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Effects of bifrontal transcranial direct current stimulation on brain metabolites and clinical outcome - Investigations using multimodal imaging methods

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1. List of abbreviations

^{1}H-NMR	Hydrogen Nuclear Magnetic Resonance
^{13}C-NMR	Carbon Nucelar Magnetic Resonance
^{31}P-NMR	Phosphate Nuclear Magnetic Resonance
CT	Computer-tomography
DLPFC	Dorsolateral Prefrontal Cortex
ECT	Electroconvulsive Therapy
FDA	Food and Drug Administration
GABA	Gamma Aminobutric Acid
Glu	Glutamate
Glx	Glutamate & Glutamine
HC	Healthy Controls
LTP	Long-term Potentiation
M1	Motor Cortex
MDD	Major Depression Disorder
MEGA-PRESS	MEshcher-GArwood Point RESolved Spectroscopy
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NIBS	Non-Invasive Brain Stimulation

PFC	Prefrontal Cortex
ROI	Region of Interest
rsFC	resting state functional connectivity
SCZ	Schizophrenia
SSRI	Serotonin Selective Reuptake Inhibitor
T	Tesla
tDCS	transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation

2. List of publications

Original Research Articles

- **Mezger, E**., Rauchmann, B. S., Brunoni, A. R., Bulubas, L., Thielscher, A., Werle, J., ... & Keeser, D. (2021). Effects of bifrontal transcranial direct current stimulation on brain glutamate levels and resting state connectivity: multimodal MRI data for the cathodal stimulation site. *European archives of psychiatry and clinical neuroscience, 271(1), 111-122.*

- **Mezger, E**., Brunoni, Andre R., Hasan, A., Häckert, J., …, Padberg, F., & Palm, U. (2020). tDCS for auditory verbal hallucinations in a case of schizophrenia and left frontal lesion: efield simulation and clinical results. *Neurocase*

Others

- Paulo J. C. Suen, Sarah Doll, Marcelo C. Batistuzzo, Geraldo Busatto, Lais B. Razza, Frank Padberg, **Eva Mezger**, Lucia Bulubas, Daniel Keeser, Zhi-De Deng & Andre R. Brunoni (2021). Association between tDCS computational modeling and clinical outcomes in depression: data from the ELECT-TDCS trial. *European archives of psychiatry and clinical neuroscience*, *271*(1), 101-110.
- **Eva Mezger**, Lucia Bulubas, Andre Brunoni, Birgit Ertl-Wagner, Sophia Stoecklein, Stephan Goerigk, Frank Padberg*, Daniel Keeser* (2021). Effects of bifrontal tDCS on brain metabolites in patients with major depression disorder, schizophrenia, and matched healthy controls. *Submitted to Biological Psychiatry.*
- Bulubas, L., Padberg, F., **Mezger, E**., Suen, P., Bueno, P. V., Duran, F., ... & Brunoni, A. R. (2021). Prefrontal resting-state connectivity and antidepressant response: no

associations in the ELECT-TDCS trial. *European Archives of Psychiatry and Clinical Neuroscience, 271(1), 123-134.*

- Edgard Morya, Kátia Monte-Silva, Marom Bikson, Zeinab Esmaeilpour, Claudinei Eduardo Biazoli Jr, Andre Fonseca, Tommaso Bocci, Faranak Farzan, Raaj Chatterjee, Jeffrey M. Hausdorff, Daniel Gomes da Silva Machado, André Russowsky Brunoni, **Eva Mezger,**…, Abrahão Fontes Baptista & Alexandre Hideki Okano (2019). Beyond the target area: an integrative view of tDCS-induced motor cortex modulation in patients and athletes. *Journal of neuroengineering and rehabilitation*, *16*(1), 1-29.
- Nora Behler, Bianka Leitner, **Eva Mezger**, Elif Weidinger, Richard Musil, Bernhard Blum, Beatrice Kirsch, Linda Wulf, Lisa Löhrs, Christine Winter, Frank Padberg and Ulrich Palm (2018). Cathodal tDCS over motor cortex does not improve Tourette syndrome: lessons learned from a case series. Frontiers in behavioral neuroscience, 12, 194.
- da Silva, R. D. M. F., Batistuzzo, M. C., Shavitt, R. G., Miguel, E. C., Stern, E., **Mezger, E**., ... & Brunoni, A. R. (2019). Transcranial direct current stimulation in obsessive-compulsive disorder: an update in electric field modeling and investigations for optimal electrode montage. *Expert review of neurotherapeutics*, *19*(10), 1025-1035.

Oral presentations

- **Eva Mezger**, Boris-Stephan Rauchmann, Andre R Brunoni, Lucia Bulubas, Axel Thielscher, Jana Werle, Matin Mortazavi, Karali Temmuz, Sophia Stöcklein, Birgit Ertl-Wagner, Stephan Goerigk, Frank Padberg*, Daniel Keeser* (2019). Effects of prefrontal cathodal tDCS on glutamate and resting state connectivity: Combining electrical stimulation and field modeling using multimodal MRI. *13th ICME International Conference on Complex Medical Engineering.*
- **Eva Mezger**, Boris-Stephan Rauchmann, Andre R Brunoni, Lucia Bulubas, Axel Thielscher, Jana Werle, Matin Mortazavi, Karali Temmuz, Sophia Stöcklein, Birgit Ertl-Wagner, Stephan Goerigk, Frank Padberg*, Daniel Keeser* (2019). Effects of

prefrontal cathodal tDCS on glutamate and resting state connectivity: Combining electrical stimulation and field modeling using multimodal MRI. *Research Festival 2019, Dept. of Psychiatry and Psychotherapy Munich, Germany*

Poster presentations

- **Eva Mezger**, Lucia Bulubas, Andre Brunoni , Birgit Ertl Wagner , Sophia Stoecklein, Stephan Goerigk , Frank Padberg, Daniel Keeser (2020). Effects of bifrontal tDCS on brain metabolites in patients with major depression disorder and healthy controls. *Organization for Human Brain Mapping (OHBM), Online Conference*
- **Eva Mezger**, Boris-Stephan Rauchmann, Andre R Brunoni, Lucia Bulubas, Axel Thielscher, Jana Werle, Matin Mortazavi, Karali Temmuz, Sophia Stöcklein, Birgit Ertl-Wagner, Stephan Goerigk, Frank Padberg*, Daniel Keeser* (2020). Effects of prefrontal cathodal tDCS on brain glutamate levels and resting state connectivity: a randomized, sham-controlled, cross-over trial in healthy volunteers. *DGKN+NIBS 2020, online conference.*
- **Eva Mezger**, Lucia Bulubas, Andre Brunoni, Birgit Ertl-Wagner, Sophia Stoecklein, Frank Padberg, Daniel Keeser (2019). Effects of bifrontal tDCS on brain metabolites in healthy participants and patients with major depression. *Organization for Human Brain Mapping (OHBM), Rome, Italy.*
- **Eva Mezger**, Boris Rauchmann, Jana Wörsching, Matin Mortazavi, Andre R Brunoni, Birgit Ertl-Wagner, Frank Padberg, Daniel Keeser (2018). Modulation of brain metabolites and resting state functional MRI connectivity by transcranial direct current stimulation (tDCS) over the left dorsolateral prefrontal cortex in healthy subjects - the double-blinded hypothesis. *Organization for Human Brain Mapping (OHBM), Singapore.*

3. Candidate's contribution to the publications

3.1. Paper 1 - Effects of bifrontal transcranial direct current stimulation on brain glutamate levels and resting state connectivity: multimodal MRI data for the cathodal stimulation site

For this publication, the candidate worked with Dr. Daniel Keeser (DK) and Prof. Frank Padberg (FP) on the formulation of an amendment for the ethics committee to be able to perform a study using magnetic resonance spectroscopy (MRS) in combination with transcranial direct current stimulation (tDCS) in healthy participants. Together with DK and Dr Boris Rauchmann (BR) the candidate established an MRS sequence for a 3T Siemens Skyra scanner at the Klinikum Großhadern, Munich including several tests before starting the official study. After approval of the ethics application the candidate started to recruit healthy participants for the study. Preliminary talks were held to exclude any magnetic resonance imaging (MRI) or tDCS contradictions. TDCS application and MRI, including rsfcMRI and MRS measurements, of all participants were performed by the candidate. After the candidates were scanned the candidate analysed MRS data using Gannet 3.0 (http://www.gabamrs.com) and LCModel (Linear Combination Model, Version 2.1-1A, (Provencher, 1993)). Furthermore, in collaboration with DK, the candidate prepared the data sets so that the analysis for rsfcMRI (not the focus of this dissertation) and localization of peak electric field (e-field) could be run using DK's pre-established automated pipelines for rsfcMRI and structural analysis; this included eventual transformation of DICOM file format into nifty format and overall data cleaning. The candidate performed electric field simulations using SIMNIBS for all participants and generated all figures of electrical field distribution in the supplementary material. The candidate, DK, and FP participated in weekly discussions regarding conception and design of the combination of MRS, electrical field and rsfcMRI analysis.

The candidate cleaned the data sets including further characteristics of participants, such as age and gender, and examined the dataset using descriptive statistics to receive an overview of the sample. In collaboration with Dr. Stephan Goerigk (SG), who provided excellent

guidance, the candidate performed the statistical analyses in this publication. Together with 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. All authors revised the manuscript, provided intellectual content, and approved the final version. Furthermore, the candidate has presented results of this publication at several conferences.

