rather small (36% and 26%, respectively). As participants can sense tDCS effects on their skin, the “sham tDCS” needs to include some electric current flow and this condition is rarely standardized between studies. Some authors hypothesize that these smallest currents already induce neurobiological changes in the brain (Fonteneau et al., 2019) and thus account for the effects in the sham groups.

Furthermore, several other parameters of tDCS protocols are not sufficiently standardized yet. For example, patients with schizophrenia were shown to respond better to 1 mA tDCS than to 2 mA tDCS during a working memory task (Papazova et al., 2018). Hence, while the currently available clinical data is promising, further understanding of tDCS parameters and their effects on neurobiological processes in the brain is necessary to provide a more patient- and symptom-adapted treatment option and to increase the overall response rates of tDCS/NTBS.

5.1.3 Action mechanisms of TDCS

In the end of the 20th century, the (dorsolateral) prefrontal cortex (DL)PFC emerged as the target region for depressive symptoms. This was based, among others, on findings from patients with brain tumors in the PFC, who often suffered from depressive mood after tumor removal (George et al., 1994). In conjunction with the impaired prefrontal glucose metabolism and cerebral blood flow that was described in patients with depression, the PFC dysfunction hypothesis was postulated (George et al., 1994) and the PFC has been used as target region for NTBS and tDCS in depression since.

While the application of NTBS expanded beyond depression, the PFC remained a promising target candidate. In particular evidence from resting-state networks – functionally coupled areas of the brain that can be detected from resting-state functional magnetic resonance imaging (fMRI) – linked the (DL)PFC to several networks impaired in depression (Li et al., 2018), schizophrenia (Lawrie et al., 2002) and involved in cognitive processes in schizophrenia (Minzenberg et al., 2009) and elsewhere (Shin et al., 2015).

There are several pathways how tDCS is suggested to exert its effects on human behavior. In a simplified model, tDCS modulates the excitability of the brain in a polarity-specific manner – anodal tDCS activates (or, more precisely, facilitates activation), cathodal tDCS inhibits brain activation (Nitsche and Paulus, 2000). However, the final effects of stimulation depend on several other factors – non-linear effects were attributed to different stimulation intensities and durations (Papazova et al., 2018; Shin et al., 2015). In addition, when both electrodes are placed over brain regions (as opposed to an extracephalic electrode montage), an activation of possibly antagonist functional networks might lead to interfering effects of stimulation (Kantrowitz et al., 2019).

Stimulation effects beyond the stimulation period, lasting from several minutes up to several hours after stimulation, are generally attributed to synaptic plasticity (Shin et al., 2015; Stagg and Nitsche, 2011). Long-term potentiation and long-term depression (LTP/LTD), the mechanisms behind synaptic plasticity, are carried mostly by NMDA receptor-dependent glutamatergic as well as GABA-ergic interneurons and were linked to tDCS effects (more precisely, tDCS experts speak of LTP/LTD-like processes) (Shin et al., 2015; Stagg and Nitsche, 2011). Neuromodulators, such as dopamine and serotonin, critical in the pathogenesis of depression and schizophrenia, were also linked to tDCS effects (Nitsche et al., 2009; Nitsche et al., 2006; Stagg and Nitsche, 2011).

5.2 Project's hypotheses

NTBS and tDCS target neurobiological processes behind psychiatric disorders such as depression and schizophrenia with the aim to alleviate symptoms of these disorders. The (DL)PFC is a common target for mood and cognitive symptoms, characteristic of both these disorders.

However, several gaps in knowledge about tDCS prevent this promising modality from tapping its full potential. Neurobiological markers that involve either the targets or the pathways of tDCS treatment could help predict clinical responses and individualize tDCS treatment. These biomarkers are derived from several modalities, ranging from neuroimaging to electrophysiology, genetics, or peripheral blood markers.

In this project, we aimed at filling the gaps of tDCS application in psychiatric disorders. Our first aim was to characterize the influence of potential neurobiological markers, derived from structural and functional MRI investigations, on the antidepressant response of patients with depression from the ELECT-TDCS trial. Meta-analyses identified reduced gray matter in medial and dorsolateral PFC and the anterior cingulate cortex (ACC) in patients with depression as opposed to healthy controls; volumes of these regions were further associated with response to pharmacotherapy and normalized after successful treatment (Bora et al., 2012; Chen et al., 2007; Smith et al., 2013). Functional connectivity between ACC and regions of the PFC was also shown to be impaired in depression and associated with treatment response to pharmacotherapy and TMS (Fu et al., 2013; Kaiser et al., 2015; Weigand et al., 2018). Yet, no such investigations exist for tDCS. Nor were structural and functional markers investigated within the same sample of patients with depression treated with tDCS.

Our second aim was to describe the influence exerted by a tDCS protocol applied in the STARTS trial, that was originally designed to improve negative symptoms of patients with schizophrenia, on cognitive functioning in these patients. While evidence for tDCS effects on primary symptoms of affective and non-affective psychoses is relatively rich, comorbid symptoms such as cognitive deficits receive less attention. In healthy and neuropsychiatric populations, tDCS is a popular task to enhance cognitive performance, even though its effects are limited (Hill et al., 2016). Valuable

insight about further pathways involved in tDCS mechanisms can be gained from investigating its effects on cognition.

5.3 Project's conclusions

5.3.1 Summary of results and their relevance for the field

The publications which resulted from this PhD project contribute towards understanding of the effects of tDCS in psychiatric disorders. From baseline neuroimaging data sets from ELECT-TDCS, a trial investigating efficacy of tDCS for treatment of depression, we have identified regions of the prefrontal cortex where gray matter volume was directly associated with treatment response. In particular, larger gray matter volume of a broad, left prefrontal cortex region was associated with stronger improvement of depression scores in the active tDCS group, compared to sham. This was not found for the right prefrontal cortex nor the bilateral anterior cingulate cortex volumes. In exploratory analyses of distinct prefrontal cortex subregions, several medial regions that reflected distributions of stronger electric field simulations were associated with the response, however this comparison did not last after correction for multiple comparisons.

Interestingly, the analysis of resting-state functional connectivity has revealed no associations of within-region nor whole-brain connectivity of these anatomically predefined regions with the antidepressant response in the ELECT-TDCS. This implies that function does not necessarily follow structure and that tDCS might be differentially related to structural and functional biomarkers. Including the whole array of individual neuroimaging (and other) biomarkers might improve the predictive value for tDCS effects. A deeper understanding of this novel therapeutical opportunity is still needed.

In STARTS, while the patients benefitted from the specific tDCS protocol in terms of negative symptoms, including flattened affect, loss of interest, and emotional withdrawal, no beneficial effects of active tDCS over sham tDCS on the cognitive performance up to 12 weeks post treatment were observed. However, subtle improvement of executive functions and delayed memory were observed in the sham tDCS group. With these findings, our study nicely reflects several problems of this field: While a tDCS protocol might be effective for specific symptoms (auditory hallucinations or the mood) or specific populations (healthy), these effects might not be transferable to other areas of application without adaptation of further parameters of stimulation. The "dosage" of the stimulation should be considered as much as the "timing", "brain state", and the "targeting" of both electrodes. Importantly, the control condition should be considered in terms of own neurobiological effects, which might be present despite the minimal electric current flows.

In summary, we provide evidence for cortical structures involved in tDCS effects and suggest a decoupling of function from structure. While cognition seems to be one of the pathways for tDCS to exert its effects, recruitment of this pathways is highly dependent of stimulation parameters.

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7. Acknowledgments

I would like to express my deepest appreciation to my committee, Prof. Dr. Frank Padberg, Prof. Alkomiet Hasan, Dr. Daniel Keeser, and Prof. Dr. Andre Brunoni for welcoming me in their teams and supporting me all the way through these projects and beyond. Special thanks go to Prof. Dr. Leandro Valiengo, for offering participation in the STARTS trial. I would like to extend my deepest gratitude to Prof. Dr. Andrea Schmitt, in particular for her excellent training sessions and professional advice, and Prof. Dr. Peter Falkai and Prof. Dr. Elisabeth Binder, without whom the format of the IMPRS-TP PhD/residency format would not have been possible. I am also grateful to the Else-Kröner-Fresenius Foundation for their financial support of the IMPRS-TP PhD/residency format. Special thanks to the coordinators of the IMPRS-TP Research School and the Munich Medical Research School for their support and advice.

I'm extremely grateful to the different teams I was/am part of in my research - Eva Mezger, my fellow PhD student, for the hundreds of hours we have spent together for these and other projects and much more; Linda Wulf, Jana Werle, Esther Dechantsreiter, Tabea Nenov-Matt, Anja Wilkening, Gerrit Burkhardt, Ulrike Kumpf as the brain stimulation team(s), Boris Papazov, Yuki Mizutani, and Kai-Yen Chang as the MRI team, Stephan Goerigk and his statistics expertise, Temuz Karali for the IT expertise, the schizophrenia team, including, but not limited to Mattia Campana, Elias Wagner, Lisa Löhrs, Ben Pross, Duygu Güler, and Irina Papazova, and, of course, our first EKFS-IMPRS-TP batch for paving the way together - Janos Kalman, Laura Albantakis, David Popovic, Ömer Öztürk, Florian Raabe.

These projects (and all other projects in medical hospitals) would not have been possible without the help of dedicated students – my deepest gratitude goes to all our HiWis and bachelor/master students, medical doctoral students, and PhD students.

Furthermore, my deepest appreciation goes to all patients willing to participate in a (clinical) study and to each person on the clinical teams, starting from nurses on our wards to the doctors and other professionals supporting (clinical) research.

Last but not least, my deepest love goes to my daughter Emilia who was and is the best reason to defer my PhD and to my partner, family and friends for supporting me through life.

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10. Original publications

10.1 Paper I - Antidepressant effects of tDCS are associated with prefrontal gray matter volumes at baseline: Evidence from the ELECT-TDCS trial

Brain Stimulation 12 (2019) 1197–1204

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Antidepressant effects of tDCS are associated with prefrontal gray matter volumes at baseline: Evidence from the ELECT-TDCS trial

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ARTICLE INFO

Article history:
Received 8 December 2018
Received in revised form
10 April 2019
Accepted 5 May 2019
Available online 8 May 2019

Keywords:
Antidepressant response
Magnetic resonance imaging
Major depressive disorder
Noninvasive brain stimulation
Structural neuroanatomy
Transcranial direct current stimulation

ABSTRACT

Background: Transcranial direct current stimulation (tDCS) is a promising intervention for major depression. However, its clinical effects are heterogeneous. We investigated, in a subsample of the randomized, clinical trial Escitalopram versus Electrical Direct Current Therapy for Depression Study (ELECT-TDCS), whether the volumes of left and right prefrontal cortex (PFC) and anterior cingulate cortex (ACC) were associated with prefrontal tDCS response.
Methods: Baseline structural T1 weighted MRI data were analyzed from 52 patients (15 males). Patients were randomized to the following conditions: escitalopram 20 mg/day, bifrontal tDCS (2 mA, 30min, 22 sessions), or placebo. Antidepressant outcomes were assessed over a treatment period of 10 weeks. Voxel-based gray matter volumes of PFC and ACC were determined using state-of-the-art parcellation approaches.
Results: According to our *a priori* hypothesis, in the left dorsal PFC, larger gray matter volumes were associated with depression improvement in the tDCS group ($n = 15$) compared to sham ($n = 21$) (Cohen's $d = 0.3$, 95% confidence interval [0.01; 0.6], $p = 0.04$). Neither right PFC nor ACC volumes were associated with depression improvement. Exploratory analyses of distinct PFC subregions were performed, but no area was associated with tDCS response after correction for multiple comparisons.
Conclusion: Left PFC baseline gray matter volume was associated with tDCS antidepressant effects. This brain region and its subdivisions should be investigated further as a potential neurobiological predictor for prefrontal tDCS treatment in depression and might be correlated with tDCS antidepressant mechanisms of action.

Abbreviations: ACC, anterior cingulate cortex; BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; ECT, electroconvulsive therapy; ELECT-TDCS, Escitalopram versus Electrical Direct-Current Therapy for Depression; HDRS-17, Hamilton Depression Rating Scale; MDD, major depression; NTBS, noninvasive transcranial brain stimulation; OLE, Omni-Lateral-Electrode; pACC, pregenual anterior cingulate cortex; PFC, prefrontal cortex; RCT, randomized controlled trial; ROI, region of interest; rTMS, repetitive transcranial magnetic stimulation; sgACC, subgenual anterior cingulate cortex; tDCS, transcranial direct current stimulation.

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https://doi.org/10.1016/j.brs.2019.05.006
1935-861X/

Introduction

Major depressive disorder (MDD) is a prevalent, morbid disease [1]. Pharmacological options are limited, as almost 30% of patients fail to achieve remission after four or more interventions [2] and some manifest only short-term benefits [3]. This highlights the need for novel treatment options, such as transcranial direct current stimulation (tDCS) [4]. The technique is based on the application of low, direct currents via electrodes placed over the scalp to change cortical activity according to the parameters of stimulation [5]. For MDD, tDCS electrodes are applied over prefrontal cortex (PFC) regions considering repetitive transcranial magnetic stimulation (rTMS) antidepressant efficacy over this region [6] and the PFC dysfunction observed in this disorder [7].

Although several randomized controlled clinical trials (RCT) on tDCS for MDD were performed, results have been heterogeneous. For instance, several studies including a large, multicentric RCT failed to show tDCS efficacy over placebo [8,9]. In our Escitalopram versus Electrical Current Therapy for Treating Depression Clinical Study (ELECT-TDCS) trial, a non-inferiority, sham-controlled study, we found that, although superior to placebo, tDCS was not non-inferior to escitalopram [10]. Moreover, meta-analyses were able to demonstrate a moderate antidepressant effect of prefrontal tDCS [11,12].

Taken together, these findings suggest that tDCS may be effective for MDD but shall be optimized further — for instance by investigating putative neurobiological markers of response to prefrontal tDCS. Of potential biomarkers worth examining, MRI-based biomarkers are particularly promising for neuromodulation techniques as brain areas implicated in the pathophysiology of the disorder are stimulated — in other words, they are potential target candidates. In MDD, MRI-based meta-analyses found reductions in prefrontal gray matter volumes such as the bilateral anterior cingulate cortices (ACC), the limbic cortex, and the dorsolateral prefrontal cortex (DLPFC) [13–17]. In MDD patients who received pharmacotherapy, pre-treatment gray matter volumes of the ACC [18–21] and subregions of the PFC [19] predicted antidepressant response. Moreover, clinical improvement was associated with the increase of gray matter volumes in these regions [22,23]. For rTMS, for example, decreased global metabolism of left DLPFC and left ACC [24], or functional coupling between the DLPFC and ACC [25], predicted the antidepressant response.