3.2. Paper 2 - Association between tDCS computational modeling and clinical outcomes in depression: data from the ELECT-TDCS trial

This publication is based on the randomized, controlled "Trial of Electrical Direct-Current Therapy versus Escitalopram for Depression" published by Prof. Andrei Russowsky Brunoni (ARB) and colleagues (A. R. Brunoni et al., 2017) in the New England Journal of Medicine in 2017. This large-scale trial included 245 subjects treated in a three-armed design by a combination of sham and verum pharmacotherapy and tDCS. The results could not show non-inferiority of the brain stimulation treatment to pharmacotherapy. Secondary analyses revealed that brain stimulation was superior to placebo treatment. ARB was a visiting scholar at our department from 2018 to 2019 and the candidate collaborated with ARB on the analysis of the imaging data sets collected in the ELECT TDCS trial.

For this publication the candidate participated in weekly meetings with FP, DK and ARB to discuss conception and design of secondary analysis of the ELECT-tDCS trial and provided expert guidance to the Sao Paulo research group on simulation of electrical fields using SIMNIBS. In collaboration with DK pre-established automated pipelines for structural analysis followed by determining localizations of peak electrical fields within the brain choosing a ROI-based approach using the Sallet atlas (Sallet et al., 2013b) were performed. Additionally, the candidate contributed by revising the final manuscript, providing intellectual content and the approval of the final version.

4. General Introduction

Electrical stimulation for the treatment of patients has been already used for centuries starting with experiences of pain relief using an electrical torpedo fish (Sarmiento et al., 2016), continuing with the investigation of electrical stimulation of muscle cells by Luigi Galvani and finally using electrical stimulation to improve mood of depressed patients by the end of the 18th century (Sarmiento et al., 2016). By the mid of the 20th century scientists started to systematically explore electrical stimulation applied to the cortex of animals modulating neuronal activity (Isitan et al., 2020). Inspired by these results, transcranial electrical stimulation (TES) of the human brain was first investigated using single, brief high-voltage electrical shocks, which generated motor-evoked potentials (MEP), yet were highly uncomfortable (Zago et al., 2021). Later, new stimulation protocols were investigated, whereas the short, high-voltage shocks were replaced by low-intensity continuous direct currents named transcranial direct current stimulation (tDCS). TDCS is a safe, easy to use, easy portable and cost-effective non-invasive brain stimulation (NIBS) technique (A. R. Brunoni, Ferrucci, et al., 2012; A. R. Brunoni, Nitsche, et al., 2012). It does not directly stimulate neurons but shifts the resting membrane potential of neurons to a more depolarized state, hence, increasing the probability of generating an action potential, or to a more hyperpolarized state, hence, decreasing the probability of generating an action potential (Chase et al., 2020).

Reported tDCS effects on the brain have been variable and mixed. To gain more knowledge about de – and hyperpolarization of neurons tDCS was investigated in combination with numerous imaging methods such as magnetic resonance imaging (MRI). MRI is a popular technique in neuroscience enabling non-invasive investigations of brain structures, metabolism and functions in both animals and humans. With the technical progress in medicine, a basic imaging technique has become highly relevant for clinical research named magnetic resonance spectroscopy (MRS). MRS or nuclear magnetic resonance (NMR) spectroscopy (van der Graaf, 2010) allows to non-invasively measure brain metabolites in vivo. This technique has been long known as an analytical method in chemistry identifying the

structures of molecules. Due to the improvement of MRI systems with higher magnetic fields such as 3 and 7 Tesla (Robitaille & Abduljalil, 1998; Schild, 2005), the measurement of metabolite concentrations using MRS was established (van der Graaf, 2010). This opportunity opened a new field of research investigating the direct molecular effect of tDCS stimulation on the change of brain metabolite concentrations, amongst others the two highly prominent neurotransmitters for excitation and inhibition, glutamate (Glu) and gamma aminobutric acid (GABA). As the tDCS current reaching neurons is mostly diminished through the scalp (Chase et al., 2020), electric field (e-field) simulations not only give insights in the current strength reaching the neuronal cells, but also its distribution based on individual anatomical images (MacKenbach et al., 2020). The combination of these methods has the potential to open a new era of precision medicine and more targeted treatment.

2. Transcranial direct current stimulation (tDCS)

2.1. Principle of action

TDCS is a non-invasive brain stimulation (NIBS) technique, which modulates brain excitability during and for some time after stimulation (Nitsche & Paulus, 2001). Anodal (excitatory) and cathodal (inhibitiory) effects of tDCS stimulation on neuronal cells are reported to be based on changes of their resting-state membrane potential to depolarization or hyperpolarization (Das et al., 2016; Pelletier & Cicchetti, 2015). This effect results from the current flow between electrodes with positive ions flowing from the anode (positive pole) to the cathode (negative pole) (Das et al., 2016).

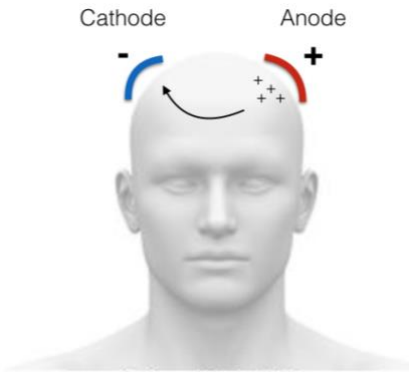

Figure 1: positive anode and negative cathode over the scull. Current flows from anode to cathode.

An increase of positive load in the extracellular space results in a hyperpolarization of the axon when located horizontally to the scull (Fig. 2 a). The cortex mainly consists of pyramidal cells lying perpendicular to the scull leading to a hyperpolarisation of neuronal dendrites. A so-called “current sink” evolves that attracts negative ions resulting in a depolarization of the soma and the axon hillock increasing the possibility of generating an action potential (Pelletier & Cicchetti, 2015).

a. b.

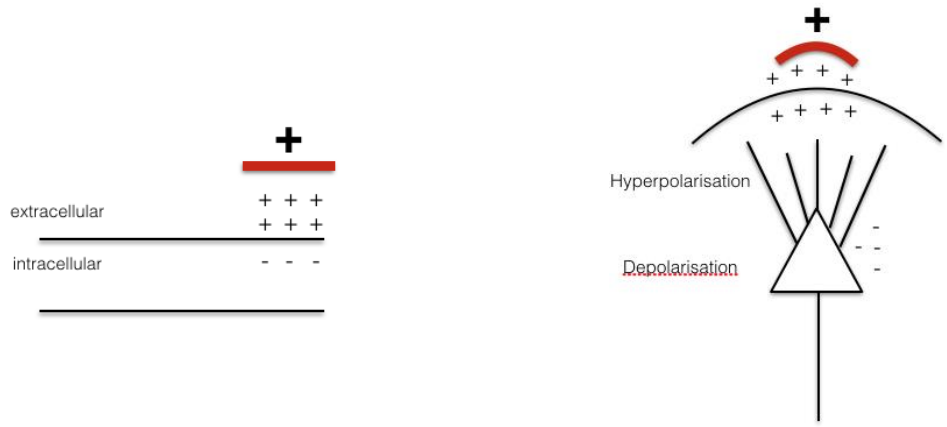

Figure 2: a. anodal stimulation increases positive load outside the cell, leading to hyperpolarization. b. anodal stimulation over the cortex leads to hyperpolarization of dendrites of pyramidal neurons. The increased positive load leads to a current sink, that attracts negative ions. This leads to the depolarization of the pyramidal neurons at the soma.

Poststimulation effects show that tDCS does not only have acute effects, but additionally activates molecular mechanisms, amongst others leading to changes in neurotransmitter concentrations (Antonenko et al., 2017; Bachtiar et al., 2015, 2018; Clark et al., 2011; Hone-Blanchet et al., 2016; Hunter et al., 2015; Knechtel et al., 2014; Stagg, Bachtiar, et al., 2011; Stagg, Jayaram, et al., 2011).

2.2. tDCS in research

Compared to other NIBS techniques such as Electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS), tDCS is a cost-effective, safe, and easy to use NIBS technique. Possible side effects are skin irritations, headache, tingling, itching and erythema, which only remain for minutes to a few hours after the procedure. Due to its many advantages, it is a promising technique for clinical application in neurologic as well as psychiatric disorders. Until the end of 2022, NIBS were not regulated for a specific disorder in the EU, whereas, depending on the use case, devices needed to be marked with regular CE, as a medical class I, or a medical class II device. By the end of 2022 a reclassification of NIBS to class III (category of highest risk) was introduced, which provoked indignation in the NIBS community challenging this decision (Baeken et al., 2023; Onarheim, 2023).