However, associations of baseline MRI-based biomarkers and tDCS antidepressant effects have not been investigated to date [26]. This was investigated in a subsample of ELECT-TDCS. According to the available literature at study design [27], we hypothesized that the gray matter volumes of the left and right DLPFC and the ACC would be directly related to depression improvement in the tDCS vs. placebo groups. The study hypotheses were specified *a priori* in a study design publication [27] and in our study protocol [10]. We also explored other group comparisons and whether specific subregions of ACC and DLPFC, based on novel neuroanatomical parcellations published after ELECT-TDCS was initiated, were associated with tDCS antidepressant response.

Methods and materials

Overview

ELECT-TDCS is a single-center, randomized, double-controlled, and double-blinded non-inferiority trial; the full study design and results are described in detail elsewhere [10,27]. In this ancillary study of ELECT-TDCS, a subsample who received MRI scans at baseline was investigated [10]. Patients with MDD were recruited from the University Hospital (University of São Paulo, São Paulo) and computer-based randomized to three groups: active tDCS plus placebo medication (tDCS group), sham tDCS plus escitalopram (escitalopram group), and sham tDCS plus placebo medication (placebo group). Randomization was performed in a 3:3:2 ratio, corresponding to the groups tDCS, escitalopram, and placebo, respectively, using randomly permuted blocks with random block sizes. The randomization scheme was generated using the Web site www.randomization.com that employs the Wichmann-Hill random number generator.

The study is in accordance with the Declaration of Helsinki and was approved by the Local and National Ethics Committee (CAAE:10173712.3.0000.0076); it is registered in clinicaltrials.gov (NCT01894815: https://clinicaltrials.gov/ct2/show/NCT01894815?term=NCT01894815&rank=1). Written, informed content was obtained from all patients before inclusion. Patients were enrolled between October 2013 and July 2016.

Patients

We included patients with MDD according to the Diagnostic Statistical Manual of Mental Disorder, fifth edition (DSM-5) according to the following inclusion criteria: $\geq$ 17 points on the Hamilton Depression Rating Scale (HDRS-17), a low risk of suicide, at least 8 years of schooling (to ensure sufficient skills in reading and writing and the ability to give informed consent), and adherence to study protocol. Exclusion criteria were bipolar disorder, brain injury, pregnancy, specific contraindications to tDCS (e.g., cranial plates), current or previous escitalopram use, and previous or concomitant participation in other tDCS trials. Patients with anxiety disorders as comorbidity were not excluded. Trained psychiatrists and psychologists, blinded to the assigned treatment, performed the clinical assessments.

Patients under antidepressant therapy underwent drug washout and remained antidepressant free for at least 5 drug half-lives. Benzodiazepines were allowed up to 20 mg/day diazepam-equivalent.

Interventions

The patients underwent 10 weeks of prefrontal tDCS (1×1 tDCS-CT, SoterixMedical, New York, NY) — 3 weeks of daily tDCS, except the weekends, and 7 weeks of weekly tDCS — resulting in a total of 22 sessions. Anode and cathode electrodes were placed over the left and right DLPFC, respectively, using the "Omni-Lateral-Electrode" (OLE) system [28]. During active sessions, 2 mA direct current was administered for 30 min. During sham treatment, the current was turned off automatically after 30 s according to the randomization code.

Patients in the drug group received 10 mg/day of escitalopram (Reconter, Libbs Pharmaceutical Company, São Paulo, Brazil) during the first 3 weeks, and 20 mg/day thereafter. The placebo medication was visually indistinguishable, tasted exactly like the escitalopram pills and both were administered in the same bottles.

Magnetic resonance imaging

All images were acquired in 3 T MR system (Achieva, Philips Healthcare, Netherlands). Volumetric images were based on T1-weighted sequences using a 3D FFE pulse sequence with the following parameters: FOV $240 \times 240 \times 180$ mm^3, spatial resolution 1×1x1 mm^3, TR 7 ms, TE 3.2 ms, FA 8°, 180 sagittal slices. MR acquisitions were performed up to 8 days before baseline and were performed at the Department of Radiology (Hospital das Clínicas da Universidade de São Paulo, São Paulo) during the weekends.

We used FSL 5.0.10 (http://www.fmrib.ox.ac.uk/fsl/index.html), AFNI (Analyses of Functional Images, http://afni.nimh.nih.gov/afni)

and in-house scripts [29] for pre-processing steps [30]. Following quality check and after brain extraction, the T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid using FAST [31]. After FLIRT and FNIRT, a linear and non-linear registration method [32], the images were warped into the MNI standard space. All data sets were deidentified by using patient-specific codes in the DICOM header information and the face was removed with the help of *pydeface* to guarantee the privacy of the patients. In-house scripts [33] and volumetric data are available under request.

Gray matter volume

According to our *a priori* hypothesis, a region-of-interest (ROI) based approach was applied to calculate the volumes of DLPFC and ACC. As the DLPFC cannot be located within the classical anatomical boundaries, we used the Sallet et al. atlas [34]. This atlas provides a parcellation of the dorsal frontal cortex based on functional and tractography data from a cross-species approach in humans and primates, and divides the dorsal frontal cortex into 10 subregions (clusters), which are attributed to Brodmann areas (BAs) and their later adaptations [35,36]. This atlas was chosen as it allows to identify ROIs in proximity of the dorsolateral PFC area, while incorporating anatomical and functional data equally. Although the Sallet et al. atlas also maps premotor areas, they were not included in our analyses as these areas were not previously specified in our hypothesis. Therefore, we calculated the volume of a dorsal PFC ROI, which includes only the anterior PFC regions and corresponds to BAs 8, 9, 10, and 46 (Fig. S1).

For the definition of the ACC ROI, we used the parcellation of the Brainnetome atlas (http://atlas.brainnetome.org/bnatlas.html), a whole-brain multimodal parcellation atlas based on anatomical, diffusion tensor imaging, resting state functional MRI connectivity, and behavioral datasets [37] (Fig. S2).

As significant effects in these hypotheses-driven regions were observed, we analyzed subregions of the PFC and ACC in an exploratory way to identify regions possibly driving these effects. In the PFC, we investigated 7 clusters according to Sallet et al. (2013): cluster 3 (corresponding to BA9), cluster 4 (BA10), cluster 5 (BA9/46D), cluster 6 (BA9/46 V), cluster 7 (BA46), cluster 8 (BA8A), and cluster 10 (BA8B). Moreover, two further group ROIs not initially proposed by Sallet et al. that include clusters 3, 4 and 5 (corresponding to BAs 9, 10 and 9/46D, i.e. "BA9,10,9/46D") and clusters 6 and 7 (BAs 9/46 V and 46, i.e. "BA9/46 V,46") were analyzed to account for the non-focality of prefrontal tDCS (Fig. S1).

Considering the different roles of the subgenual (sgACC) and pregenual ACC (pACC) in predicting antidepressant response, particularly in rTMS literature [26,38–41], we further explored the volumes of these subregions, also using the sgACC ("A32sg") and pACC ("A32p") ROIs from the Brainnetome atlas (Fig. S2).

All gray matter volume calculations were corrected for the intracranial volumes between subjects.

Statistical analysis

We used R 3.4.3 [[42], https://www.R-project.org/], RStudio 1.1.383 [[43], http://www.rstudio.com/], and the package ggplot2 2.2.1 [44] to create line charts. We used the packages lme4 1.1–14 [45] and lmerTest 3.0–1 [46] to perform linear mixed effects analyses to explore which brain regions were associated with depression improvement. MRIcron was used to visualize ROIs as an overlay on the ch2better standard template [47].

The primary outcome was the HDRS-17 score evaluated at each time point as stated in the original study (baseline, 3, 6, 8, and 10 weeks, respectively). The primary investigated regions were the bilateral dorsal PFC and the bilateral ACC ROIs. To assess group differences, four separate linear mixed effects models were calculated for each one of these regions with the primary outcome (HDRS-17) as dependent variable; group, gray matter volume of ROI, time point, and their interaction were used as fixed variables, and individual intercepts and slopes as random effects (see supplemental material). Significant findings were only observed in the interaction of the three fixed variables. Hence, we further report values only based on this interaction. The group differences in ROI volume–outcome interactions were evaluated using the slope, Cohen's d (estimated from the model residual standard deviation) [48], their 95% confidence intervals, and significance levels. Cohen's d values of 0.2, 0.5, and 0.8 indicate small, medium, and large effect sizes, respectively. In addition, we report results from the same mixed effects model focused solely on the tDCS group. As these regions were hypotheses-driven, we did not apply a correction for multiple testings.

Finally, we exploratively investigated the volumes of subdivisions of the DLPFC and ACC applying the same models. This approach resulted in 11 additional models that were carried out for each hemisphere. We provide results of this exploratory analysis in the form of *uncorrected* results, as well as Bonferroni *corrected* p-values.

Results

Out of the original sample, 68 patients received MRI at baseline. The most important reasons for the omitted use of MRI were (1) the delayed start of the MRI collection, which only started after 30% of the sample had already been recruited, (2) patient refusal, as MRI collection was not obligatory for trial participation and occurred only at the weekends, and (3) lack of slots available for performing MRI up to 8 days before baseline. Other minor reasons included contraindications for MRI, the impossibility of performing MRIs during holidays, and technical reasons (for instance, MRI not available due to MRI maintenance).

Moreover, MRI scans of 16 patients were excluded after an initial quality check (absence of T1 anatomical sequences, abnormal anatomical findings, and poor quality due to head motion). Finally, MRI data of 52 patients were included, with 15, 16, and 21 patients in tDCS, escitalopram, and placebo groups, respectively.

Demographic and clinical characteristics of the 52 patients were distributed equally among the three treatment groups, with the exception for benzodiazepine use (Table 1). Volumes of MRI ROIs at baseline did not significantly differ between the three groups (Table 1). Mirroring the results of the main trial, our subgroup did not differ from the original sample, neither in most of the characteristics, nor in the outcomes (Table S1).

Hypotheses-driven regions

Prefrontal cortex

In the left PFC, larger gray matter volumes were associated with depression improvement in the tDCS compared to sham group (Cohen's $d = 0.3$, 95% confidence interval [0.01; 0.6], $p = 0.04$; Table S3, Fig. 1). Within the tDCS group, there was a trend for a direct association between PFC volume at baseline and further reduction of HDRS scores (factor $= 0.8 \pm 0.4$, $d = 0.5$, [-0.009; 1.0], $p = 0.055$).

In the right PFC, no significant difference between placebo vs tDCS ($d = 0.1$ [-0.1,0.4], $p = 0.4$; tDCS only: $d = 0.2$ [-0.3,0.7], $p = 0.4$) was observed.

Anterior cingulate cortex

In the left ACC, no significant differences between placebo vs tDCS group on depression improvement were found ($d = -0.01$

Table 1
Patient group characteristics.

n	tDCS	Escitalopram	Placebo	p
	15	16	21	
Males (%)	7 (46.7)	3 (18.8)	5 (23.8)	0.2
Age	43.8 ± 11.2	42.0 ± 13.4	36.4 ± 10.9	0.1
HDRS[a] at baseline	21.8 ± 4.1	21.2 ± 3.4	21.7 ± 3.8	0.9
HDRS[a] at week 3	14.6 ± 6.2	10.9 ± 3.9	12.7 ± 5.8	0.2
HDRS[a] at week 6	13.8 ± 6.8	11.5 ± 3.5	11.9 ± 5.1	0.4
HDRS[a] at week 8	10.2 ± 7.7	9.6 ± 3.5	14.4 ± 8.2	0.08
HDRS[a] at week 10	14.6 ± 9.9	11.1 ± 4.7	15.8 ± 8.3	0.2
Total HDRS[a] improvement	7.2 ± 11.3	10.1 ± 5.6	5.8 ± 8.5	0.3
Characteristics of current depressive episode[b]				
- Chronic (%)	6 (40.0)	7 (43.8)	11 (52.4)	0.7
- Severe (%)	3 (20.0)	3 (18.8)	5 (23.8)	0.9
- Recurrent (%)	10 (66.7)	14 (87.5)	12 (57.1)	0.1
- Resistant (%)	4 (26.7)	3 (18.8)	5 (23.8)	0.9
Years of schooling	13.8 ± 4.8	13.3 ± 5.2	15.2 ± 4.2	0.4
Benzodiazepine use (%)	5 (33.3)	0 (0.0)	6 (28.6)	0.04
Low income (%)	10 (66.7)	13 (81.2)	14 (66.7)	0.6
BMI	26.8 ± 3.4	28.0 ± 7.1	25.9 ± 5.0	0.5
Gray matter volumes (cm^3) at baseline				
Left PFC	24.3 ± 1.5	23.2 ± 2.4	23.8 ± 2.3	0.38
Right PFC	24.4 ± 1.4	23.5 ± 2.4	23.9 ± 2.1	0.53
Left ACC	7.2 ± 0.7	6.9 ± 0.9	7.3 ± 0.8	0.36
Right ACC	6.1 ± 0.5	5.7 ± 0.7	6.0 ± 0.7	0.15

Distribution of characteristics, clinical outcomes, and baseline gray matter volumes of the four main regions among intervention groups. Values are displayed as count and percentage for categorical variables, visible by (%), or mean ± standard deviation. Differences between groups were tested using ANOVA or chi-square test.
PFC = prefrontal cortex, ACC = anterior cingulate cortex.
[a] Scores on the 17-item Hamilton Depression Rating Scale (HDRS-17; 0 to 52, the higher the more severe depressed).
[b] These variables include characteristics of current episode: chronicity (≥12-month duration), severity (a score of 24 or more on HDRS-17), recurrence (>3 previous episodes), and treatment resistance (≥1 treatment failure in the current episode or >4 treatment failures over the lifetime).

[-0.3,0.3], p = 0.9; tDCS only: $d = 0.02$ [-0.5,0.5], p = 0.9). Accordingly, there were also no differences observed in the right ACC (d = 0.2 [-0.07,0.5], p = 0.1; tDCS only: $d = 0.4$ [-0.09,0.9], p = 0.1; Table S3).

Exploratory outcomes

Prefrontal cortex

For placebo vs tDCS, left BA9,10,9/46D ($d = 0.3$ [0.04; 0.6], $p_{uncorr} = 0.03$, $p_{corr} = 0.6$), left BA10 ($d = 0.3$ [0.04; 0.6], $p_{uncorr} = 0.02$, $p_{corr} = 0.5$), and right BA9 ($d = 0.3$ [0.03; 0.6], $p_{uncorr} = 0.03$, $p_{corr} = 0.7$, Fig. 1) were associated with depression improvement. A trend was observed for left BA46 ($d = 0.2$ [-0.04; 0.5], p = 0.09) (Table S3, Fig. S3). Small effect sizes were found for these regions (Fig. 2). For other regions, including the right hemisphere, placebo vs tDCS was not significant.