Basic research mainly in healthy participants and animals revealed an effect of tDCS on a variety of neurotransmitter systems including dopamine, acetylcholine, serotonin, Glu and GABA, on ion channels such as sodium and calcium (for review see Medeiros et al., 2012) and on receptors contributing to the information transfer between neuronal cells (Cavaleiro et al., 2020; Miller et al., 2014; Monte-Silva et al., 2013). A review of Moffa et al from 2020 gives an overview about the status of tDCS research in clinical settings. tDCS is investigated in neurological disorders such as pain, Parkinson's disease, stroke, and epilepsy as well as in psychiatric disorders such as major depression disorder (MDD), obsessive compulsive disorder, Tourette syndrome, schizophrenia, and drug addiction. There is quite some evidence of tDCS being effective in MDD compared to other disorders although stimulation parameters

such as the current density, the duration of stimulation and the recurrence of stimulations have been variable hampering a common statement about the “best” treatment protocol for tDCS in MDD.

Decades of research in the field of MDD generated an evidence base for the dysfunction in several cortical and subcortical areas, mainly the dorsolateral and ventromedial cortices, as well as the amygdala and the hippocampus (A. R. Brunoni, Ferrucci, et al., 2012). TDCS treatment is mainly based on the principle of the left dorsolateral prefrontal cortex (DLPFC) being hypoactive and the right DLPFC being hyperactive (Grimm et al., 2008). This is why the anode is normally placed over the right DLPFC in studies investigating the effects of tDCS on depression symptoms, whereas the location of the cathode varies (e.g. right DLPFC, orbitofrontal area, right shoulder) (Lefaucheur et al., 2017; Moffa et al., 2018; Razza et al., 2020). TDCS shall restore the balance between left and right DLPFC with a special regard to increasing the activity in the left DLPFC (Razza et al., 2023). Promising data have been published using prefrontal tDCS in patients with MDD (Arul-Anandam & Loo, 2009; A. R. Brunoni et al., 2016; Ferrucci et al., 2009). Several reviews have already investigated clinical outcomes of tDCS in MDD, most of them coming to the same results of active tDCS being superior to sham tDCS for response, remission, and depression improvement (A. R. Brunoni et al., 2016; Moffa et al., 2020; Razza et al., 2020, 2021; Zhang et al., 2021). Still, results of single studies vary and have rather small sample sizes indicating that individual characteristics might play a major role in treatment success for patients (Jog et al., 2019).

3. Magnetic resonance spectroscopy (MRS)

3.1. Magnetic resonance spectroscopy in research

Just like tDCS being a non-invasive method to study the brain, MRS has emerged as a powerful non-invasive method to measure brain metabolites including GABA and Glu. H^1-NMR spectroscopy is the most abundant MRS technique in research, measuring the magnetic

properties of hydrogen-atoms within molecules, whereas ^{31}P-NMR spectroscopy acquires organic compounds that contain phosphates such as ATP and ^{13}C-NMR spectroscopy measuring the amount of carbon atoms often acquired in studies.

Besides the well-known “monoamine-hypothesis” stating the imbalance of the monoamines serotonin, norepinephrine, and dopamine as the pathophysiological basis for depression (B. Liu et al., 2017), the neurotransmitters Glu and GABA have been consistently investigated in the development of MDD during the last years with promising results (Sarawagi et al., 2021). Glu is the main excitatory neurotransmitter in the brain and is crucial for long-term potentiation (LTP), neuroplasticity, learning and many more cognitive processes. GABA, as the most important antagonist to Glu, is also highly involved in neuroplasticity, learning and memory, and additionally in aggressive-defensive behaviour, impulsivity, and attention(Siegel et al., 2012). A reduction of Glu and GABA has been observed in depressed patients and animal studies, alongside reduced expression of Glu and GABA receptors and receptor subunits as well as altered energy metabolism (Abdallah et al., 2014; Deschwanden et al., 2011; Duman et al., 2019; Esterlis et al., 2018; Hasler et al., 2007; Rajkowska et al., 2005). This goes in line with a LTP deficiency in MDD patients proposing reduced neuroplasticity in certain brain regions consolidating clinical symptoms such as enhanced fear acquisition (Nissen et al., 2010). Taken together, these results collectively suggest that glutamatergic dysfunctions might contribute to the pathophysiology of depression.

As MRS is an easy-to-use, non-invasive method to investigate in vivo brain metabolite concentrations, Moriguchi et al performed a review of 49 studies exploring Glu, glutamine, and Glu+glutamine (Glx) concentrations in patients with depression using MRS (Moriguchi et al., 2019). The analysis revealed significantly lower levels of Glx in the medial frontal cortex in patients with depression compared to healthy controls. More recent studies report similar findings of reduced Glu in the ACC (Benson et al., 2020) though others report no changes (Persson et al., 2021; Smith et al., 2021) or increases of Glu (McGirr et al., 2015). Varying results might originate from different MRS protocols, different medication statuses of the

patients or stages and severities of the illness. More large-scale studies with similar protocols are needed to increase the knowledge about the molecular underpinnings of depression.

4. Electrical field simulation

4.1. Electrical field simulation in research

Due to the high variability between results, specifically of non-invasive brain stimulation studies, the interest in the spread and the strength of e-fields within the brain has increased. The stimulation of a specific brain region does not only impact locally but spreads into other, network related or deeper, brain regions (MacKenbach et al., 2020). Brain structures, morphology and anatomy are distinct between individuals and therefore a one-size-fits-all solution is not likely to be found as a treatment option for patients (Saturnino et al., 2019). Experiments in this field are constantly increasing to understand the impact of tDCS induced e-fields on neuronal networks. In a study applying transcranial alternating current stimulation (tACS) to epilepsy patients during surgical treatment significant effects of cortical e-fields, even with relatively low currents such as 1-2 mA were reported (Huang et al., 2017). Electrical fields induced by tDCS range from 0.4 mV/mm (1 mA stimulation) to 0.8 mV/mm (2 mA stimulation) and can substantially impact neural mechanisms such as the polarization of cellular membranes and the timing of action potentials (Hyeon Seo & Sung Chan Jun, 2018; A. Liu et al., 2018). Additionally, the impact on brain oscillations might influence behavioral states that have been reported as being abnormal in neurological disorders (Reato et al., 2013).

Not only current strength, but also localization and type of electrodes as well as individual anatomy are crucial for the formation of the e-field (MacKenbach et al., 2020). There are different montages, such as the conventional low-definition rectangular pad montage (widely used in psychiatric research studies), the high-definition disc montage (one central round electrode, the anode, and a concentric ring, the cathode, at a distant site) and the high-

definition montage (one ring electrode, the anode, with four electrodes, the cathodes, placed in a circle around the anode) to apply the electrical current to a specific brain region. Increasing the inter-electrode distance will enhance electrical field strength but comes at cost of reduced focality, whereas disc and high-definition montages seem to establish a better focal stimulation compared to the conventional low-definition rectangular pad (MacKenbach et al., 2020). Whether increased or reduced focality, the orientation of neurons in relation to the electrical field seems to be crucial for the effect of tDCS (Rahman et al., 2013). The massive gyrification of the human cortex makes it difficult to target specific neuronal structures because of the high variability of neuronal orientations between individuals (Laakso et al., 2016, 2019; Opitz et al., 2015). This might be an additional reason for the mixed results reported from studies in this field.

tDCS in MDD rat models have shown high e-field strength directly below the anode increasing the excitability of the cortex (Liebetanz et al., 2006). This is underpinned by other animal research studies (Asan et al., 2018; Tanaka et al., 2020). In human studies, results have been mixed, which might mainly result from varying tDCS protocols. A study of Mizutani-Tiebel et al. showed varying electrical fields in MDD, schizophrenia and healthy controls (HC), while peak values were mainly measured between electrodes, but not underneath the anode. E-field strength in MDD patients was significantly lower compared to HC, which might result from reduced cortical thickness in clinical samples (Mackin et al., 2013; Mizutani-Tiebel et al., 2022). In the meta-analysis of Razza et al it is pointed out, that a high variability of tDCS protocols leads to high variability in e-field location and strength, which might considerably influence treatment outcome (Farhat et al., 2022; Mutz et al., 2018, 2019; Razza et al., 2023; Wischnewski et al., 2021; Zhang et al., 2021). The comparison of 20 studies and 1008 patients revealed a negative association between e-field strength in frontal and medial parts of the right DLPFC and antidepressant response, whereas no effect of e-field strength was seen on the left DLPFC and bilateral subgenual ACC (Razza et al., 2023). The high variability of tDCS protocols and interindividual differences that are not accounted for, make a conclusion of study results difficult. Individualized treatment for patients, generating the peak e-field in the left

DLPFC across a study group would be needed to further investigate the association between e-field strength and depression symptoms (Albizu et al., 2020).

5. Effects of tDCS on neurotransmission

5.1. tDCS and MRS in research

The combination of tDCS and MRS allows insights in molecular changes by measuring metabolite concentrations before, during or after tDCS. The focus of this dissertation lies on neurometabolites such as Glu, Glx and GABA since the ability to measure these may enhance our knowledge about basic principles of tDCS contributing to new treatment procedures in clinical settings.