The comparisons of escitalopram vs tDCS showed trends only for the left BA9,10,9/46D ($d = 0.2$ [-0.03; 0.5], p = 0.08), left BA10 ($d = 0.2$ [-0.03; 0.5], p = 0.08), and right BA9 ($d = 0.2$ [-0.03; 0.5], p = 0.08) (Table S3, Fig. S3). No significant effects were detected for placebo vs escitalopram (Table S4).

In addition, we found no differences between the other groups (placebo vs escitalopram) in the left ($d = 0.09$ [-0.2; 0.4], p = 0.5) nor the right PFC ($d = 0.05$ [-0.2; 0.3], p = 0.7; Table S4).

Anterior cingulate cortex

No significant differences were observed for the left or right sgACC and pACC for any pairwise comparisons (Table S3&S4, Fig. S4).

Discussion

In this ancillary MRI study from the ELECT-TDCS trial [10], we investigated whether the baseline volumes of DLPFC and ACC, prefrontal brain regions that are associated with MDD pathophysiology and putatively involved in the mechanisms of action of tDCS [27], were associated with antidepressant improvement in 52 depressed subjects receiving tDCS, escitalopram, or placebo. As predicted *a priori*, our findings and visual evaluations strongly suggest a direct association between left PFC gray matter volume and tDCS antidepressant effects.

The left PFC has been associated with MDD pathophysiology in the past [19,20,22,49,50] and is general target region for NTBS (rTMS and tDCS) in MDD [6,11,51]. Some studies suggested that left PFC is relatively hypoactive compared to the right PFC, explaining why this region is targeted [7,52], but this was not confirmed by others [53].

Moreover, as "conventional" tDCS provides non-focal stimulation, large prefrontal areas are stimulated, and it is unclear whether the antidepressant effect is carried by the whole PFC region, its subregions, or more complex network interactions. For rTMS in MDD, the quality of DLPFC targeting, e.g. by applying neuronavigation [54], or more lateral and anterior targeting [55] predicted the antidepressant response to rTMS. In particular the functional connectivity between DLPFC target regions and the ACC has been discussed as mediator of this predictive effects [25,41], supporting the role of the DLPFC specifically. For prefrontal tDCS, very recent studies show that its effects on neurocognitive performance in healthy subjects and on symptoms in MDD patients may depend on structural (i.e. cortical thickness) or functional characteristics of DLPFC regions [70,71].

The left dorso*medial* PFC, a region that provides hub connections to several functional networks, is impaired in MDD [56] and should be discussed as target engagement candidate mediating antidepressant effects of tDCS as well. Indeed, recent rTMS trials in MDD have stimulated the left dorsomedial cortex, with positive results [57]. In fact, a recent electric field modeling study that simulated the electrode positioning used in ELECT-TDCS showed peak current densities in lateral and medial PFC areas [58].

Furthermore, in past modeling studies [64] and intracranial recordings [65,66], administration of 2 mA currents, as used in our study, led to different individual electric field intensities. Inter-individual differences in gray matter structural anatomy [67,68] could have contributed to different responses between individuals after prefrontal tDCS. Thus, it is possible that brain volume and tDCS-induced currents in the brain are correlated and that continuous treatment over weeks with higher electrical charge contributes to larger clinical responses. In future studies, individual dose-response relationships at targets for therapeutic effects should be further elaborated. The standard use of 1 mA or 2 mA should be overcome, favoring individual intensity tailoring based on neuroanatomical and functional findings. Possibly, intensities higher than 2 mA might produce greater clinical effects in MDD that shows a reduced gray matter volume in the PFC [18–21].

Our exploratory investigations suggest a direct association between antidepressant effects of tDCS and the volume of several subregions of the PFC, particularly a smaller area corresponding to BAs 9, 10 and 46 in the left hemisphere, a region corresponding to left BA 10 and another region in the right hemisphere that corresponds to BA9. These regions were located medially to the "classic" DLPFC location and potentially underline the role of medial regions for tDCS antidepressant effects, as stated in the previous paragraph. On the other hand, our results suggest also an involvement of the right PFC where, in the present study, cathodal tDCS was applied. Nonetheless, these exploratory investigations were no longer

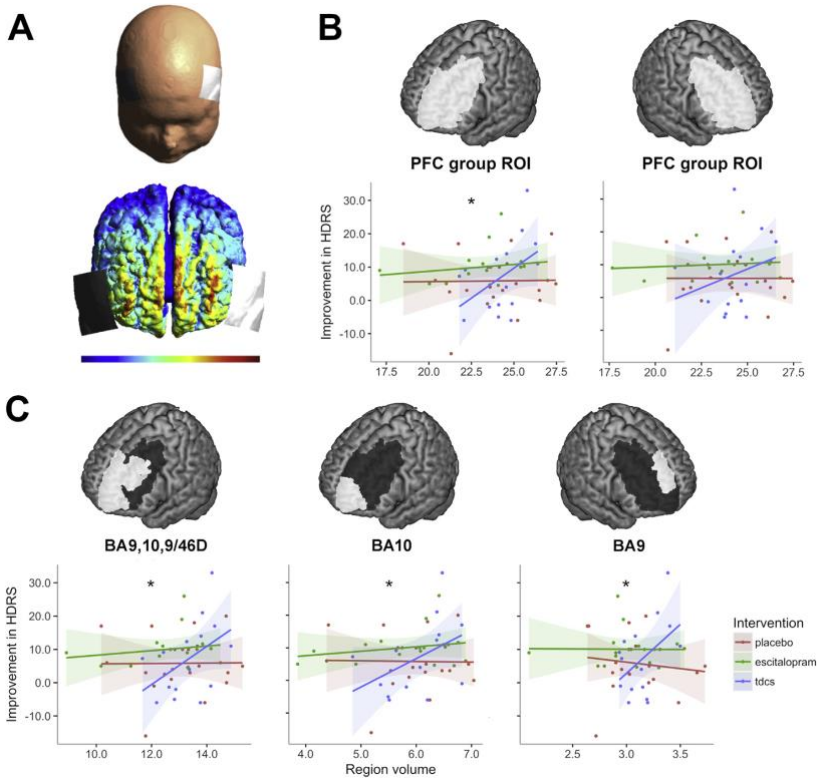

Fig. 1. Effect of prefrontal cortex volume on depression improvement
Figure 1 A illustrates the location of the stimulation electrodes and the distribution of the electric field on a study-specific group template (EEG-based F5–F6 location was used as approximation of the originally used Omni-Lateral-Electrode [OLE] position, as they show good accordance [28] and can be directly implemented in the electric field modeling software SIMNIBS [69]).
B&C show the outcomes according to the *a priori* hypothesis and the significant findings from the exploratory analyses, respectively. Shown are locations of the prefrontal cortex (PFC) regions when investigating the interactions of region-of-interest (ROI) volumes (cm^3) with depression improvement from baseline to week 10 on the Hamilton Depression Rating Scale (HDRS). A direct correlation was observed in the active tDCS group (blue) in opposition to the two control groups (placebo shown in red, escitalopram in green). Lines show regression graphs with 95% confidence intervals. The star (*) indicates statistical significance for comparison of tDCS vs placebo group. BA = Brodmann area. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

significant after Bonferroni corrections and hence, should be understood as exploratory for further hypothesis generation in future studies.

Contrary to our initial hypothesis, ACC volumes were not associated with the antidepressant response, whereas a recent meta-analysis showed that ACC volume was a robust predictor of the clinical response to antidepressant medication [18–20,26,59]. A possible explanation is that tDCS electrodes are directly placed over the scalp, hence rather modulating cortex regions at the convexity such as the DLPFC than inner cortical structures such as the ACC, which can be more properly targeted via invasive methods such as deep brain stimulation [60]. In addition, previous studies have shown that antidepressant effects may be related to functional or metabolic states of the ACC, rather than its structure [25,38–40,59,61] and future studies should better investigate the state effects rather than accept it as a trait.

Our findings cannot be presently compared with other tDCS studies in MDD as, to the best of our knowledge, this is the first controlled clinical trial investigating MRI parameters associated with antidepressant response to prefrontal tDCS. In fact, our results are relatively novel also considering other antidepressant therapies, as placebo-controlled trials investigating structural predictors of antidepressant response are insufficient and results from studies investigating different therapeutics are heterogeneous [26]. Short and long-term response to fluoxetine, for example, was associated with larger hippocampus volumes [62,63] and faster response was associated with larger volumes in several areas, such as the ACC, insula, or the left PFC [20]. In

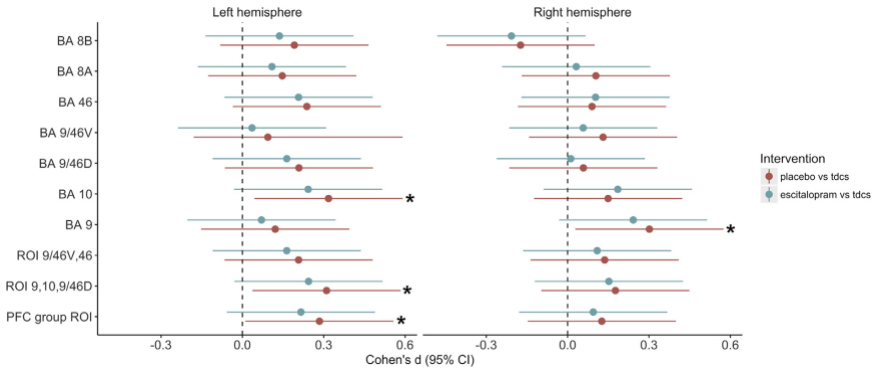

Fig. 2. Effect size of group differences. This figure shows the Cohen's *d* and the 95% confidence intervals of the difference of interactions of prefrontal cortex (PFC) volumes with depression improvement between the tDCS group and placebo group, and escitalopram group, respectively. Cohen's *d* values of 0.2, 0.5, and 0.8 indicate small, medium, and large effects, respectively. The star indicates statistical significance. BA = Brodmann area, ROI = region of interest.

our sub-sample, no association between volumes and response to escitalopram were observed. Nonetheless, as a direct association between PFC volume and tDCS response was observed, relatively small PFC volumes could be indicative of preferring escitalopram over tDCS. However, we did not identify PFC volume as a predictor of differential response between tDCS and escitalopram.

Limitations and strengths

Some study limitations should be underscored. First, for several reasons, MRI scans could only be obtained in a subsample of the original trial. Therefore, some non-statistically significant findings might be false-negative results owing to low statistical power. Nonetheless, our results should be regarded as exploratory and hypothesis-driven for future trials. Second, other neuroimaging approaches, such as resting-state functional MRI connectivity, or individual distributions of electric fields, were not explored in the present study. Although resting-state fMRI was collected at baseline, these data have not yet been analyzed and will be explored further.

Study strengths include: first, our hypotheses were defined *a priori*, enhancing the validity of our findings; second, the study employed a parallel, three-arm design, allowing comparisons of tDCS with both placebo and escitalopram; third, patients were not using any treatment at the beginning of the study, which could have been a potential source of confounding, and; fourth, we used novel approaches for defining PFC and ACC subregions, as the ROIs were based on the Sallet et al. [34] and Brainnetome atlases [37], which delimit brain regions based on anatomical and functional aspects, in contrast to standard atlases included in neuroimaging software packages.

Conclusion

Our findings provide a neurobiological underpinning for antidepressant effects of prefrontal tDCS in patients with MDD, as we showed that the response was associated with the volume of a left-sided PFC region at baseline for tDCS, but not escitalopram and placebo. Nonetheless, our results should be regarded as exploratory and hypothesis-generating for further study trials. In addition, our findings provided first evidence that baseline MRI measurements may be used for identifying patient groups that benefit from tDCS, which can be useful in future studies investigating multimodal predictors of tDCS response.

Funding

ELECT-TDCS funding: São Paulo Research State Foundation (FAPESP) and others. Registration: ClinicalTrials.gov NCT01894815.

Acknowledgments & DISCLOSURES

This study was primarily supported by a São Paulo Research State Foundation (FAPESP) grant (2012/20911-5). ARB is a recipient of a CAPES/Alexander von Humboldt fellowship award for experienced researchers and receives speakers' fee from Neurocademy GmbH (Munich, Germany). The Laboratory of Neuroscience receives financial support from the Beneficent Association Alzira Denise Hertzog da Silva and the CAPES / INCT program "National Institute of Biomarkers in Psychiatry" (INBioN). FP is a member of the European Scientific Advisory Board of Brainsway Inc., Jerusalem, Israel, and has received speaker's honoraria from Mag&More GmbH and the neuroCare Group. His lab has received support with equipment from neuroConn GmbH, Ilmenau, Germany, and Mag&More GmbH and Brainsway Inc., Jerusalem, Israel. This work was also supported by the German Center for Brain Stimulation (GCBS) research consortium (Work Package 5) [grant number 01EE1403E], funded by the Federal Ministry of Education and Research (BMBF). The work of LB is part of a PhD/residency program of the Ludwig-Maximilians University (LMU) and the International Max Planck Research School for Translational Psychiatry (IMPRS-TP) financially supported by the Else Kröner Fresenius Foundation. PVB, FD, GB, EAJ, IMB, PAL, WG, SG, and DK reported no biomedical financial interests or potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.05.006.

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10.2 Paper II - Prefrontal resting-state connectivity and antidepressant response: no associations in the ELECT-TDCS trial

European Archives of Psychiatry and Clinical Neuroscience
https://doi.org/10.1007/s00406-020-01187-y

ORIGINAL PAPER

Prefrontal resting-state connectivity and antidepressant response: no associations in the ELECT-TDCS trial

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Received: 30 April 2020 / Accepted: 20 August 2020

Abstract
Functional and structural MRI of prefrontal cortex (PFC) may provide putative biomarkers for predicting the treatment response to transcranial direct current stimulation (tDCS) in depression. A recent MRI study from ELECT-TDCS (Escitalopram versus Electrical Direct-Current Theror Depression Study) showed that depression improvement after tDCS was associated with gray matter volumes of PFC subregions. Based thereon, we investigated whether antidepressant effects of tDCS are similarly associated with baseline resting-state functional connectivity (rsFC). A subgroup of 51 patients underwent baseline rsFC-MRI. All patients of ELECT-TDCS were randomized to three treatment arms for 10 weeks (anodal-left, cathodal-right PFC tDCS plus placebo medication; escitalopram 10 mg/day for 3 weeks and 20 mg/day thereafter plus sham tDCS; and placebo medication plus sham tDCS). RsFC was calculated for various PFC regions and analyzed in relation to the individual antidepressant response. There was no significant association between baseline PFC connectivity of essential structural regions, nor any other PFC regions (after correction for multiple comparisons) and patients' individual antidepressant response. This study did not reveal an association between antidepressants effects of tDCS and baseline rsFC, unlike the gray matter volume findings. Thus, the antidepressant effects of tDCS may be differentially related to structural and functional MRI measurements.

Keywords Antidepressant response · Resting state functional connectivity (rsFC-MRI) · Major depressive disorder (MDD) · Non-invasive transcranial brain stimulation (NTBS) · Prefrontal cortex · Transcranial direct current stimulation (tDCS)

Abbreviations

ACC	Anterior cingulate cortex
BA	Brodmann area
DMN	Default mode network
MPFC	Medial prefrontal cortex
DLPFC	Dorsal lateral prefrontal cortex
ELECT-TDCS	Escitalopram versus Electrical direct-current therapy for depression
HDRS-17	Hamilton depression rating scale
NA	Negative affect
PANAS	Positive and negative affect scale
PA	Positive affect
PCC	Posterior cingulate cortex
PFC	Prefrontal cortex
ROI	Region of interest
rsFC	Resting state functional connectivity
TMS	Repetitive transcranial magnetic stimulation
tDCS	Transcranial direct current stimulation

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00406-020-01187-y) contains supplementary material, which is available to authorized users.

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Introduction

MRI derived resting-state functional connectivity (rsFC) is a promising approach for subtyping major depressive disorder (MDD) and the antidepressant response to several therapies [for reviews, consider 1–7]. A particular rsFC network

Published online: 02 September 2020

associated with MDD is the default mode network (DMN), comprising the ventral and medial prefrontal (MPFC), the posterior cingulate (PCC) and lateral parietal cortices, [8–10]. Connectivity of regions of the DMN, such as the prefrontal cortex (PFC), the MPFC, and the dorsal lateral PFC (DLPFC), was associated with the depressive episode [2, 3, 11] and a marker of treatment response in depression, either to medication and/or psychotherapy [4, 12–15], electroconvulsive therapy [16–19], or transcranial magnetic stimulation (TMS) [20–24].

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulatory brain stimulation method that has been increasingly applied since the 2000s [25]. It is hypothesized to modify resting membrane potentials leading to excitatory and inhibitory effects on underlying brain regions [25, 26]. Clinical outcomes of prefrontal tDCS as add-on or monotherapy for depression are promising but heterogeneous [27–33]. This comes partially from heterogeneous treatment protocols in terms of numbers of sessions and treatment periods [33]; however, individual factors may also contribute to this variance.

One factor that may explain heterogeneous tDCS responses is the gray matter morphology of the tDCS target region, the left PFC, as was shown in our earlier complementary analysis of baseline MRI data from the Escitalopram versus Electrical Direct-Current Therapy for Depression (ELECT-TDCS) trial by revealing a positive correlation between gray matter volumes of PFC subregions and the antidepressant response to tDCS when compared to placebo [34]. Similar associations of cortical thickness in this region and tDCS effects on cognition were found in a study applying a decision-making paradigm [35]. This relationship between structural morphology and tDCS effects could be explained by the fact that the intensity of the electric current induced by tDCS at the cortical level depends on *the individual* brain structure *and conductivity of the respective tissues including* cerebrospinal fluid and skull [36, 37]. Thus, the variation of these factors could theoretically explain a variation of behavioral effects.

As tDCS was shown to modulate rsFC of the DMN and frontal-parietal networks, involving regions in the PFC [38–40] and task activation of the left DLPFC, with the latter being suggested as a biomarker of antidepressant response following tDCS combined with psychotherapy [41], modulation of rsFC in the PFC and associated networks is being considered as a major mechanism behind tDCS effects. However, direct tDCS effects on rsFC show high interindividual variability [42], therefore there is a need to further investigate tDCS effects based on baseline rsFC among patients with MDD. Furthermore, no study has yet investigated the relationship of PFC's structural anatomy and rsFC with regards to the clinical outcome of depressed patients in the same sample, in particular one comparable to that from the ELECT-TDCS trial, which included a control group receiving sham tDCS and placebo medication. The results of our first ancillary study of MRI data from the ELECT-TDCS trial identified mainly the left PFC and in addition three subregions of the left and right PFC that were associated with tDCS response in terms of baseline gray matter volumes [34]. TDCS response was evaluated with changes in the Hamilton Depression Rating Scale (HDRS-17), which showed superior effects of tDCS over placebo in the main trial [43]. Here, we investigate whether baseline rsFC-MRI in these four a priori defined structural regions is associated with the antidepressant response to tDCS [34]. We then performed exploratory analyses of the full parcellation of the dorsal PFC to identify associations of rsFC and the antidepressant response to tDCS [44]. Additional analyses investigated whether rsFC could predict changes to negative and positive affect.

Methods and materials

Study design

This is an ancillary study of ELECT-TDCS, a randomized, double-blinded, sham-controlled, non-inferiority trial conducted between October 2013 and July 2016 at the University Hospital of the University of São Paulo. The full study design and results are described in detail elsewhere [27, 43]; in short, patients with MDD were treated over 10 weeks with (1) active tDCS and placebo medication, (2) sham tDCS and escitalopram, or (3) sham tDCS plus placebo medication. The primary outcome failed to showed non-inferiority of tDCS treatment compared to escitalopram treatment, but a superior effect of tDCS compared to placebo was observed in the secondary analyses [27]. Following our previous study on the relationship between improvement of depression after tDCS and MRI-based PFC gray matter volumes at baseline [35], we investigated in the current study whether MRI-based rsFC shows a similar association for PFC subregions.

Ethics approval

ELECT-TDCS was designed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards and approved by the Local and National Ethics Committee (CAAE:10173712.3.0000.0076). All participants signed an informed consent form prior to inclusion (clinicaltrials.gov NCT01894815).

Patients

MDD was diagnosed according to the Diagnostic Statistical Manual of Mental Disorder, fifth edition (DSM-5). Patients with ≥ 17 points on the HDRS-17, a low risk of suicide, at least 8 years of school education (to ensure sufficient skills in reading and writing and the ability to give informed consent), and those who were able to follow the study protocol were included. Exclusion criteria were bipolar disorder, brain injury, pregnancy, specific contraindications to tDCS (e.g., cranial plates), current or previous use of escitalopram, and past or concomitant participation in other tDCS trials. Patients with anxiety disorders as comorbidity were not excluded. A drug washout was performed in patients who received antidepressants before study onset, and a drug-free period of at least 5 drug half-lives was kept. Benzodiazepines were allowed up to 20 mg/day diazepam-equivalent.

Interventions

After randomization, active or sham tDCS were conducted with 22 sessions (3 weeks daily tDCS Monday to Friday, 7 weeks tDCS once a week) as required by the respective condition. Active tDCS was applied at 2 mA for 30 min using a 1 × 1 tDCS-CT device (SoterixMedical, New York, NY) with the "Omni-Lateral-Electrode" (OLE) electrode montage (anode over left, cathode over right DLPFC) [45]. The same set up and duration was used for sham tDCS, except that the current was automatically turned off after 30 s.

The drug comparison was escitalopram, an effective antidepressant drug [46] (Reconter, Libbs Pharmaceutical Company, São Paulo, Brazil). The initial dose of 10 mg/day escitalopram was administered for 3 weeks to reduce possible adverse effects and blinding breaking. After 3 weeks, escitalopram was titrated up to 20 mg/day in all patients. Placebo medication was administered over full 10 weeks; the placebo pill looked and tasted exactly like the escitalopram pills and they were distributed in same bottles.

MR acquisition and analysis

A 3 T MR system (Achieva, Philips Healthcare, Netherlands) was used. Structural images were acquired with a T1-weighted, 3D FFE pulse sequence (FOV 240 × 240x180 mm^3, spatial resolution 1 × 1x1 mm^3, TR 7 ms, TE 3.2 ms, FA 8°, 180 sagittal slices). Functional connectivity was acquired in resting state using an EPI single shot (FOV 240 × 240x144 mm^3, spatial resolution 3 × 3x4 mm^3, TR 2000 ms, TE 30 ms, imaging matrix 80 × 79, FA 80°, 32 slices, 200 volumes). MRI scans performed at the Institute of Radiology (Hospital das Clínicas da Universidade de São Paulo, São Paulo) up to 8 days before the start of the trial.

We adhered to our automated pipelines for pre-processing and analysis of functional data sets, for details, see (https://doi.org/10.5281/zenodo.3530897) [38, 42, 47–49]. Some of the crucial steps consisted of the automated conversion of DICOM format files into NIFTI under anonymization of the header information, relying on a patient-specific codes, and quality check using the XNAT app (https://doc.brain-stimulation.de/xnat-app-upload/). Low- and high-bandpass filtered (0.1–0.009 Hz), slice timed, and motion-corrected time series were transformed to subject-space using the linear and non-linear transformation from the FSL software package (FSL 5.0.10 (https://www.fmrib.ox.ac.uk/fsl/index.html). Motion and mean signal intensity of the white matter and cerebrospinal fluid were used as nuisance regressors before the residuals were exported using AFNI ((https://afni.nimh.nih.gov/afni). These residuals were demeaned, averaged, and smoothed before averaged time series were extracted and correlated to the whole-brain residual masks. The regions of interest (ROIs) from which averaged time series were extracted (four in the primary analysis, ten in exploratory analysis) are described in the next paragraph. These correlation maps were then transformed into z-values using Fisher's R-to-Z transformation and thresholded into positive and negative correlations using thresholds of $z > 0.3$ and $z < -0.3$, which equals a conservative significance level of $p \leq 0.0027$ [49]. The z masks were transformed into MNI standard space and averaged z values as well as numbers of voxels over threshold of $z = 0.3$ were extracted from the respective ROIs (i.e. correlations within these ROIs, "regional rsFC"), as well as the whole brain mask (i.e. correlations of ROI to the whole brain, "global rsFC"). While z values give averaged and transformed correlation intensity, the numbers of activated voxels give the spatial extent of correlations, i.e. how many of those voxels in the regional mask show these suprathreshold correlations [49]. As we have shown the spatial extent of connectivity to be a reliable outcome interest [42, 49], this is what we used as the primary outcome of interest (for scatter plots showing numbers of activated voxels and z-values, see Suppl. Figure 3).

Regions in the prefrontal cortex

Our primary hypothesis was to investigate the rsFC in four left and right PFC regions, for which we have shown an association of gray matter volumes and reduction of HDRS-17 scores after 10 weeks of tDCS treatment in a previous ancillary study of the ELECT-TDCS trial [34]. We have chosen the ROI-based approach and the restriction to predefined hypotheses due to our limited sample size; more refined approaches, such as individual component analysis, would

be even more vulnerable to this limitation of our study. The prefrontal cortex regions were defined according to a previously published parcellation of the dorsal frontal cortex based on functional and tractography data from a cross-species approach in humans and primates by Sallet et al. [50]. It divides the dorsal frontal cortex into ten subregions (clusters), which are attributed to Brodmann areas (BAs) and their later adaptations [51, 52]. This atlas was chosen as it allows to identify regions in proximity of the dorsolateral PFC area, taking anatomical and functional data equally into account. The specific ROIs from the primary hypothesis were: the whole left dorsal prefrontal cortex region ("Left PFC") and its subregions—left BA9, BA10, and BA9/46D (anterior subregion), left BA10 (single anterior subregion), and right BA9 (medial single subregion, see supplemental information). In a second exploratory analysis, all 10 PFC regions from our previous analysis were analyzed [34] (see Suppl. Figure 1).

Analysis methods and outcome variables

Linear mixed-effects models (LMM) were calculated to identify the associations of baseline MRI-based rsFC ("regional" connectivity within the respective ROIs and "global" connectivity from ROI to the whole brain) and improvement of depression with treatment group and time point as fixed effects, and individual intercepts as random effects (R 3.6.0 [53, https://www.R-project.org/], RStudio 1.1.463 [54, https://www.rstudio.com/], and packages ggplot2 3.2.1 [55], lme4 1.1–21 [56], and lmerTest 3.1–1 [57]) MRIcron was used for visualization [58]. The primary outcome, i.e. "improvement of depression" was defined as the change in HDRS-17 score; the secondary analyses were performed using the positive (PA) and negative affect (NA) symptom subscale from the Positive and Negative Affect Scale (PANAS) as dependent variables. There were five timepoints (week 0, 3, 6, 8, 10) for the HDRS-17 score and four timepoints (week 0, 3, 6, 10) for the PANAS. If for a specific time point, the outcome measure was missing, a linear model based on the baseline, age, and gender was generated to predict the missing value, following the procedures used in the original manuscript [27]. In terms of HDRS, up to 25% of the sample were missing values (13 cases per week 6 and 8, 10 cases per week 10), for PA and NA, these were 12 missing points at week 6 and 10 at week 10.

The association of rsFC and antidepressant response was considered significant if $p < 0.05$ for the comparison of tDCS vs. placebo group of the triple-interaction of treatment group, baseline rsFC, and time point, a model used in our previous work [34]. The group differences in rsFC–outcome interactions were then evaluated using the slope, Cohen's *d* (estimated from the model residual standard deviation) [59], their 95% confidence intervals, and significance levels. Correction for multiple comparisons was performed using Bonferroni corrections. Cohen's *d* values of 0.2, 0.5, and 0.8 indicate small, medium, and large effect sizes, respectively. A power analysis was not performed a-priori due to the ancillary nature of our investigation, yet we calculated post-hoc estimates of achievable effect sizes and sample sizes based on out model parameters (see supplementary information, Figs. 6 and 7).

Results

Patient characteristics and description of clinical outcomes in this subsample

Of the 245 patients included in the original ELECT-TDCS trial, patients were not included in the current analysis due to missing MRI baseline data a) due to the delayed start of the MRI collection after 30% of the sample had already been recruited, b) due to patient refusal, as MRI collection was not mandatory, c) patient exclusion due to MRI contraindications, d) or scheduling issues (such as lack of slots available for performing MRI up to 8 days before baseline, during holidays, or non-availability of the MRI scanner due to maintenance; $n = 177$). Furthermore, datasets were excluded due to low quality (high head motion, abnormal anatomy; $n = 16$). One dataset included in the previous structural analysis [34] did not include an EPI sequence, resulting in 51 datasets available for this rsFC analysis. Significant differences between treatment groups were seen for benzodiazepine use and anxiety levels, as well as the smoking status (Table 1). Reduction of depression scores were largest for the escitalopram group ($n = 16$), followed by the tDCS ($n = 15$) and placebo ($n = 20$) groups. For PA and NA, reductions were largest for tDCS group, followed by escitalopram and placebo groups (Table 1). Differences were not statistically significant.