The measurement of GABA and Glu concentration in combination with tDCS was increasingly investigated during the last years (Antonenko et al., 2017; Antonenko, Thielscher, Saturnino, et al., 2019; Bachtiar et al., 2015, 2018; Barron et al., 2016; Bunai et al., 2021; Filmer et al., 2019; Guan et al., 2020; Hone-Blanchet et al., 2016; Kim et al., 2014; Knechtel et al., 2014; Nwaroh et al., 2019; Patel et al., 2019; Rango et al., 2008; Reidler et al., 2012; Shinde et al., 2023; Stagg et al., 2009; Talsma et al., 2018; Tremblay et al., 2014). Anodal stimulation of M1 showed decreased GABA levels at stimulation site (Bachtiar et al., 2015; Stagg, 2014; Stagg et al., 2009; Stagg, Jayaram, et al., 2011) whereas cathodal stimulation showed decreased Glu+glutamine (Glx) and GABA levels in the same region (Stagg et al., 2009). Only a few studies have assessed the impact of tDCS on metabolites in the parietal, temporal and cerebellar cortex (Bachtiar et al., 2015; Barron et al., 2016; Dwyer et al., 2019; Heimrath et al., 2020; Hunter et al., 2015; Koolschijn et al., 2019). Three studies showed a significant decrease of GABA during anodal tDCS over the occipital-temporal lobe and the right temporal cortex (Barron et al., 2016; Koolschijn et al., 2019) and one of them also reported an increase in Glu after stimulation (Koolschijn et al., 2019). Another study showed

an increased GABA to Glu relation under the stimulation site after anodal and cathodal stimulation of the auditory cortex (Heimrath et al., 2020), whereas no changes in GABA nor Glx levels have been observed after anodal or sham stimulation in the left posterior superior temporal gyrus (Dwyer et al., 2019). Numbers of studies combining tDCS and MRS on the prefrontal cortex have been increased in the last 5-10 years in healthy subjects (Bunai et al., 2021; Filmer et al., 2019; Hone-Blanchet et al., 2016; Knechtel et al., 2014; Shinde et al., 2023; Talsma et al., 2018) due to more profound evidence of this region being involved in many psychiatric disorders (Koenigs & Grafman, 2009), though results are still variable.

Guan et al. and Shinde et al. reported decreased Glx values in the left DLPFC and ACC, respectively, after active tDCS stimulation over the left prefrontal cortex (Guan et al., 2020; Shinde et al., 2023). Shinde et al. additionally reported a decrease of GABA in the ACC, whereas Guan et al. couldn't reproduce such findings in the left DLPFC. This might be related to different stimulation parameters used, while Shinde et al. stimulated with 5 mA for 9 min and Bunai et al. with 2 mA for 30 min. Hone-Blanchet et al. and Talsma et al. both stimulated the left DLPFC with 1 mA for 30 and 20 min, respectively, while the first study reported an increase in Glx in the PFC and the Striatum (Hone-Blanchet et al., 2016) and the second one did not find any changes in the left DLPFC following anodal stimulation (Talsma et al., 2018). The same negative result was shown by Knechtel et al. stimulating the left DLPFC with 2 mA for 20 min (Knechtel et al., 2014). In the study of Bunai et al. an increase of GABA was reported in the left striatum and a decrease in the right striatum and the left DLPFC following bilateral DLPFC stimulation (Bunai et al., 2021). An overview of the publications can be found in table 1.

Table 1: prefrontal tDCS and MRS studies in healthy subjects.

Author	*tDCS*	*Metabolites*	*Brain region*	*Results*
Rango 2008	Anode (right M1), cathode (left shoulder) 1.5 mA, 15 min	mI	Right M1	↑ mI
Stagg 2009	Anode (left M1), cathode (right supraorbital ridge) Cathode (left M1), anode (right supraorbital ridge)	Glu, GABA	Left M1	Anodal: ↓ GABA Cathodal: ↓ Glu
Reidler 2012	Anode (left M1), cathode (right supraorbital ridge) 2 mA, 20 min	NAA, Gln	thalamus, ACC, M1, occipital cortex	Anodal: positive correlation between pain threshold and NAA (ACC); negative correlation between pain threshold and Gln (thalamus)
Kim 2014	Anode (left M1), cathode (right supraorbital ridge) Cathode (left M1), anode (right supraorbital ridge) 1.5 mA, 15 min	GABA	Left M1, right M1	Anodal: ↓ GABA Cathodal: No changes detected.
Knechtel 2014	Anode (F3; cathode: supraorbital ridge) 2 mA, 20 min	Glx	Left DLPFC	No changes detected.
Bachtiar 2015	Anode (left M1), cathode (right supraorbital ridge) 1 mA 20 min	GABA	Left M1	↓ GABA
Tremblay 2016	Anode (left M1), cathode (right M1) Cathode (left M1), anode (right M1) 1 mA, 20 min	tCr, tNAA, mI, GABA, Glx	Left M1	No changes detected.
Barron 2016	Anode (right temporal cortex), cathode (left supraorbital ridge)	GABA, Glu	Right temporal cortex	↓ GABA during tDCS ↑ Glu after stimulation
Hone-Blanchet 2016	Anode (left DLPFC) Cathode (right DLPFC) 1 mA, 30 min	NAA, Glx, GABA	PFC, Striatum	↑ NAA, Glx in PFC and Striatum No changes in GABA detected.
Antonenko 2017	Anode (left SM1), cathode (right supraorbital ridge) 1 mA, 15 min	GABA	Left M1	↓ GABA
Bachtiar 2018	Anode (left M1), cathode (right M1) 1 mA, 10 min	GABA, Glu	Left M1, right M1	Anodal: ↓ GABA (left & right M1) Cathodal: ↓ GABA (left & right M1)
Talsma 2018	Anode (left DLPFC), cathode (right supraorbital ridge) Cathode (left DLPFC), anode (right supraorbital ridge) 1 mA, 20 min	GABA, Glu	Left DLPFC	No changes detected.

Patel 2019	Anode (left M1), cathode (right supraorbital ridge) 1 mA, 10 min	GABA	Left M1	↓ GABA
Antonenko 2019	Anode (left M1), cathode (right supraorbital ridge) 1 mA, 15 min	GABA, Glu	Precentral gyrus	Anodal: ↓ GABA & Glu Cathodal: ↓ GABA & Glu
Filmer 2019	Anode (1 cm posterior left DLPFC), cathode (1 cm posterior right DLPFC) 0.7 mA, 9 min	GABA, Glu	Left PFC, bilateral visual cortex	Baseline GABA is associated with higher level of disruption to response selection training gains.
Nwaroh 2020	Anode (right M1), cathode (left supraorbital ridge) 1 mA, 20 min	GABA, Glx	Left M1, right M1	No changes detected.
Guan 2020	Anode (left DLPFC), Cathode (FP2) 2 mA, 30 min	GABA, Glx	Left DLPFC	↓ Glx (2 and 4 weeks post tDCS) No changes in GABA were detected.
Bunai 2021	Anode (left DLPFC), cathode (right DLPFC) 2 mA, 20 min	GABA	Left DLPFC, bilateral striatum	↑ GABA (left striatum) ↓ GABA (right striatum & left DLPFC)
Shinde 2023	Anode (left supraorbital), cathode (right mastoid) 5 mA, 9 min	GABA, Glx	ACC	↓ GABA, Glu (ACC)

Today, medication and psychotherapy are considered to be the first-line treatment for patients with psychiatric disorders, though, many show treatment resistance by not responding to medication, psychotherapy, or both. Thus, alternative treatment strategies are warranted. NIBS is used for several psychiatric disorders amongst others MDD (Bajbouj et al., 2018; A. Brunoni et al., 2016; A. R. Brunoni et al., 2013; Burkhardt et al., 2023; Kumpf et al., 2023; Palm et al., 2016). As already stated earlier, dysfunctional Glu neurotransmission and a resulting imbalance of excitation and inhibition is reported in MDD patients, the so-called Glutamate-Hypothesis (Huang et al., 2017; Sanacora et al., 2012). A rebalance using tDCS might have positive effects on depressive symptoms shown by several studies (A. R. Brunoni et al., 2013, 2016; Chrysikou et al., 2017; Fregni, Boggio, Nitsche, & Rigonatti, 2006; Loo et al., 2018), such as tDCS being superior or non-inferior to pharmacotherapy (A. R. Brunoni et al., 2013, 2016), the improvement of mood (Loo et al., 2018) and working memory (Fregni, Boggio, Nitsche,

Marcolin, et al., 2006), the upregulation of ventromedial activity and the reassessment of negative emotional stimuli (Chrysikou et al., 2020) as well as the elevation of Glx concentration after stimulation (Chrysikou et al., 2017).

To the best of our knowledge, there has been no study yet investigating prefrontal tDCS and brain metabolite changes in MDD. TDCS and MRS have been studied in neurological disorders such as stroke (O'Shea et al., 2014), pain (Foerster et al., 2015; Simis et al., 2015), neuropathic pain (Auvichayapat et al., 2018; Wilke et al., 2016), muscle spasticity (Auvichayapat et al., 2017), aphasia (Harris et al., 2019) and migraine (Pohl et al., 2023). In general, results are variable, while some studies report an increase in Glx following anodal stimulation (Auvichayapat et al., 2017, 2018) whereas others report a decrease in Glx following anodal stimulation (Carlson et al., 2018; Foerster et al., 2015). Two studies investigated GABA concentrations being decreased after active stimulation (Harris et al., 2019; Pohl et al., 2023). In stroke patients baseline GABA values were associated with clinical outcome (O'Shea et al., 2014). In psychiatric research studies combining tDCS and MRS measurements have been conducted for SCZ (Lee et al., 2018), gambling disorder (Dickler et al., 2018) and opioid use disorder (Kumar et al., 2022). Only one study reported an increase of GABA after cathodal stimulation (Dickler et al., 2018). The heterogeneity of results might result from varying stimulation protocols and locations as well as voxel locations across patient groups. In general, tDCS indeed seems to have an effect on brain metabolites, but more studies in the field of neurological and psychiatric disorders are needed to come to a meaningful conclusion.

6. Research question: Effects of tDCS using multimodal imaging techniques

TDCS is a widely studied non-invasive brain stimulation being a cost-effective, rather safe, and easy applicable method to treat neurologic and psychiatric disorders. There are several studies showing symptom improvement in MDD applying tDCS over the prefrontal cortex with the anode over the left DLPFC and the cathode over a contralateral region (Arul-Anandam & Loo, 2009; A. R. Brunoni et al., 2016; Ferrucci et al., 2009), however little is known about the exact underlying mechanisms and its effects on the brain. The use of multimodal imaging methods together with tDCS stimulation may shed light on these still open questions.

MRS is a relatively new method quantifying brain metabolite concentrations in a pre-defined region. Concentrations of the two neurotransmitters, Glu and GABA, are quantified by MRS leading to a better understanding of tDCS excitation and inhibition effects on the brain. Until now, studies mainly investigated tDCS effects on the motor cortex whereas a smaller number investigated the prefrontal cortex, all applying variable stimulation protocols. Reporting different results about anodal (excitatory) and cathodal (inhibitory) stimulation this leads to an ongoing debate about the basic mechanisms of tDCS. Outcomes of tDCS in healthy participants and patients have been variable, which might, amongst others, originate from individual anatomy and physiology of the brain. This is why e-field simulations of distribution and strength of the current applied to the brain is increasingly investigated in more recent studies. Simulations of the e-field contribute to a better understanding about the brain regions the current might be most effective in.

This dissertation shall pursue the following questions:

1. Does prefrontal tDCS exert acute effects on brain metabolites such as Glu, Glx or GABA and is there a change of the effects during or after tDCS?

2. How are these effects linked to other parameters such as gender, psychopathology or e-field distribution and strength?
3. How does e-field distribution and strength associate with clinical outcomes in depression?

In order to answer these questions, we have conducted two studies. In the first pilot study we investigated the effect of tDCS on brain metabolites in the right DLPFC under the cathode in healthy participants and expected a reduction of Glu and GABA.

The second study was performed to investigate the association between e-field strength and clinical outcomes in a cohort of MDD patients expecting an interdependency of both.

7. Summary

NIBS techniques are gaining increased interest as treatment in clinical settings, especially for psychiatric disorders. As an add on or in cases of medication resistance, NIBS techniques, among others tDCS stimulation, have shown to improve symptom severity (A. R. Brunoni et al., 2016). However, underlying mechanisms are still under investigation and results from studies are variable.

To develop a better knowledge about the basic mechanisms of tDCS, the combination with multimodal imaging techniques is promising. Using MRS, the expected activation under the anode and inhibition under the cathode can be underpinned by quantifying metabolites such as Glu and GABA under the electrodes. In a double-blinded, cross-over subject design we analysed 19 healthy participants undergoing active and sham stimulation inside the scanner with MRS (10 min) and rsfcMRI (6 min) measurements before and after, and two MRS (20 min) measurements during stimulation. Bilateral tDCS stimulation was applied for 20 min with 2 mA over the DLPFC, as mostly used in clinical settings. Exploratively, we simulated e-fields of each individual using a ROI-based approach to calculate e-field strength in certain brain regions according to the Sallet atlas (Sallet et al., 2013a). Changes of metabolite concentrations over time with exploratory regard to gender differences have been measured and showed a marked reduction of Glu after active stimulation under the cathode that was mainly carried by women. Electrical field strength and metabolite changes showed stronger Glu changes with higher electrical field strength in women.

Following the increasing interest in e-field simulation acting as a more precise indicator for the targeted brain area by tDCS, we further investigated the association between e-field strength and behavioral changes in a depression patient cohort from the ELECT-tDCS trial (A. R. Brunoni et al., 2017). In the ELECT-tDCS trial patients were randomized to three groups for 10 weeks, one receiving bifrontal tDCS stimulation plus placebo medication, one receiving

Escitalopram 10 mg/day for 3 weeks followed by 20 mg/day plus sham tDCS and another group receiving placebo medication plus sham tDCS. Brunoni at al. reported tDCS combined with placebo medication being inferior to a treatment with escitalopram, but superiority to placebo medication plus placebo tDCS (A. R. Brunoni et al., 2017). For 16 depressed patients' anatomical images were used to simulate the e-field with 5x5 cm electrodes, a current of 2 mA and anode and cathode targeting the left and right DLPFC respectively. Similarly to the first study, a ROI-based approach was used to measure e-field strength in specific brain regions, such as the DLPFC and ACC. E-field strength was shown to be inversely associated with negative affect in left and right ACC as well as in left and right DLPFC, meaning the higher the e-field strength, the lower the negative affect scores. The same result was found for high e-field values in the left ACC being associated with lower depression scores.

Findings of both studies are in line with previous publications showing the potential benefits of tDCS on the behavioral and cellular level in health and disease (A. R. Brunoni et al., 2016, 2017; Hone-Blanchet et al., 2016; Lee et al., 2018). While the first study was more focused on biochemical changes and the underlying effect of tDCS, the second study investigated clinical effects of stimulation in an MDD cohort. In previous studies an association between metabolite concentration and behavioral measures in depression patients has been reported (Benson et al., 2020; Chen et al., 2022; Gonsalves et al., 2022; Luykx et al., 2012; Tadayonnejad et al., 2018). Gonsalves and Chen report improved clinical outcomes in depression associated with metabolite alterations in the left DLPFC and the ACC. Benson and colleagues found an inverse correlation between Glu values in the dACC and anhedonia ratings. Even pre-treatment metabolite concentrations might have an influence on clinical outcome as shown in a machine-learning study predicting personalized treatment outcome in depression dependent on pre metabolite concentrations in the left hippocampus (Ali et al., 2022).

Most studies *either* investigate tDCS effects on electrical fields, metabolite changes *or* clinical outcomes, but only a few multimodal studies have been carried out (Antonenko et al.,

2017; Antonenko, Thielscher, & Bicalho, 2019; Bachtiar et al., 2018; Hunter et al., 2015). The association between metabolite changes and electrical field simulations in depression patients hasn't been investigated so far, however, there are results about healthy individuals. Antonenko and colleagues have found a reduction of GABA following active stimulation in the left SM1 whereas metabolite changes were significantly linked to e-field strength within the left precentral gyrus (Antonenko, Thielscher, & Bicalho, 2019). In line with these findings another study reported higher e-field strengths being associated to greater decreases in GABA in M1. This association was also influenced by grey matter volume in the MRS voxel (Nandi et al., 2022). In MDD patients, Glu increase in the left DLPFC after stimulation was significantly correlated with improved treatment outcome (Luborzewski et al., 2007). In our study, we have found significant effects of tDCS on brain metabolites mainly driven by women. Performing studies with both genders might influence results due to different hormonal status (Grachev & Apkarian, 2000). In the 20th century studies were often performed with male participants only because including women implied additional work such as documentation of menstrual cycle and contraceptives (Holdcroft, 2007). Today, most studies are keen to include different genders in equilibrium in their studies, though, there is still a lack of gender-specific analysis.

To the best of our knowledge, no study so far has investigated the relation between metabolite changes, e-field simulation, and behavioral changes in MDD or HC during or after tDCS. A greater number of multimodal studies are required to understand underlying mechanisms of tDCS for clinical treatment. As already mentioned, results of studies are diverse which might result from several different factors such as stimulation protocols, MR protocols and anatomical as well as biological differences of the individual participants. Even larger studies with big sample sizes show varying results. In the last years three large scale studies with over 100 participants have been performed (A. R. Brunoni et al., 2017; Burkhardt et al., 2023; Loo et al., 2018), reporting a decrease in depression scores after tDCS or escitalopram treatment compared to placebo (A. R. Brunoni et al., 2017), improvement of mood after four weeks of tDCS treatment regardless of active or sham stimulation (Loo et al.,

2018) and no improvements on depression scores after six weeks of tDCS treatment (Burkhardt et al., 2023). Brunoni and Burkhardt and colleagues applied tDCS over the prefrontal cortex with the anode over the left and the cathode over the right DLPFC. Loo and colleagues chose the same location for the anode but placed the cathode over the lateral right frontal area. As already mentioned previously, different electrode montages produce high variability in e-field distribution and strength, which might be associated to behavioral changes.

Taken together, notwithstanding the variable study results, all studies contribute to achieve a better picture of tDCS mechanisms on a molecular as well as a clinical level. Large scale studies are needed with a multimodal approach to minimize more inter study variabilities such as current strength, electrode positioning, MRS voxel positioning, analysis procedures, etc. At the same time, it is important to profoundly investigate individual treatment strategies because brain anatomy such as curvature, grey and white matter distribution as well as neuron orientation seem to significantly impact the current flow through tDCS.