Functional connectivity of essential structural regions and clinical improvement

In the four a-priori defined regions (left PFC; combined left BA9, BA10, BA9/46D; left BA10; and right BA9) the improvement of patients' HDRS-17 scores after 10 weeks of tDCS treatment was not associated with baseline rsFC ($p > 0.05$, global nor regional rsFC, Fig. 1, Table 2).

Further analysis showed that there was no association between baseline rsFC in these regions and changes in specific symptom domains such as PA and NA (Suppl. Figure 2, Table 3).

Table 1 Patient characteristics

	Escitalopram	tDCS	Placebo	*p*
n	16	15	20	
Age	42.31 (13.23)	43.33 (11.06)	36.90 (10.98)	.220
Males (%)	3 (18.8)	7 (46.7)	5 (25.0)	.200
Study years	14.20 (3.88)	14.64 (3.69)	15.42 (4.32)	.669
Smoking (%)	3 (18.8)	7 (46.7)	1 (5.3)	.014
Benzos (%)	0 (0.0)	5 (33.3)	6 (30.0)	.039
BMI	27.95 (7.12)	26.75 (3.36)	25.96 (5.16)	.556
Recurrent MD (%)	14 (87.5)	10 (66.7)	12 (60.0)	.183
Nr. of episodes	10.03 (12.48)	4.91 (2.23)	7.48 (13.67)	.448
Chronic (%)	7 (43.8)	6 (40.0)	10 (50.0)	.834
Melancholic (%)	24.81 (11.82)	27.33 (13.23)	24.90 (8.97)	.775
Anxiety (%)	8 (50.0)	9 (60.0)	4 (20.0)	.041
Response	4 (25.0)	9 (60.0)	7 (35.0)	.121
HDRS change	10.07 (5.63)	7.16 (11.26)	5.63 (8.64)	.322
PA change	3.94 (9.62)	6.53 (8.45)	3.65 (8.55)	.602
NA change	6.61 (9.18)	7.99 (8.66)	4.77 (7.73)	.535

Clinical characteristics of the treatment groups; if not specified, mean and standard deviation are shown, otherwise number and percentage (%). Differences between groups were tested using ANOVA or chi-square test

Recurrence was defined as > 3 previous episodes; chronicity as ≥ 12-month duration, response was defined as a > 50% reduction from the baseline 17-item Hamilton Depression Rating Scale (HDRS-17) score; HDRS = scores on the HDRS-17 (scores range from 0 to 52); PA = positive affect scores and NA = negative affect scores on the Positive and Negative Affect Schedule (PANAS; scores range from 10 to 50). Change in depression/affect scores refers to the difference from week 10 to baseline, which is calculated so that a larger change corresponds to a larger improvement of depressive symptoms

Exploratory analyses

In the exploratory analyses, baseline regional (within ROI) rsFC of the right-sided BA9/46 V,46 and the right-sided BA46 regions was associated with improvement of depression on the HDRS-17, when compared to the placebo group, showing a positive association of baseline regional rsFC and depression improvement (right BA9/46 V,46: slope = − 4.92, std.error = 2.13, $p = 0.02$, Cohen $d = -0.32$ CI [− 0.60; − 0.05]; right BA46: slope = − 12.38, std.error = 5.29, $p = 0.02$, Cohen $d = -0.33$ CI [− 0.60;− 0.05]; Fig. 2). Global rsFC of a right-sided BA9/46D region was associated with larger improvement of NA when compared to the placebo group, showing a negative association of baseline rsFC and NA improvement (slope = 0.26, std.error = 0.10, $p = 0.01$, Cohen $d = 0.35$ CI [0.07; 0.62]; Fig. 2), although it should be stated that this effect might be driven by the placebo group. Yet none of these effects sustained after Bonferroni corrections for multiple comparisons (p > 0.50). In general, the baseline rsFC and gray matter volume showed no associations ($p > 0.05$, Suppl. Figure 4; their distributions are shown in Suppl. Figure 5).

Discussion

In this ancillary investigation of rsFC-MRI data from the ELECT-TDCS trial, we did not identify any association between prefrontal regional and global functional connectivity and improvement of depressive symptoms after tDCS treatment. This study adds to our first ancillary investigation of structural MRI data from the ELECT-TDCS trial [34].

Lack of an association of baseline rsFC and antidepressant effects in the ELECT-TDCS trial

In our first ancillary study of structural MRI data from the ELECT-TDCS study, we showed that gray matter volumes of a larger, left-sided PFC region were associated with clinical improvement of MDD after 10 weeks of tDCS treatment [34]. This effect was carried by bilateral MPFC regions, that showed higher electric field intensities based on computational models from MRI data [34]. Thus, we followed this finding using rsFC-MRI data for the same PFC subregions according to the Sallet et al. atlas [50], however, we were not able to detect a similar association between antidepressant effects and functional connectivity in these regions.

There are several possible explanations for obtaining significant results for structural, but not for rsFC-MRI data.

While there is some evidence that, at least in unimodal regions, such as primary sensory and motor regions the functional connectivity is constrained by the structural connectivity [60], in other regions, however, this relationship is not that clear [60, 61]. Several reviews and one meta-analysis on structural *and* functional imaging concluded on their property to show region-specific and modality-specific predictions of antidepressant response [1, 4–6]. For some regions, such as the hippocampus, they provided data in support of a link between structural or rsFC characteristics and the antidepressant response (following rTMS treatment in the case of hippocampus), yet for most regions, there were no such associations [5], Whether this is due to a true absence of a structure–function relationship, or rather due to the limited number of studies comparing structure and function within the same regions, or a publication bias towards significant findings is less clear. A recent review explained this apparent "uncoupling" of structure and function on the level of their respective connectivities; they hypothesized that current models are not sufficient to predict FC from structural connectivity due to the lack of biological data and suggested to enrich structural network reconstructions with cellular and molecular metadata to improve the models of structure–function relationships [61].

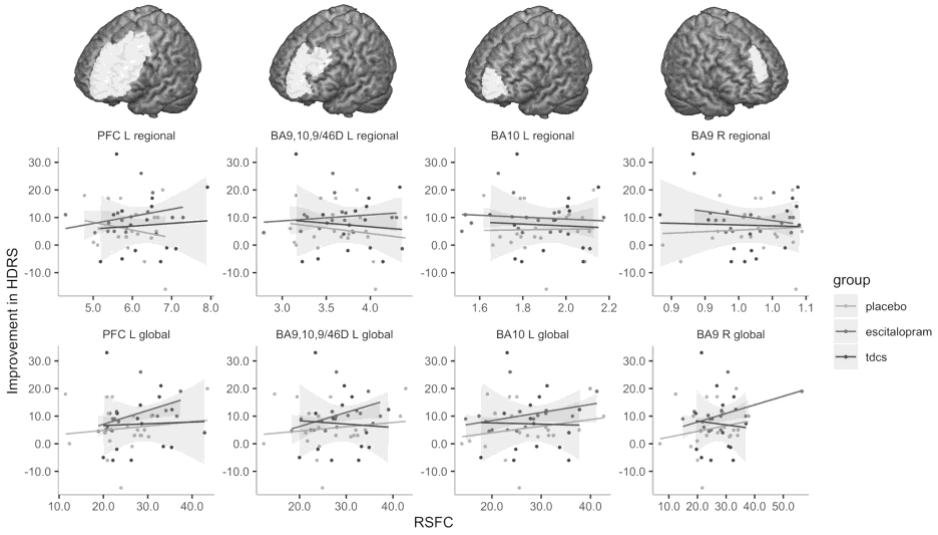

Fig. 1 No associations of resting-state functional connectivity in essential structural prefrontal cortex regions and improvement of depression after tDCS. This figure shows no significant associations of baseline regional (within ROI) and global (ROI to the whole brain) resting-state functional connectivity (rsFC) and improvement of depression on the 17-item Hamilton Depression Rating Scale (HDRS-17) in the treatment arm that received transcranial direct current stimulation (tDCS), as compared to the two control arms, in essential structural regions (shown in the top row). For visualization purposes, the regression lines show associations with change in HDRS scores; statistics were calculated using mixed linear effects models with HDRS as the outcome variable, group, rsFC, and timepoint as fixed, and individual intercepts and slopes as random effects. *RSFC* represents numbers of activated voxels $\times 10^3$. *BA* Brodmann area, *PFC* prefrontal cortex

Table 2 Associations between baseline resting-state functional connectivity in prefrontal regions and the antidepressant response to tDCS

Region	Slope	Std.error	*p*.value	Cohen.*d*	95% CI
Global rsFC					
Left PFC	0.04	0.10	0.68	0.06	[− 0.22; 0.33]
Left BA9, BA10, and BA9/46D	0.05	0.10	0.64	0.07	[− 0.21; 0.34]
Left BA10	0.05	0.10	0.65	0.06	[− 0.21; 0.34]
Right BA9	0.06	0.10	0.55	0.08	[− 0.19; 0.36]
Region	Slope	Std.error	*p*.value	Cohen.*d*	[min; max]
Regional rsFC					
Left PFC	− 0.34	0.96	0.72	− 0.05	[− 0.32; 0.22]
Left BA9, BA10, and BA9/46D	0.05	1.77	0.98	0.00	[− 0.27; 0.28]
Left BA10	2.91	4.14	0.48	0.10	[− 0.18; 0.37]
Right BA9	9.16	10.83	0.40	0.12	[− 0.16; 0.39]

Contrast tDCS vs. placebo is shown here, derived from linear mixed-effects models showing the effects of group interaction, resting-state functional connectivity (rsFC; global, i.e. from region to the whole brain, or regional, i.e. within the region), and timepoint on change of the 17-item Hamilton Depression Rating Scale (HDRS-17) score. The group differences in rsFC–outcome interactions were evaluated using the slope, standard error, significance levels, and Cohen's d (estimated from the model residual standard deviation; d of 0.3 represents moderate effect size) and its 95% confidence intervals (95% CI [lower bound; upper bound])

Table 3 Associations of baseline resting-state functional connectivity in prefrontal regions and positive/negative affect change after tDCS

Region	Estimate	Std.error	*p*.value	Cohen.d	95% CI
A) Positive affect					
Global rsFC					
Left PFC	− 0.10	0.13	0.44	− 0.11	[− 0.38;0.17]
Left BA9, BA10, and BA9/46D	− 0.14	0.14	0.35	− 0.13	[− 0.41;0.14]
Left BA10	− 0.22	0.14	0.13	− 0.21	[− 0.49;0.06]
Right BA9	− 0.18	0.14	0.21	− 0.18	[− 0.45;0.10]
Regional rsFC					
Left PFC	0.27	1.34	0.84	0.03	[− 0.25;0.30]
Left BA9, BA10, and BA9/46D	0.63	2.47	0.80	0.04	[− 0.24;0.31]
Left BA10	− 7.15	5.70	0.21	− 0.18	[− 0.45;0.10]
Right BA9	− 10.93	15.01	0.47	− 0.10	[− 0.38;0.17]
B) Negative affect					
Global rsFC					
Left PFC	0.10	0.13	0.46	0.10	[− 0.17;0.38]
Left BA9, BA10, and BA9/46D	0.17	0.14	0.22	0.17	[− 0.10;0.45]
Left BA10	0.25	0.14	0.08	0.25	[− 0.03;0.52]
Right BA9	0.13	0.14	0.36	0.13	[− 0.14;0.40]
Regional rsFC					
Left PFC	− 0.63	1.31	0.63	− 0.07	[− 0.34;0.21]
Left BA9, BA10, and BA9/46D	− 1.07	2.38	0.65	− 0.06	[− 0.34;0.21]
Left BA10	1.44	5.42	0.79	0.04	[− 0.24; 0.31]
Right BA9	3.02	14.89	0.84	0.03	[− 0.25; 0.30]

Contrast tDCS vs. placebo is shown here, derived from linear mixed-effects models showing the effects of the group interaction, resting-state functional MRI connectivity (rsFC; global, i.e. from region to the whole brain, or regional, i.e. within the region), and timepoint of change of the positive and negative affect scores derived from the Positive and Negative Affect Schedule (PANAS). The group differences in rsFC–outcome interactions were evaluated using the slope, standard error, the significance levels, and Cohen's *d* (estimated from the model residual standard deviation; *d* of 0.3 represents moderate effect size) and its 95% confidence intervals (95% CI [lower bound; upper bound])

Second, methodological aspects could provide an explanation for our inability to identify the association of structure and function with the antidepressant response in the ELECT-TDCS trial. Studies apply different measures to assess structure and function. "Structure" is commonly assessed on the cortical level by voxel-based morphometry, surface-based measurement of cortical thickness, calculation of gray matter volumes in volumetric space, or in terms of structural connectivity by investigating the integrity of white matter tracts. Likewise, "function" may be expressed as functional connectivity measured in the resting state or functional activation of regions during a task, intended at activating regions responsible for specific functions (e.g. working memory). Theoretical constructs of rsFC measures themselves differ among studies; often they are defined as functional connectivity in resting-state networks as a whole or depict regions with increased or decreased connectivity within these networks. This variation of methods and underlying constructs makes it difficult to compare results between studies, and the type of measure may bias findings towards decoupling (or coupling?) of structure and function. For example, the non-linear relationship of structure and function mentioned in the previous paragraph is based on measures of structural and functional connectivity [60, 61], while the meta-analysis referred to task-based functional activation and voxel-based morphometry [4].