8. Zusammenfassung

Non-invasive Hirnstimulationsverfahren (NIBS) gewinnen zunehmend an Interesse für die Behandlung im klinischen Umfeld, insbesondere bei psychiatrischen Störungen. Als Ergänzung für konventionelle Therapien oder bei Medikamentenresistenzen wirken sich NIBS, unter anderem die transkranielle Gleichstrom Stimulation (tDCS), nachweislich positiv auf den Schweregrad der Symptome aus. Die zugrundeliegenden Mechanismen sind jedoch noch nicht gänzlich erforscht und Studien berichten divergente Ergebnisse.

Um mehr über die grundlegenden Mechanismen der tDCS zu erfahren, ist die Kombination mit multimodalen Bildgebungsverfahren vielversprechend. Mit der Magnetresonanzspektrokopie (MRS) kann eine erwartete Aktivierung unter der Anode und eine Inhibierung unter der Kathode durch die Quantifizierung von Metaboliten wie Glutamat und GABA untersucht werden. In einem doppelt verblindeten cross-over Studiendesign untersuchten wir 19 gesunde Teilnehmer, die sich einer aktiven und einer Placebo Stimulation im Scanner unterzogen, mit MRS (10 min) und funktioneller MRT-Ruhezustandsmessungen (6 min) vor und nach, sowie zwei MRS Messungen (20 min) während der Stimulation. Der dorsolaterale Präfrontalkortex (DLPFC) wurde bilateral für 20 Minuten mit 2 mA stimuliert, wie es in der klinischen Praxis üblich ist. Explorativ simulierten wir die Verteilung des elektrischen Feldes im Gehirn jeder Person mit einem ROI-basierten Ansatz, um die Feldstärke in bestimmten Hirnregionen gemäß dem Sallet-Atlas (Sallet et al., 2013a) zu berechnen. Veränderungen der Metaboliten Konzentrationen im Laufe der Zeit mit explorativem Blick auf Geschlechtsunterschiede wurden analysiert und zeigten eine deutliche Reduktion von Glutamat nach aktiver Stimulation unter der Kathode, die hauptsächlich von Frauen getragen wurde. Ebenso ergaben die Berechnungen eine stärkere Glutamat Veränderungen bei höheren Feldstärken bei Frauen.

Aufgrund des zunehmenden Interesses an der Simulation der elektrischen Felder als präziseren Indikator für den durch die tDCS anvisierten Hirnbereich, haben wir den Zusammenhang zwischen der elektrischen Feldstärke und den Verhaltensänderungen in

einer Patientenkohorte mit Depression aus der ELECT-tDCS-Studie (A. R. Brunoni et al., 2017) untersucht. In der ELECT-tDCS-Studie wurden Patienten für 10 Wochen in drei Gruppen randomisiert: eine Gruppe erhielt eine bifrontale tDCS-Stimulation plus Placebo-Medikation, eine zweite Gruppe erhielt Escitalopram 10 mg/Tag für 3 Wochen, gefolgt von 20 mg/Tag und Placebo tDCS; und eine dritte Gruppe erhielt eine Placebo Medikation und Placebo tDCS. Brunoni et al. berichtete zwar, dass tDCS in Kombination mit einer Placebomedikation einer Behandlung mit Escitalopram unterlegen war, aber einer Placebomedikation und Placebo tDCS überlegen war (A. R. Brunoni et al., 2017). Bei 16 depressiven Patienten wurden anatomische Bilder weiterverarbeitet, um das elektrische Feld der 5x5 cm großen Elektroden und einer Stromstärke von 2 mA im Gehirn zu simulieren, wobei Anode und Kathode über dem linken bzw. rechten DLPFC platziert wurden. Ähnlich wie in der ersten Studie wurde ein ROI-basierter Ansatz verwendet, um die Feldstärke in bestimmten Hirnregionen, wie dem DLPFC und dem ACC, zu messen. Es zeigte sich, dass die Feldstärke im linken und rechten ACC sowie im linken und rechten DLPFC negativ mit den Werten des negativen Affekts korrelierten, d. h. je höher die Feldstärke, desto niedriger die Werte des negativen Affekts. Das gleiche Ergebnis wurde für hohe Feldstärken im linken ACC gefunden, die mit niedrigeren Depressionswerten korrelierten.

Die Ergebnisse beider Studien stehen im Einklang mit früheren Veröffentlichungen, die den potenziellen Nutzen von tDCS auf Verhaltens- und Zellebene bei Gesunden als auch bei Patienten belegen. Während sich die erste Studie mehr auf biochemische Veränderungen, ausgelöst durch tDCS konzentriert, untersucht die zweite Studie die klinischen Auswirkungen der Stimulation in einer Depressionskohorte. In früheren Studien wurde ein Zusammenhang zwischen der Konzentration von Metaboliten und dem Verhalten bei Depressionspatienten festgestellt (Benson et al., 2020; Chen et al., 2022; Gonsalves et al., 2022; Luykx et al., 2012; Tadayonnejad et al., 2018). Gonsalves und Chen berichten über verbesserte klinische Ergebnisse bei Depressionen im Zusammenhang mit Metabolitenveränderungen im linken DLPFC und im ACC. Benson et al. fand eine negative Korrelation zwischen Glutamat Werten

im dACC und Anhedonie Werten. Sogar Metabolitenkonzentrationen vor einer Behandlung könnten Einfluss auf das klinische Ergebnis haben. Das zeigte eine *machine-learning* Studie die personalisierte Behandlungsergebnisse bei Depressionen in Abhängigkeit von den Metabolitenkonzentrationen vor der Behandlung im linken Hippocampus prognostizierte (Ali et al., 2022).

Die meisten Studien untersuchen *entweder* die Auswirkungen von tDCS auf elektrische Felder, Metabolitenveränderungen *oder* klinische Ergebnisse, aber es wurden nur wenige multimodale Studien durchgeführt (Antonenko et al., 2017; Antonenko, Thielscher, & Bicalho, 2019; Bachtiar et al., 2018; Hunter et al., 2015). Der Zusammenhang zwischen Metabolitenveränderungen und elektrischen Feldsimulationen bei Depressionspatienten ist bisher nicht untersucht worden, es gibt jedoch Ergebnisse von gesunden Probanden. Antonenko et al. zeigte eine Verringerung von GABA nach aktiver Stimulation im linken SM1, wobei Metabolitenveränderungen signifikant mit der Feldstärke im linken präzentralen Gyrus verbunden waren (Antonenko, Thielscher, & Bicalho, 2019). Im Einklang mit diesen Ergebnissen wurde in einer weiteren Studie berichtet, dass höhere Feldstärken mit einer stärkeren Abnahme von GABA in M1 verbunden seien, wobei auch das Volumen der grauen Substanz ein Einfluss zu haben scheint (Nandi et al., 2022). Außerdem konnte bei Patienten mit Depression ein Glutamat Anstieg im linken DLPFC nach der Stimulation mit einem signifikant verbesserten Behandlungsergebnis in Verbindung gebracht werden (Luborzewski et al., 2007).

In unserer Studie haben wir signifikante Auswirkungen der tDCS auf die Hirnmetaboliten gefunden, die hauptsächlich von Frauen getragen wurden. Die Durchführung von Studien mit beiden Geschlechtern können Ergebnisse aufgrund des unterschiedlichen hormonellen Status beeinflussen (Grachev & Apkarian, 2000). Im 20. Jahrhundert wurden Studien häufig nur mit männlichen Teilnehmern durchgeführt, da die Einbeziehung von Frauen mit zusätzlichem Aufwand verbunden war, unter anderem mit der Dokumentation des Menstruationszyklus und der Anwendung von Verhütungsmitteln (Holdcroft, 2007). Heute sind

die meisten Studien bestrebt, verschiedene Geschlechter gleichberechtigt in ihre Untersuchungen einzubeziehen, doch fehlt es noch immer an geschlechtsspezifischen Analysen.

Soweit uns bekannt, hat bisher keine Studie den Zusammenhang zwischen Metabolitenveränderungen, elektrischen Feld Simulationen und Verhaltensänderungen bei Depressionen oder Gesunden während oder nach der tDCS untersucht. Eine größere Anzahl an multimodalen Studien sind erforderlich, um die zugrunde liegenden Mechanismen der tDCS für die klinische Behandlung zu verstehen. Wie bereits erwähnt, unterscheiden sich die meisten Studienergebnisse ungemein, was auf verschiedene Faktoren wie Stimulationsprotokolle, MR-Protokolle und anatomische sowie biologische Unterschiede der einzelnen Teilnehmer zurückzuführen sein könnte. Selbst größere Studien mit großen Stichproben zeigen unterschiedliche Ergebnisse. In den letzten Jahren wurden drei Studien mit mehr als 100 Teilnehmern durchgeführt (A. R. Brunoni et al., 2017; Burkhardt et al., 2023; Loo et al., 2018). Brunoni et al. zeigte eine Verbesserung der Depressionssymptome nach einer tDCS- oder Escitalopram-Behandlung im Vergleich zu Placebo (A. R. Brunoni et al., 2017), wobei Loo et al. eine Verbesserung der Stimmung nach vierwöchiger tDCS-Behandlung unabhängig von aktiver oder Placebo-Stimulation (Loo et al., 2018) berichtete. Burkhardt et al. wiederum konnte keine Verbesserung der Depressionssymptome nach sechswöchiger tDCS-Behandlung (Burkhardt et al., 2023) zeigen. Brunoni und Burkhardt et al. platzierten tDCS Elektroden über dem präfrontalen Kortex mit der Anode über dem linken und der Kathode über dem rechten DLPFC. Loo und Kollegen wählten die gleiche Stelle für die Anode, platzierten die Kathode jedoch über dem rechten frontal lateralen Kortex. Wie bereits erwähnt, führen unterschiedliche Elektrodenanordnungen zu einer hohen Variabilität in der Verteilung und Stärke des elektrischen Feldes, was Auswirkungen auf den Erfolg der Behandlung haben könnte.

Trotz der unterschiedlichen Studienergebnisse tragen alle Studien dazu bei, ein besseres Bild über die grundlegenden Mechanismen der tDCS sowohl auf molekularer als

auch auf klinischer Ebene zu erhalten. Studien mit großen Stichproben und multimodalem Ansatz sind erforderlich, um die große Variabilität, wie z. B. Stromstärke, Elektrodenpositionierung, MRS-Voxelpositionierung oder Analyseverfahren, zwischen Studien zu minimieren. Gleichzeitig ist es wichtig, individuelle Behandlungsstrategien zu explorieren, da die Anatomie des Gehirns wie z. B. die Gyrifizierung, die Verteilung der grauen und weißen Substanz sowie die Ausrichtung der Neuronen, den Stromfluss durch tDCS erheblich zu beeinflussen scheint.

9. References

Abdallah, C. G., Jiang, L., De Feyter, H. M., Fasula, M., Krystal, J. H., Rothman, D. L., Mason, G. F., & Sanacora, G. (2014). Glutamate metabolism in major depressive disorder. *American Journal of Psychiatry*, *171*(12), 1320–1327. https://doi.org/10.1176/appi.ajp.2014.14010067

Albizu, A., Fang, R., Indahlastari, A., O'Shea, A., Stolte, S. E., See, K. B., Boutzoukas, E. M., Kraft, J. N., Nissim, N. R., & Woods, A. J. (2020). Machine learning and individual variability in electric field characteristics predict tDCS treatment response. *Brain Stimulation*, *13*(6), 1753–1764. https://doi.org/10.1016/j.brs.2020.10.001

Ali, F. Z., Wengler, K., He, X., Nguyen, M. H., Parsey, R. V, & DeLorenzo, C. (2022). Gradient boosting decision-tree-based algorithm with neuroimaging for personalized treatment in depression. *Neuroscience Informatics*, 100110.

Antonenko, D., Schubert, F., Bohm, F., Ittermann, B., Aydin, S., Hayek, D., Grittner, U., & Flöel, A. (2017). tDCS-Induced Modulation of GABA Levels and Resting-State Functional Connectivity in Older Adults. *The Journal of Neuroscience*, *37*(15), 4065–4073. https://doi.org/10.1523/JNEUROSCI.0079-17.2017

Antonenko, D., Thielscher, A., & Bicalho, G. (2019). *Brain Stimulation Towards precise brain stimulation : Is electric fi eld simulation related to neuromodulation ? xxxx*. https://doi.org/10.1016/j.brs.2019.03.072

Antonenko, D., Thielscher, A., Saturnino, G. B., Aydin, S., Ittermann, B., Grittner, U., & Flöel, A. (2019). Towards precise brain stimulation: Is electric field simulation related to neuromodulation? *Brain Stimulation*, *12*(5), 1159–1168. https://doi.org/10.1016/j.brs.2019.03.072

Arul-Anandam, A. P., & Loo, C. (2009). Transcranial direct current stimulation: A new tool for the treatment of depression? *Journal of Affective Disorders*, *117*(3), 137–145. https://doi.org/10.1016/j.jad.2009.01.016

Asan, A. S., Gok, S., & Sahin, M. (2018). Electric fields induced By transcutaneous And intracranial current injections In The Rat brain. *2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, 2252–2255.

Auvichayapat, P., Aree-Uea, B., Auvichayapat, N., Phuttharak, W., Janyacharoen, T., Tunkamnerdthai, O., Boonphongsathian, W., Ngernyam, N., & Keeratitanont, K. (2017). Transient changes in brain metabolites after transcranial direct current stimulation in spastic cerebral palsy: a pilot study. *Frontiers in Neurology*, *8*, 366.

Auvichayapat, P., Keeratitanont, K., Janyachareon, T., & Auvichayapat, N. (2018). The effects of transcranial direct current stimulation on metabolite changes at the anterior cingulate cortex in neuropathic pain: a pilot study. *Journal of Pain Research*, 2301–2309.

Bachtiar, V., Johnstone, A., Berrington, A., Lemke, C., Johansen-Berg, H., Emir, U., & Stagg, C. J. (2018). Modulating regional motor cortical excitability with noninvasive brain stimulation results in neurochemical changes in bilateral motor cortices. *Journal of Neuroscience*, *38*(33), 7327–7336. https://doi.org/10.1523/JNEUROSCI.2853-17.2018

Bachtiar, V., Near, J., Johansen-Berg, H., & Stagg, C. J. (2015). Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation. *ELife*, *4*(September 2015), 1–9. https://doi.org/10.7554/eLife.08789

Baeken, C., Arns, M., Brunelin, J., Chanes, L., Filipcic, I., Ganho-Ávila, A., Hirnstein, M., Rachid, F., Sack, A. T., O'shea, J., D'urso, G., & Antal, A. (2023). European reclassification of non-invasive brain stimulation as class III medical devices: A call to action. In *Brain Stimulation* (Vol. 16, Issue 2, pp. 564–566). Elsevier Inc. https://doi.org/10.1016/j.brs.2023.02.012

Bajbouj, M., Aust, S., Spies, J., Herrera-Melendez, A. L., Mayer, S. V., Peters, M., Plewnia, C., Fallgatter, A. J., Frase, L., Normann, C., Behler, N., Wulf, L., Brakemeier, E. L., & Padberg, F. (2018). PsychotherapyPlus: augmentation of cognitive behavioral therapy (CBT) with prefrontal transcranial direct current stimulation (tDCS) in major depressive disorder—study design and methodology of a multicenter double-blind randomized placebo-controlled trial. *European Archives of Psychiatry and Clinical Neuroscience*, *268*(8), 797–808. https://doi.org/10.1007/s00406-017-0859-x

Barron, H. C., Vogels, T. P., Emir, U. E., Makin, T. R., O'Shea, J., Clare, S., Jbabdi, S., Dolan, R. J., & Behrens, T. E. J. (2016). Unmasking Latent Inhibitory Connections in Human Cortex to Reveal Dormant Cortical Memories. *Neuron*, *90*(1), 191–203. https://doi.org/10.1016/j.neuron.2016.02.031

Benson, K. L., Bottary, R., Schoerning, L., Baer, L., Gonenc, A., Eric Jensen, J., & Winkelman, J. W. (2020). 1H MRS Measurement of Cortical GABA and Glutamate in Primary Insomnia and Major Depressive Disorder: Relationship to Sleep Quality and Depression Severity. *Journal of Affective Disorders*, *274*, 624–631. https://doi.org/10.1016/j.jad.2020.05.026

Brunoni, A., Nitsche, M., & Loo, C. (2016). *Transcranial direct current stimulation in neuropsychiatric disorders*. Springer International Publishing.

Brunoni, A. R., Ferrucci, R., Fregni, F., Boggio, P. S., & Priori, A. (2012). Transcranial direct current stimulation for the treatment of major depressive disorder: A summary of preclinical, clinical and translational findings. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *39*(1), 9–16. https://doi.org/10.1016/j.pnpbp.2012.05.016

Brunoni, A. R., Moffa, A. H., Fregni, F., Palm, U., Padberg, F., Blumberger, D. M., Daskalakis, Z. J., Bennabi, D., Haffen, E., Alonzo, A., & Loo, C. K. (2016). Transcranial direct current stimulation for acute major depressive episodes: meta-analysis of individual patient data. *The British Journal of Psychiatry*, *208*(6), 522 LP – 531. http://bjp.rcpsych.org/content/208/6/522.abstract

Brunoni, A. R., Moffa, A. H., Sampaio, B., Borrione, L., Moreno, M. L., Fernandes, R. A., Veronezi, B. P., Nogueira, B. S., Aparicio, L. V. M., Razza, L. B., Chamorro, R., Tort, L. C., Fraguas, R., Lotufo, P. A., Gattaz, W. F., Fregni, F., & Bensenor, I. M. (2017). Trial of electrical direct-current therapy versus escitalopram for depression. *New England Journal of Medicine*, *376*(26), 2523–2533. https://doi.org/10.1056/NEJMoa1612999

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., Edwards, D. J., Valero-Cabre, A., Rotenberg, A., Pascual-Leone, A., Ferrucci, R., Priori, A., Boggio, P. S., & Fregni, F. (2012). Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions. *Brain Stimulation*, *5*(3), 175–195. https://doi.org/10.1016/j.brs.2011.03.002

Brunoni, A. R., Valiengo, L., Baccaro, A., Zanao, T. A., de Oliveira, J. F., Goulart, A., Boggio, P. S., Lotufo, P. A., Bensenor, I. M., & Fregni, F. (2013). The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial,

randomized, controlled trial. *JAMA Psychiatry*, *70*(4), 383–391. https://doi.org/10.1001/2013.jamapsychiatry.32