Though such theoretical considerations are tempting, a simple explanation for the lack of significant rsFC findings in spite of our previous findings for PFC grey matter volumes are type II errors. A major limitation in this study was the sample size which was the reason for staying with our a priori hypotheses and not advancing to independent component analyses or other more refined approaches. As our analyses are largely vulnerable to type II errors, the negative findings in our study do not prove the absence of an association between antidepressant effects and functional connectivity data. This is particularly relevant as both samples were practically identical (i.e. 51 and 52 patients from the ELECT-TDCS trial; one patient with missing EPI data, thus explaining the difference). The effects sizes of the tDCS vs. placebo model were rather small to moderate, tending

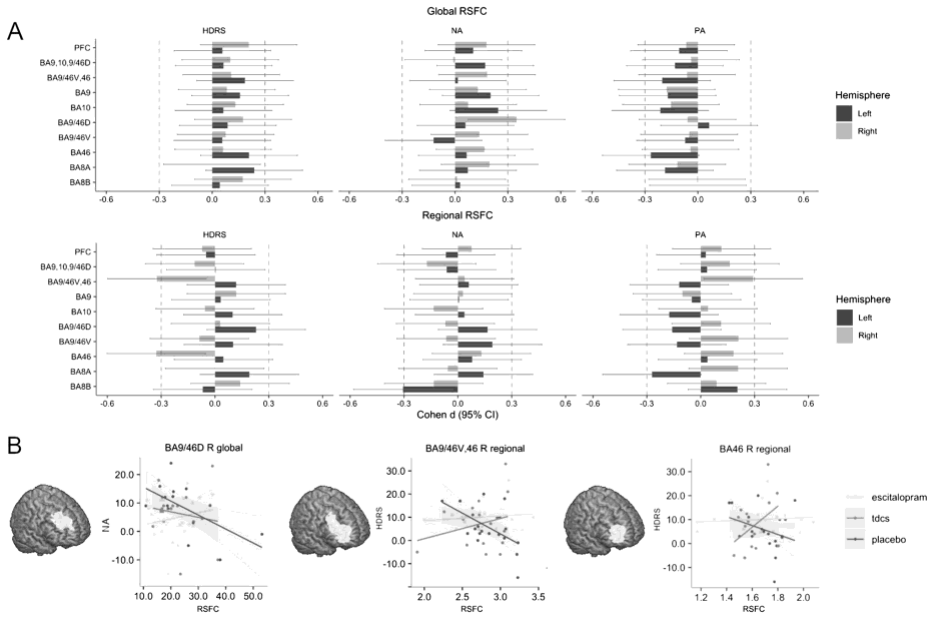

Fig.2 Effects of resting-state functional connectivity on depression improvement and positive/negative affect change after tDCS, exploratory analysis of prefrontal cortex regions. **a** describes the extent to which each region of the PFC contributes to tDCS effects on depression (change in 17-item Hamilton Depression Rating Scale [HDRS-17] score, left) or negative (NA, middle) and positive affect (PA, right) symptom change, as assessed by the Positive and Negative Affect Schedule (PANAS). Effect sizes (Cohen *d* and 95% confidence intervals [CI]) refer to the output of interest, the triple-interaction of the tDCS versus placebo treatment group, baseline rsFC, and time point, extracted from linear mixed effect models. Effect size of 0.3 represents small to medium-sized effects; regions which larger effect sizes are extracted in the bottom row and show associations of baseline rsFC with the change in respective symptom score among the treatment groups (**b**). Note that HDRS-17 and NA are positively, while PA is negatively associated with sereneness of depression, which explains the different patterns observed for these scores. *RSFC* represents numbers of activated voxels $\times 10^3$. *BA* Brodmann area, *PFC* prefrontal cortex

towards smaller effect sizes for functional connectivity analyses [34].

Effects of non-invasive transcranial brain stimulation (NTBS) may depend on resting state functional connectivity

For tDCS, data on rsFC MRI predicting tDCS effects on a cognitive, behavioral or even clinical level are very limited, though tDCS can modulate brain activity while showing behavioral effects; e.g. bifrontal tDCS was shown to improve performance in a working memory task and reduce left MPFC and ACC delta activity [62]. While no tDCS studies directly investigated baseline rsFC as a putative predictor for its effects, a recent study by Nord et al. suggested that higher baseline task activation in the left DLPFC during a working memory task might be a predictor of tDCS response [41].

In contrast, a larger body of evidence is available for TMS suggesting an impact of rsFC MRI data on TMS responses, e.g. rsFC between regions such as the ACC, MPFC, lateral parietal cortex, and the DLPFC were predictive of TMS response [21, 23]. In particular, anticorrelations of two regions, the left DLPFC and subgenual ACC predicted the clinical efficacy of left DLPFC TMS [20, 24] while for left MPFC TMS, functional connectivity for left dorsal MPFC left DLPFC, left amygdala and several other regions was associated with clinical response [63].

Being aware of the risk of overanalyzing the data, we further investigated additional PFC subregions. Interestingly, these exploratory analyses suggested an association of rsFC in *lateral* portions of the PFC with tDCS response,

although these effects did not survive the corrections for multiple comparisons. This is particularly notable as structurally relevant regions were located rather *medially* in the PFC [50]. The above-mentioned evidence from tDCS and TMS studies supports indeed the involvement of the DLPFC in stimulation effects [20, 24, 38, 41], yet is not restricted to this region. In fact, it seems unusual that the association of rsFC and antidepressant response was observed under the right-sided, cathodal stimulation electrode, as stronger antidepressants effects are attributed to excitatory stimulation, hence high-frequency TMS or anodal tDCS [26, 64].

Of note, an additional incidental visual finding in our data is the side-dependence of the regional rsFC and improvement of symptoms, with lower baseline rsFC in left-sided regions, located below the anode, and higher baseline rsFC in right-sided regions, located below the cathode, being associated with greater improvement (Fig. 2). A possible interpretation in favor of our findings might be that anodal tDCS induces excitatory, and cathodal tDCS induces inhibitory effects [25, 26], thus "normalizing" a possibly pathological rsFC in these regions. Generally speaking, a deviation in both directions of baseline rsFC might facilitate the polarity-dependent tDCS effects.

Strengths and limitations

To the best of our knowledge, this is the first study investigating whether the antidepressant response to tDCS may be associated with distinct baseline rsFC MRI patterns in PFC regions. The original trial, where the current ancillary analysis has been conducted in a subsample of subjects with MRI data, is a milestone study in the field with an elaborate three-arm design, comparing tDCS plus placebo medication, pharmacotherapy plus sham tDCS and a double-placebo condition (i.e. sham tDCS and placebo medication). While the pharmacotherapy and placebo groups are advantageous in terms of the presence of control conditions, a major limitation is the relatively small sample size in the group of interest, the tDCS group. Although the analyses might be underpowered, we formulate clear hypotheses based on previous findings from structural features, which we can investigate in further trials including larger samples. Methodologically, comparison of our results to other studies is limited due to several factors, such as differences in stimulation parameters of tDCS (1 mA vs. 2 mA, placement of electrodes), differences between different mechanisms of stimulation modalities (tDCS vs. TMS) and differences in measures of MRI parameters (derived from, for example task fMRI or metabolic PET investigations) or connectivity (looking at positive or negative correlations, ICA-based rsFC networks versus seed-based rsFC analysis). In future studies, our findings should be replicated with regards to structural features to identify multimodal mediators of tDCS response. Clinical characteristics [44] and depression subgroups [63] should also be considered.

A strength of our study is that it is based on a prior investigation of a subgroup from the same trial and it allows us to address a problem from different points of view; the influence of specific regions on the same outcome from the perspective of structural, hence long-term, or functional, hence state-dependent, parameters. In fact, although the structure of the human brain has a marked imprint on its function, this interaction is complex and rules out simple one-to-one correspondence/transmission [61].

Conclusion

While rsFC of several regions and networks centered around the DLPFC and MPFC is being discussed as a putative biomarker of TMS response in depression [7, 21, 23, 24], we did not identify a similar association of rsFC in PFC regions and tDCS response. This is of particular interest as, the tDCS response was associated with baseline gray matter volumes, indicating that tDCS may be differentially related to structural and functional biomarkers. The whole array of individual structural and functional MRI information offers a unique potential for identifying sensitive and specific MRI-based predictors of the antidepressant response. A deeper understanding of the stimulation brain interaction, however, is needed for the selection of predictive factors.

Acknowledgments This study was primarily supported by a São Paulo Research State Foundation (FAPESP) grant (2012/20911-5). ELECT-TDCS was registered under ClinicalTrials.gov, registration number NCT01894815. ARB is a recipient of a CAPES/ Alexander von Humboldt fellowship award for experienced researchers and receives speakers' fee from Neurocademy GmBH (Munich, Germany). The Laboratory of Neuroscience receives financial support from the Beneficent Association Alzira Denise Hertzog da Silva and the CAPES / INCT program "National Institute of Biomarkers in Psychiatry" (INBioN). FP is a member of the European Scientific Advisory Board of Brainsway Inc., Jerusalem, Israel, and has received speaker's honoraria from Mag&More GmbH and the neuroCare Group. His lab has received support with equipment from neuroConn GmbH, Ilmenau, Germany, and Mag&More GmbH and Brainsway Inc., Jerusalem, Israel. This work was also supported by the German Center for Brain Stimulation (GCBS) research consortium (Work Package 5) [grant number 01EE1403E], funded by the Federal Ministry of Education and Research (BMBF). The work of LB is part of a PhD/residency program of the Ludwig-Maximilians University (LMU) and the International Max Planck Research School for Translational Psychiatry (IMPRS-TP) financially supported by the Else Kröner Fresenius Foundation.

Compliance with ethical standards

Conflict of interest EM, PS, PVB, FD, GB, EAJ, IMB, PAL, WG, SG, and DK reported no biomedical financial interests or potential conflicts of interest.

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11. Apendix A

11.1 Paper III - Cognitive outcomes after tDCS in schizophrenia patients with prominent negative symptoms: Results from the placebo-controlled STARTS trial

Schizophrenia Research 235 (2021) 44–51

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Cognitive outcomes after tDCS in schizophrenia patients with prominent negative symptoms: Results from the placebo-controlled STARTS trial

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ARTICLE INFO

Keywords:
Cognition
Noninvasive brain stimulation
Transcranial direct current stimulation (tDCS)
Schizophrenia
Negative symptoms
Cognitive factors

ABSTRACT

Cognitive deficits and negative symptoms in schizophrenia are associated with poor functional outcomes and limited in terms of treatment. The Schizophrenia Treatment With Electric Transcranial Stimulation (STARTS) trial has shown efficacy of transcranial direct current stimulation (tDCS) for improving negative symptoms. In this secondary analysis, we investigate its effects on cognitive performance. In STARTS, a double-blinded, sham-controlled, randomized clinical trial, patients were treated with twice-daily, 20-min, 2-mA fronto-temporal tDCS over 5 days or sham-tDCS. In 90 patients, we evaluated the cognitive performance up to 12 weeks post-treatment. We found that active-tDCS showed no beneficial effects over sham-tDCS in any of the tests. Based on a 5-factor cognitive model, improvements of executive functions and delayed memory were observed in favor of sham-tDCS. Overall, the applied active-tDCS protocol, primarily designed to improve negative symptoms, did not promote cognitive improvement. We discuss possible protocol modification potentially required to increase tDCS effects on cognition.

ClinicalTrials.gov identifier: NCT02535676

1. Introduction

Schizophrenia presents a major burden in patients' lives (Whiteford et al., 2013). In particular, cognitive impairment is associated with poor long-term functional and social outcomes (Lin et al., 2013; Ventura et al., 2009; NEDENA Group et al., 2006), along with increased negative

Abbreviations: EMI, Emotion identification; PANSS, Positive and Negative Syndrome Scale for Schizophrenia; PCET, Penn Conditional Exclusion Test; Penn-CNB, Penn Computerized Neurocognitive Battery; PFMT, Penn Face Memory Test; PLNB, Penn Letter N-Back test; PWMT, Penn Word Memory Test; cVOLT, short Visual Object Learning Test; tDCS, transcranial direct current stimulation; STARTS, Schizophrenia Treatment With Electric Transcranial Stimulation trial.

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https://doi.org/10.1016/j.schres.2021.07.008
Received 20 March 2021; Received in revised form 7 July 2021; Accepted 10 July 2021
Available online 22 July 2021

symptoms (Lin et al., 2013; Ventura et al., 2009). Both cognitive and negative symptoms are difficult to tackle as there are no effective pharmacological treatments (Fusar-Poli et al., 2015). Cognitive dysfunctions are prominently observed in memory and executive control (Guo et al., 2019).

Transcranial direct current stimulation (tDCS) delivers continuous electric currents to underlying brain regions through electrodes placed on the head surface (Brunoni et al., 2012). It has been increasingly investigated for neurologic and psychiatric disorders, including schizophrenia (Lefaucheur et al., 2017). While precise mechanisms are still unknown, evidence points towards short-term effects induced through modulation of membrane polarisation, hence changing the excitability of underlying neurons, as well as long-term effects through processes alike long-term polarisation and depression (Hasan et al., 2011; Nitsche and Paulus, 2000; Stagg and Nitsche, 2011).

The dorsolateral prefrontal cortex is a common target to improve cognitive and negative symptoms, as is a key region of networks impaired in schizophrenia (Minzenberg et al., 2009). Several studies showed promising effects of prefrontal tDCS on negative symptoms (Brunelin et al., 2012; Chang et al., 2020; Gomes et al., 2018; Kennedy et al., 2018; Osoegawa et al., 2018; Palm et al., 2016) and (some) cognitive functions (Chang et al., 2019; Jeon et al., 2018; Narita et al., 2018; Smith et al., 2015), but these findings could not be immaculately replicated (Fitzgerald et al., 2014; Kim et al., 2019; Koops et al., 2018; Sreeraj et al., 2020). Even in healthy participants, tDCS effects on cognition can range from improvement to no effects, and eventually inducing worsening effects (Ankri et al., 2020; Galli et al., 2019; Hill et al., 2016; Sellers et al., 2015; Tremblay et al., 2014).

To further assess whether tDCS improves cognitive outcomes in schizophrenia, we describe the trajectories of cognitive performance in patients treated with tDCS as part of the STARTS trial (Schizophrenia Treatment With Electric Transcranial Stimulation) (L. da C. L. Valiengo et al., 2019). In this ancillary analysis, we use a 5-factor cognitive model, including the domains of attention, executive control (working memory, abstraction and mental flexibility), delayed, and intermediate memory (verbal, facial, and visuospatial), and social cognition as assessed via the Penn Computerized Neurocognitive Battery (Penn-CNB) (Gur, 2001; Gur et al., 2010) and the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) cognitive-disorganized factor (Higuchi et al., 2014). Furthermore, we investigate negative symptoms as moderators of cognitive improvement.

2. Methods and materials

2.1. Study design and ethical information

The STARTS trial was a two-center (Institute of Psychiatry, Clinics Hospital of the University of São Paulo Medical School, São Paulo, Brazil and Instituto Bairral de Psiquiatria, Itapira, São Paulo, Brazil), double-blinded, sham-controlled randomized clinical trial that assessed the effects of tDCS applied two times per day, over 5 consecutive days, for treating patients with schizophrenia with prominent negative symptoms. The primary outcome of the STARTS trial was change in score on the PANSS negative symptoms subscale over time, assessed at baseline, 5 days, 2 weeks, 4 weeks, 6 weeks, and 12 weeks (L. Valiengo et al., 2019b; L. da C. L. Valiengo et al., 2019).

Written, informed consent was collected from all patients before study enrollment according to the Declaration of Helsinki. The study was approved by the hospitals' ethical committees (Comitê de Ética em Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, and the ethics committee of Instituto Bairral, respectively). The study was registered on ClinicalTrials.gov (NCT02535676) and the study protocol, as well as the primary outcome, were published previously (L. Valiengo et al., 2019; L. da C. L. Valiengo et al., 2019). Here, we investigated the effects of tDCS on cognitive performance trajectories among STARTS patients as secondary outcomes of the STARTS trial.

2.2. Participants

We included 100 patients with schizophrenia diagnosed according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Portuguese version) and prominent negative symptoms, based on psychiatric assessment and the PANSS negative symptom subscale score > 20. Patients older than 55 years, with unstable disease symptoms or unstable antipsychotic medication in the last 4 weeks, unstable medical conditions, other general comorbidities, who received electroconvulsive therapy within the last 6 months, or had a history of noninvasive brain stimulation treatment were excluded. The patients were on stable doses of standard antipsychotic drug treatment for at least 4 weeks. Benzodiazepines were allowed up to 10 mg/day diazepam-equivalent.

2.3. Interventions

The tDCS treatment was applied following the protocol by (Brunelin et al., 2012); 20 min of tDCS (Neuroconn) at 2 mA were delivered over the left prefrontal cortex and the temporoparietal junction for anodal and cathodal stimulation, respectively, two times per day over the course of one week, Monday to Friday, with inter-session intervals between 180 and 210 min.

Sham tDCS consisted of ramp-up and ramp-down periods of 40 s, with a current of 2 mA with duration of 30 s between the ramp phases. Application of active and sham stimulation was double-blinded using 5-digit stimulation codes.

2.4. Neurocognitive outcomes

The primary outcome in the present study was the change of neurocognitive performance on the Penn-CNB (web-based version 1.0 June 2010, (Gur, 2001; Gur et al., 2010)) assessed at baseline and at week 6. In addition, we investigated the PANSS-based cognitive-disorganized factor (Higuchi et al., 2014) assessed at baseline, 5 days, 2 weeks, 4 weeks, 6 weeks, and 12 weeks.

2.4.1. Neurocognitive assessment

The Penn-CNB measures accuracy (number of correct responses) and speed (reaction time) on different neurobehavioral domains. These measures are used to calculate efficiency scores for each test by transforming the raw accuracy and speed values to their standard equivalents (z-scores based on means and SDs for the entire sample) and creating the sum of these values (speed was multiplied by negative 1). For detailed test descriptions, please consider publications by Gur et al. (Gur, 2001; Gur et al., 2010). In short, we implemented the following tests:

- Penn Conditional Exclusion Test (PCET) as a measure of abstraction and mental flexibility; here, the participant has to determine which object in a row does not belong to the group.
- Penn Letter N-Back test (PLNB) as a measure of working memory; here letters appear subsequently on the screen; the participant has to react either directly when the letter appears (0-back), the current screen shows the same letter as the previous screen (1-back), or as the second-previous screen (2-back).
- Penn Word Memory Test (PWMT), Penn Face Memory Test (PFMT), and short Visual Object Learning Test (sVOLT) measure verbal, face, and spatial memory, respectively, by presenting 20 target objects (words, faces, and Euclidean shapes) mixed with 20 distractors each. These tests measure immediate and delayed recall after 20 min (efficiency scores are based on the number of correct responses only for the latter).
- Emotion identification (EMI), a 40-item facial affect identification test by presenting four qualities of affect (happy, sad, anger, fear) and 8 neutral faces, as a measure of social cognition.

2.4.2. Cognitive domains

Due to the large number of cognitive tests within the Penn-CNB, five cognitive domains were created similarly to the approach by (Moore et al., 2015). These were "attention" (attention PLNB 0-back and sustained attention PLNB 1-back), "executive control" (working memory PLNB 2-back, abstraction and mental flexibility PCET), "memory" (verbal PWMT, facial PFMT, and visuospatial sVOLT, subdivided into intermediate and delayed memory), and "social cognition" (emotion identification EMI). By summing up the efficiency scores of the single tests per domain, we generated the composite scores for each domain. This approach had two advantages: (1) cognitive domain factors provide a more meaningful clinical interpretation of the findings and (2) reduction of multiple testing and statistical issues associated with it.

2.4.3. PANSS-based cognitive-disorganized factor

This factor relies on the PANSS subscale "disorganization/cognition", identified by means of a principal component analysis from a Brazilian sample of 292 patients with schizophrenia (Freitas et al., 2019; Higuchi et al., 2014). This subscale consists of: conceptual disorganization (P2), poor attention (G11), disorientation (G10), disturbance of volition (G13), difficulty in abstract thinking (N5), stereotyped thinking (N7), and mannerisms/posturing (G5) and was included because it was assessed at more timepoints than the cognitive test battery and, hence, offers a better timely resolution of cognitive improvement.

2.5. Statistical analyses

2.5.1. Neurocognitive trajectories

Analyses were performed using R (version 4.0.2, R Foundation) (Team, 2013). Results were considered significant at the $P < .05$ two-tailed threshold.

Extreme values in raw test scores outside of the 5 to 95% quantiles were winsorized (Supplementary Fig. 1). Change in the cognitive outcomes was modeled using linear mixed models (LMM) with time, group, and their interaction as fixed factors. Repeated measurements were considered as nested within patients. Interindividual variation at baseline was accounted for by including a random intercept term. Models were controlled for influences of established moderators in neurocognitive assessments. Gender, age, education, and log-transformed haloperidol equivalent dose were added as covariates. Effect sizes were reported as Cohen's d, which was derived from the model slopes using the formula by (Raudenbush and Xiao-Feng, 2001) as recommended by (Feingold, 2009). To avoid false positive findings due to multiple comparisons, tests for changes in the cognitive domains (primary outcome) were corrected for the false discovery rate (FDR). Results for single cognitive tests were reported as unadjusted complementary findings.

2.5.2. Confirmatory factor analysis

To validate the latent structure of higher order cognition factors, confirmatory factor analysis (CFA) was applied in the *lavaan* framework for latent variable modeling (Rosseel, 2012). Factor loadings of the first indicator of each latent variable were constrained to 1 to fix the scale of the factor. Since the social cognition factor was measured by only one indicator, its variance was constrained to 1. Endogenous cognition factors were assumed to covary, so were immediate and delayed memory indicators from the same memory domain. Models were fit with full information maximum likelihood estimation. Fit index combinations for model adequacy, as recommended in simulation studies by (Beauducel and Wittmann, 2005), were computed.

2.5.3. Associations with improvement in negative symptoms

Associations of the change in cognition scores with symptomatic improvement until primary study endpoint of the STARTS trial (week 6) were assessed using general linear models (GLM) with the difference score (week 6 minus baseline) of the cognitive domains as the dependent variable and treatment group, improvement in negative symptoms, and their interaction as independent variables, and vice-versa. Tests for associations including cognitive domain scores (primary outcome) were FDR-corrected.

2.6. Data availability

The datasets that support the findings of this study are available from the corresponding authors upon reasonable request.

3. Results

3.1. Overview

We included 90 patients (90% of the original sample) in this additional analysis of the STARTS trial; in 10% of the sample, neurocognitive testing could not be performed due to patients' difficulties using the computer and these patients were excluded. We included 48 patients in the active-tDCS and 42 patients in the sham-tDCS group, respectively. The active and sham-tDCS groups were comparable in main baseline and clinical characteristics (Table 1). There were no significant differences between groups in blinding integrity ($\chi^2 = 0.45$; $P = .50$). Confirmatory factor analysis showed acceptable model fit of the proposed 5 factor solution according to cutoff values suggested by Hu and Bentler (1998) (Supplementary Fig. 2, Supplementary Tables 2 and 3).

3.2. Cognitive performance in the main cognitive domains

After correction for known moderators of cognitive functioning (gender, age, education, and antipsychotics dose), significant time x group interactions were found for executive functions ($F_{(1,61.46)} = 7.38$, $p_{FDR} = 0.038$, $d = 0.50$, $CI_{95\%} = 0.14$ to 0.87) and delayed memory ($F_{(1,64.18)} = 6.20$, $p_{FDR} = 0.038$, $d = 0.52$, $CI_{95\%} = 0.11$ to 0.93) (Table 2, Fig. 1, Supplementary Fig. 3). The group mean differences indicated that these effects were carried by a slight worsening in the active-tDCS and a slight improvement in the sham-tDCS group, however, the numerical values post-treatment were identical or very similar in both groups, so possibly, these effects might have been caused by differences in baseline performance levels (Table 2). Overall, active-tDCS showed no beneficial effects on cognitive performance in neither one of the cognitive domains compared to sham-tDCS.

3.3. PANSS-based cognitive disorganized factor

The PANSS-based cognitive-disorganized factor, which was additionally measured at multiple timepoints, showed a significant decrease over time ($F_{(1,467.35)} = 4.38$, $p = .037$), but no differences in change between the treatment groups ($F_{(1,467.35)} = 0.89$, $p = .347$) (Supplementary Fig. 4).

3.4. Results for individual cognitive tasks

Significant improvements irrespective of group membership were found for PFMT ($F_{(1,64.57)} = 13.21$, $p < .001$), PLNB 0-back ($F_{(1,52.41)} = 9.07$, $p = .004$) and PLNB 2-back ($F_{(1,65.82)} = 8.29$, $p = .005$) accuracies, as well as for response times of PFMT ($F_{(1,62.71)} = 7.55$, $p = .008$), PWMT ($F_{(1,65.36)} = 11.91$, $p < .001$), EMI ($F_{(1,66.11)} = 10.13$, $p = .002$), and sVOLT ($F_{(1,65.38)} = 7.97$, $p = .006$). Significant time x group interactions were found for median response times of the PCET ($F_{(1,68.46)} = 7.41$, $p = .008$) and for accuracy on the delayed PWMT ($F_{(1,65.45)} = 9.29$, $p = .003$) in favor of sham tDCS (Supplementary Fig. 5, Supplementary Tables 4 and 5).

3.5. Associations with improvement in negative symptoms

There was a significant interaction effect between group and

Table 1
Clinical and demographic characteristics of the sample.

Characteristic	Active tDCS	Sham tDCS
Age in years, mean (SD)	34.38 (8.14)	34.83 (10.17)
Women, n (%)	8 (16.67)	10 (23.81)
Educational years, mean (SD)	11.69 (2.99)	10.76 (2.77)
Unemployed, n (%)	37 (77.08)	31 (73.81)
Not married, n (%)	39 (81.25)	36 (85.71)
Self-declared white ethnicity, n (%)	15 (31.25)	11 (26.19)
Duration of disease in years, mean (SD)	13.82 (7.95)	13.17 (8.94)
Number of hospitalizations, mean (SD)	0.9 (1.54)	1.94 (2.10)
Previous clozapine use, n (%)	18 (37.50)	15 (35.71)
Treatment resistant schizophrenia, n (%)	36 (75)	29 (69.05)
Ultra-treatment resistant schizophrenia, n (%)	23 (47.92)	18 (42.86)
Equivalent Haloperidol by Andreasen (Andreasen et al., 2010) dose, mg/day - mean (SD)	9.57 (4.51)	10.51 (8.12)
Electroconvulsive therapy (ECT), n (%)	2 (4.17)	3 (7.14)
Antipsychotic drugs (by generation)		
Typical, n (%)	3 (6.25)	1 (2.38)
Atypical, n (%)	35 (72.92)	34 (80.95)
Both, n (%)	6 (12.5)	5 (11.9)
Anticholinergic dose, mean (SD)	81.02 (215.88)	166.66 (299.13)
PANSS positive symptoms, mean (SD)	14.12 (4.02)	14.24 (3.90)
PANSS negative symptoms, mean (SD)	24.75 (3.78)	25.1 (3.54)
PANSS general symptoms, mean (SD)	34.17 (10.37)	34.88 (9.30)
PANSS total symptoms, mean (SD)	73.04 (15.83)	74.21 (14.13)
SANS, mean (SD)	59.08 (13.04)	61.81 (11.46)
AHRS, mean (SD)	9.42 (11.98)	7.62 (12.94)
CDSS, mean (SD)	2.40 (3.83)	2.26 (3.16)
GAF, mean (SD)	47.7 (11.18)	45.46 (11.73)
Patients with no auditory hallucinations per AHRS, n (%)	28 (58.33)	30 (71.43)
Patients with no major depressive episode per CDSS, n (%)	24 (50)	14 (33.33)

Note: SD standard deviation; PANSS Positive and Negative Syndrome Scale; SANS Scale for the Assessment of Negative Symptoms; AHRS Auditory Hallucinations Rating Scale; CDSS Calgary Depression Scale for Schizophrenia; There were no statistically significant differences between groups, except number of hospitalizations, which was higher in the sham group. Treatment-resistant schizophrenia was defined as lack of satisfactory clinical response to treatment with at least 2 antipsychotic drugs from different groups used with therapeutic doses and for 6 or more weeks. Ultra–treatment-resistant schizophrenia was defined as those with treatment-resistant disease who did not respond to at least 6 months of clozapine in dosages 300 mg/d or more. Information on specific antipsychotic drugs are summarized in Supplementary Table 1. Potency of anticholinergic drugs was computed according to (Rehse et al., 2016).

negative symptom improvement predicting change in the social cognition domain only ($\beta = 0.38$, $t_{(73)} = 3.55$, $p_{FDR} < 0.003$; Table 3; for single tests, see Supplementary Tables 6–7). This interaction indicated that changes in social cognition were unaffected by symptomatic changes in the tDCS group but increased linearly with negative symptom improvements in the sham group.

4. Discussion

In this ancillary analysis of the STARTS trial investigating tDCS for negative symptoms in schizophrenia, active-tDCS showed no beneficial effects on cognitive performance in neither one of the cognitive domains (attention, executive function, delayed, and intermediate memory, and social cognition) compared to sham-tDCS, nor in the PANSS-based cognitive-disorganized factor. However, in favor of the sham-tDCS group, significant effects were seen on executive function and delayed memory; with similar performance post-treatment in both groups, the clinical relevance of this finding should be considered carefully. Furthermore, these findings were carried by an increased response speed in the PCET (mental flexibility) and accuracy in the PWMT (delayed word memory) only. In the sham-tDCS group, negative symptom improvement was also associated with social cognition improvement.

Overall, our results suggest that the applied active tDCS protocol did not promote cognitive improvement. There is an ongoing discussion about tDCS effects on cognition in general (Brunoni and Vanderhasselt, 2014; Dedoncker et al., 2016; Hill et al., 2016) and in schizophrenia, specifically (Narita et al., 2019). Several other negative clinical trials reported - similarly to us - no superior effects of active tDCS over sham on working memory, processing speed, attention, executive functioning, learning, or problem solving and cognitive flexibility (Chang et al., 2020; Gomes et al., 2018; Koops et al., 2018; Palm et al., 2016). A common factor and possible explanation is the fact that in these studies, as well as in the STARTS trial, the cognitive improvement itself was not the primary outcome - this was rather negative symptoms (Chang et al., 2020; Palm et al., 2016) or auditory hallucinations (Koops et al., 2018).

Designing a specific cognition-protocol for tDCS should be the scope of future studies. In review of the current literature, significant differences among the applied tDCS protocols become visible; these protocols differ in terms of numbers of tDCS sessions (ranging from 5 stimulation in 5 days (Smith et al., 2015) over 10 sessions in 1 (Brunelin et al., 2012; Kantrowitz et al., 2019) or 2 weeks (Gomes et al., 2018; Jeon et al., 2018; Koops et al., 2018; Palm et al., 2016; Smith et al., 2020) to "accelerated" protocols using two bifrontal anodal electrodes (Chang et al., 2020) or 40 sessions in 4 weeks (Lindenmayer et al., 2019)). While meta-analytical evidence from cognition studies in schizophrenia is still lacking, from protocols aimed at improving hallucinations or negative symptoms, it seems as including more stimulation sessions is of benefit (17,25), as are, in terms of working memory and memory recollection, longer stimulation duration and higher electric currents (28,31).

Evidence from recent meta-analyses furthermore suggests that not only the "dosage", but the "timing" of tDCS are of importance. So was online tDCS more effective in patients, while offline tDCS showed

Table 2
Cognitive outcomes in cognitive domains.

	Active tDCS		Sham tDCS		Active vs. Sham		
Measure	Baseline	Week 6	Baseline	Week 6	Difference	p-Value	Effect Size
Attention	0.02 ± 1.77	0.03 ± 1.3	0.03 ± 1.92	−0.03 ± 2.07	−0.43 (1.9)	0.629	0.09 (−0.27 to 0.44)
Executive functions	0.21 ± 1.59	0.03 ± 1.6	−0.25 ± 1.74	−0.01 ± 1.75	−0.76 (1.64)	**0.009**	0.5 (0.14 to 0.87)
Immediate memory	0.43 ± 2.44	0.38 ± 2.6	−0.31 ± 2.37	−0.34 ± 2.27	0.02 (2.38)	0.884	0.02 (−0.3 to 0.35)
Delayed memory	0.44 ± 2.29	−0.24 ± 2.95	−0.49 ± 3.04	−0.24 ± 2.95	−0.89 (2.93)	**0.015**	0.52 (0.11 to 0.93)
Social cognition	0.04 ± 1.69	−0.01 ± 1.56	−0.04 ± 1.51	−0.01 ± 1.56	−0.01 (1.62)	0.451	0.14 (−0.21 to 0.5)

Note: Numbers rounded to 2 decimal places; Effect size Cohen's d; P-Value was determined for time × group interactions reflecting differences in change over time between the treatment groups; models were controlled for age, gender, education, and haloperidol-equivalent doses use; Significance of model factors computed using Type III analyses of variance with Satterthwaite's method.

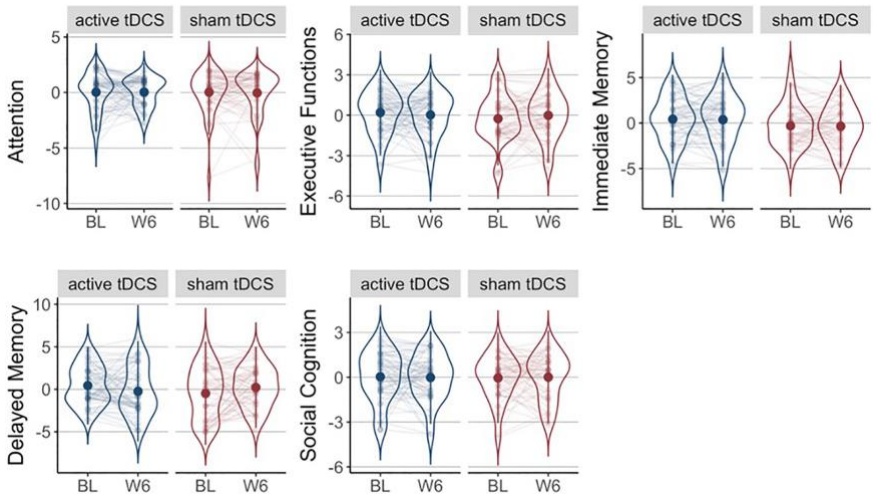

Fig. 1. Cognitive outcomes after tDCS treatment in cognitive domains
Note: Bold points indicate group means; Faded lines represent intra-individual change; Y-axis represents standardized scores; error bars within violin plots represent ±1 standard deviation.

Table 3
Bi-directional associations with negative symptoms.

	Negative symptoms predicting cognition				Cognition predicting negative symptoms			
	Symptoms		Group × symptoms		Cognition		Group × cognition	
Measure	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Attention	0.52	0.47	0.69	0.41	0.52	0.48	0.27	0.61
Executive functions	0.41	0.52	1.97	0.16	0.40	0.53	0.43	0.52
Immediate memory	0.84	0.36	2.45	0.12	0.82	0.37	0.45	0.50
Delayed memory	3.23	0.08	1.01	0.32	3.27	0.07	2.00	0.16
Social cognition	3.50	0.07	12.59	<0.001*	3.03	0.09	0.99	0.32

Note: Numbers rounded to 2 decimal places; Significance of model factors computed using Type III analyses of variance with Satterthwaite's method.

efficacy in the healthy population (Hill et al., 2016). In studies investigating specifically cognition of schizophrenic patients, the time of cognitive assessment differed majorly between studies as well, but was not directly connected to positive/negative outcomes (for example, significant outcomes were observed directly at the end of intervention (Smith et al., 2015), 2 weeks after (Smith et al., 2020), or at 12 weeks (Jeon et al., 2018), while non-significant findings were reported at the end of intervention and 3 months after (Chang et al., 2020; Gomes et al., 2018; Koops et al., 2018)). There is still a lot of uncertainty how tDCS effects are induced, but a combination of an acute component (Brunoni and Vanderhasselt, 2014; Papazova et al., 2018), facilitated through acute changes of resting membrane potentials (Nitsche and Paulus, 2000; Stagg and Nitsche, 2011), and a long-term component through changes in synaptic transmission and long term potentiation- and long term depression-like effects (Jeon et al., 2018), are discussed. A recent review indeed suggested that 3 months might be too short of a period to detect these cognitive processes, as pharmacotherapeutic effects on cognition partially require 6 months intervention periods (Désaméricq et al., 2014; Hasan et al., 2016). State-dependency is an emerging topic in brain stimulation studies overall (Hill et al., 2016) and goes hand-in-hand with individual factors that possibly interfere with brain traits, such as higher education (Berryhill and Jones, 2012), stronger impairment at baseline (Jeon et al., 2018), or higher negative symptoms at baseline specifically (Kim et al., 2019), all connected to better response to active tDCS. While this might be counterintuitive in first place, as higher cognitive impairments themselves are associated with poorer functional outcomes in schizophrenia (Lin et al., 2013; Ventura et al., 2009; NEDENA Group et al., 2006), Vercammen et al. suggested that this phenomenon goes back to a larger "cognitive reserve" at baseline, which makes the patients more likely to respond to active tDCS, possibly by tDCS facilitating recruitment of this reserve (Vercammen et al., 2011) (Hill et al., 2016).

Furthermore, while most studies use either the fronto-temporal (Brunelin et al., 2012; Chang et al., 2019; Lindenmayer et al., 2019) or bifrontal electrode montage (Gomes et al., 2018; Jeon et al., 2018; Palm et al., 2016), and electrode montage was not relevant in a recent meta-analysis (Brunoni and Vanderhasselt, 2014), evidence is limited. In a meta-analysis comparing tDCS to repetitive transcranial magnetic stimulation, the latter showed more robust effects on working memory than tDCS, possibly due to its more focal and more intensive character (Brunoni and Vanderhasselt, 2014). In order to reach deeper brain structures, alternating electrode montages or an individual stimulation intensity (1 vs. 2 mA) (Papazova et al., 2018) might be necessary.

Some have even suggested that, depending on their placement, cathodal stimulation might interfere with the effects achieved by anodal stimulation, the paradigm of interest. Chang et al. (2020) designed their

trial applying a novel approach of bi-anodal, prefrontal stimulation, with extracephalic cathodal electrodes, arguing and successfully showing that targeting the (hypothesized) bifrontal hypoactivity in the PFC is superior when aiming at improving negative symptoms and cognition (Chang et al., 2020). Hence, cathodal currents applied over the already hypoactive contralateral PFC, even when off-target (Kantrowitz et al., 2019) might result in disruptive tDCS effects on cognition directly after tDCS (Berryhill et al., 2014; Lapenta et al., 2012; Marshall et al., 2005; Orlov et al., 2017; Sellers et al., 2015). Extracephalic cathodal electrode placement might be the better option here. The temporal cathodal stimulation used in our trial might explain the -seemingly - improved cognitive functions in the sham group (although, as stated above, numerically, both groups showed similar performance post-treatment).

However, the improvement of cognition might be attributed to sham tDCS directly. A recent review discusses the possibility that some of the negative trials in the tDCS field are direct results of sham tDCS exerting neurobiological effects, in addition to the non-specific placebo effects (despite low stimulation intensities of sham tDCS in the sense of a stochastic resonance model predicting functional changes after noise injection) (Fonteneau et al., 2019) In particular the sham protocol used by us, consisting of 40-s ramp-up/down phases and a 30 s active stimulation at 2 mA, represents a rather large amount of current applied to the brain for sham tDCS. A study in healthy participants, for example, has shown effects of 1.6 s of direct current stimulation on verbal memory (Javadi et al., 2012)(Boonstra et al., 2016) Given the role of cognition in general, and social cognition in particular on functional outcomes in schizophrenia patients (Green et al., 2015; Lewandowski et al., 2020; Lin et al., 2013; Ventura et al., 2009; NEDENA Group et al., 2006), it is of clinical interest to investigate in further studies whether the observed improvement of executive function and delayed memory, and the association of negative symptom improvement with social cognition improvement is caused by active and/or sham stimulation, or any of the confounding factors discussed above (Chang et al., 2020; Kantrowitz et al., 2019; Kennedy et al., 2018).

Finally, we included also the PANSS-based cognition/disorganization scale in our analysis due to the fact that this assessment was easier and available at multiple timepoints, and due to the hypothesized advantages when detecting effects of active tDCS on cognition (Chang et al., 2020; Gomes et al., 2018). Yet, we, and others (Kantrowitz et al., 2019), could not confirm this advantage. An explanation offered by some is that this scale might rather depict verbal ability and memory than general cognitive functioning (Ehmann et al., 2004; Nielsen et al., 2014).

Limitations should be underscored. First, cognition was an ancillary outcome of the main trial, hence the tDCS treatment parameters might not have been optimal for inducing effects on cognition in terms of stimulation intensity or lack of cognitive training by online application of tDCS with a cognitive task as discussed above (Dedoncker et al., 2016; Hill et al., 2016). In particular with the negative outcome of our study, ceiling effects in the group of high-performing participants, in spite of careful data observation and usage of standardized tests, can't be ruled out completely, possibly reducing the sensitivity of our approach to identify effects of the active treatment. Cognitive changes were assessed using the Penn-CNB at baseline and week 6, which might have prevented us from finding acute improvement directly after tDCS, or at any later time point; assessment of the PANSS cognition/disorganization subscale had the advantage of several time points, yet it is not a test of cognitive performance per se. Furthermore, outcomes on different cognitive tests might vary and comparison of our results to studies using different scales might be limited. For example, the MATRICS consensus cognitive battery is a rather popular tool used in the field (Green et al., 2004)the shorter Brief Assessment of Cognition in Schizophrenia (Keefe et al., 2004) is well suitable for repeated longitudinal assessments of cognition and the Brief UCSD Performance-based Skills Assessment (Mausbach et al., 2010; Mausbach et al., 2007) was shown to be a useful measure of functional outcomes in patients with schizophrenia.

To conclude, in this ancillary study of the STARTS trial, the applied active-tDCS protocol chosen originally to improve negative symptoms in schizophrenia was not beneficial over sham-tDCS in terms of cognitive performance. Our negative results highlight the need to investigate tDCS protocols specifically aimed at improving cognitive function in general or specific cognitive domains particularly by modifying several components of currently available tDCS protocols, such as the dosage, timing, location, and state-dependency of stimulation.

Funding information

This research was primarily supported by grant 12T-011 from Stanley Medical Research Institute and was partly developed during the São Paulo Research State Foundation and Bavarian Academic Center for Latin America bilateral meetings (grant 17/50223-7). The Laboratory of Neuroscience receives financial support from the Beneficent Association Alzira Denise Hertzog da Silva and the Coordination for the Improvement of Higher Education Personnel and National Institute of Science and Technology program "National Institute of Biomarkers in Psychiatry" (INBioN) (grant 14/50873-3). This work was also supported by grant 01EE1403E from the German Center for Brain Stimulation research consortium funded by the German Federal Ministry of Education and Research.

The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; nor in the decision to submit the article for publication.

CRediT authorship contribution statement

JBC, BSP, HE, WFG, ARB, and LV designed and conducted the study, including patient recruitment and data collection. LB, SG, JSG, AKB, FP, ARB, and LV performed the data analysis and interpretation of data. LB and SG prepared the manuscript draft with important intellectual input from all other authors. All authors approved the final manuscript.

Declaration of competing interest

The work of LB is part of a PhD/residency program of the Ludwig-Maximilians University (LMU) and the International Max Planck Research School for Translational Psychiatry (IMPRS-TP) financially supported by the Else Kröner Fresenius Foundation. SG, JSG, AKB, JBC, BSP, WFG, and LV declare no conflicts of interest. HE received research grants from FAPESP and honoraria for travel support, advisory board participation, or speaking from Aché, Cristália, Daiichi-Sankyo, Janssen, Novartis Roche and Teva. FP has received speaker's honoraria and support with equipment from Mag&More GmbH, Munich, Germany and the neuroCare Group, Munich, Germany as well as from Brainsway Inc., Jerusalem, Israel. ARB is Chief Medical Advisor of Flow Neuroscience (Malmö, Sweden) and has a small equity in this company. This role started in June 2019.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2021.07.008.

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