Bunai, T., Hirosawa, T., Kikuchi, M., Fukai, M., Yokokura, M., Ito, S., Takata, Y., Terada, T., & Ouchi, Y. (2021). tDCS-induced modulation of GABA concentration and dopamine release in the human brain: A combination study of magnetic resonance spectroscopy and positron emission tomography. *Brain Stimulation*, *14*(1), 154–160. https://doi.org/10.1016/j.brs.2020.12.010

Burkhardt, G., Kumpf, U., Crispin, A., Goerigk, S., Andre, E., Plewnia, C., Brendel, B., Fallgatter, A., Langguth, B., Abdelnaim, M., Hebel, T., Normann, C., Frase, L., Zwanzger, P., Diemer, J., Kammer, T., Schönfeldt-Lecuona, C., Kamp, D., Bajbouj, M., … Padberg, F. (2023). Transcranial direct current stimulation as an additional treatment to selective serotonin reuptake inhibitors in adults with major depressive disorder in Germany (DepressionDC): a triple-blind, randomised, sham-controlled, multicentre trial. *The Lancet*, *402*(10401), 545–554. https://doi.org/10.1016/S0140-6736(23)00640-2

Carlson, H. L., Ciechanski, P., Harris, A. D., MacMaster, F. P., & Kirton, A. (2018). Changes in spectroscopic biomarkers after transcranial direct current stimulation in children with perinatal stroke. *Brain Stimulation*, *11*(1), 94–103.

Cavaleiro, C., Martins, J., Gonçalves, J., Castelo-Branco, M., & Marsili, L. (2020). Memory and Cognition-Related Neuroplasticity Enhancement by Transcranial Direct Current Stimulation in Rodents: A Systematic Review. In *Neural Plasticity* (Vol. 2020). Hindawi Limited. https://doi.org/10.1155/2020/4795267

Chase, H. W., Boudewyn, M. A., Carter, C. S., & Phillips, M. L. (2020). Transcranial direct current stimulation: a roadmap for research, from mechanism of action to clinical implementation. In *Molecular Psychiatry* (Vol. 25, Issue 2, pp. 397–407). Springer Nature. https://doi.org/10.1038/s41380-019-0499-9

Chen, Y., Li, X., Wang, L., Tian, S., Chen, Y., Wang, F., Gu, K., Wang, Y., Xu, G., Zhang, S., Liu, J., Wang, H., Jia, Z., Li, L., Wang, X., Xie, F., Wang, X., Wang, S., Xue, C., … Qian, L. (2022). Effects of Repetitive Transcranial Magnetic Stimulation on Cognitive Function in Patients With Stress-Related Depression: A Randomized Double-Blind fMRI and 1H-MRS Study. *Frontiers in Neurology*, *13*. https://doi.org/10.3389/fneur.2022.844606

Chrysikou, E. G., Hanson, G. K., Ramey, C. H., Martin, L. E., Choi, I.-Y., Lee, P., & Brooks, W. M. (2017). Proceedings #7. Transcranial direct current stimulation effects on prefrontal cortex in major depressive disorder (MDD) measured by in vivo proton magnetic resonance spectroscopy (1 H MRS). *Brain Stimulation*, *10*(4), e53–e54. https://doi.org/10.1016/j.brs.2017.04.100

Chrysikou, E. G., Wing, E. K., & van Dam, W. O. (2020). Transcranial Direct Current Stimulation Over the Prefrontal Cortex in Depression Modulates Cortical Excitability in Emotion Regulation Regions as Measured by Concurrent Functional Magnetic Resonance Imaging: An Exploratory Study. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, 1–10. https://doi.org/10.1016/j.bpsc.2019.12.004

Clark, V. P., Coffman, B. A., Trumbo, M. C., & Gasparovic, C. (2011). Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: A 1H magnetic resonance spectroscopy study. *Neuroscience Letters*, *500*(1), 67–71. https://doi.org/10.1016/j.neulet.2011.05.244

Das, S., Holland, P., Frens, M. A., & Donchin, O. (2016). Impact of transcranial direct current stimulation (tDCS) on neuronal functions. *Frontiers in Neuroscience*, *10*(NOV), 1–7. https://doi.org/10.3389/fnins.2016.00550

Deschwanden, A., Karolewicz, B., Feyissa, A. M., Treyer, V., Ametamey, S. M., Johayem, A., Burger, C., Auberson, Y. P., Sovago, J., & Stockmeier, C. A. (2011). Reduced metabotropic glutamate receptor 5 density in major depression determined by [11C] ABP688 PET and postmortem study. *American Journal of Psychiatry*, *168*(7), 727–734.

Dickler, M., Lenglos, C., Renauld, E., Ferland, F., Edden, R. A., Leblond, J., & Fecteau, S. (2018). Online effects of transcranial direct current stimulation on prefrontal metabolites in gambling disorder. *Neuropharmacology*, *131*, 51–57. https://doi.org/10.1016/j.neuropharm.2017.12.002

Duman, R. S., Sanacora, G., & Krystal, J. H. (2019). Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. *Neuron*, *102*(1), 75–90. https://doi.org/10.1016/j.neuron.2019.03.013

Dwyer, G. E., Craven, A. R., Hirnstein, M., Kompus, K., Assmus, J., Ersland, L., Hugdahl, K., & Grüner, R. (2019). No effects of anodal tDCS on local GABA and GLx levels in the left posterior superior temporal gyrus. *Frontiers in Neurology*, *10*(JAN), 1–10. https://doi.org/10.3389/fneur.2018.01145

Esterlis, I., Holmes, S. E., Sharma, P., Krystal, J. H., & DeLorenzo, C. (2018). Metabotropic glutamatergic receptor 5 and stress disorders: knowledge gained from receptor imaging studies. *Biological Psychiatry*, *84*(2), 95–105.

Farhat, L. C., Carvalho, A. F., Solmi, M., & Brunoni, A. R. (2022). Evidence-based umbrella review of cognitive effects of prefrontal tDCS. In *Social Cognitive and Affective Neuroscience* (Vol. 17, Issue 1, pp. 43–60). Oxford University Press. https://doi.org/10.1093/scan/nsaa084

Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., Barbieri, S., & Priori, A. (2009). Transcranial direct current stimulation in severe, drug-resistant major depression. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2009.02.015

Filmer, H. L., Ehrhardt, S. E., Bollmann, S., Mattingley, J. B., & Dux, P. E. (2019). Accounting for individual differences in the response to tDCS with baseline levels of neurochemical excitability. *Cortex*, *115*, 324–334. https://doi.org/10.1016/j.cortex.2019.02.012

Foerster, B. R., Nascimento, T. D., DeBoer, M., Bender, M. A., Rice, I. C., Truong, D. Q., Bikson, M., Clauw, D. J., Zubieta, J. K., Harris, R. E., & DaSilva, A. F. (2015). Excitatory and Inhibitory Brain Metabolites as Targets and Predictors of Effective Motor Cortex tDCS Therapy in Fibromyalgia. *Arthritis Rheumatol*, *33*(4), 395–401. https://doi.org/10.1038/nbt.3121.ChIP-nexus

Fregni, F., Boggio, P. S., Nitsche, M. A., Marcolin, M. A., Rigonatti, S. P., & Pascual-Leone, A. (2006). Letters to the Editor Treatment of major depression with transcranial direct current stimulation Ephedrine-induced emergence of bipolar symptoms. *Bipolar Disorder*, 203–204.

Fregni, F., Boggio, P. S., Nitsche, M. A., & Rigonatti, S. P. (2006). Cognitive Effects of Repeated Sessions of tDCS in patients with depression. *Depression and Anxiety*, *23*(1), 482–484. https://doi.org/10.1002/da

Gonsalves, M. A., White, T. L., Barredo, J., Fukuda, A. M., Joyce, H. E., Harris, A. D., & Carpenter, L. L. (2022). Repetitive Transcranial Magnetic Stimulation-Associated Changes in Neocortical Metabolites in Major Depression: A Systematic Review. In *NeuroImage: Clinical* (Vol. 35). Elsevier Inc. https://doi.org/10.1016/j.nicl.2022.103049

Grachev, I. D., & Apkarian, a V. (2000). Chemical heterogeneity of the living human brain: a proton MR spectroscopy study on the effects of sex, age, and brain region. *NeuroImage*, *11*(5 Pt 1), 554–563. https://doi.org/10.1006/nimg.2000.0557

Grimm, S., Beck, J., Schuepbach, D., Hell, D., Boesiger, P., Bermpohl, F., Niehaus, L., Boeker, H., & Northoff, G. (2008). Imbalance between Left and Right Dorsolateral Prefrontal Cortex in Major Depression Is Linked to Negative Emotional Judgment: An fMRI Study in Severe Major Depressive Disorder. *Biological Psychiatry*, *63*(4), 369–376. https://doi.org/10.1016/j.biopsych.2007.05.033

Guan, H., Zheng, Y., Wang, M., Zhang, Y., Weng, M., Chen, W., & Zhang, S. (2020). Effect of Long-term Transcranial Direct Current Stimulation on Glx and GABA: A Pilot Study. *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*, *2020-July*, 3561–3564. https://doi.org/10.1109/EMBC44109.2020.9175996

Harris, A. D., Wang, Z., Ficek, B., Webster, K., Edden, R. A. E., & Tsapkini, K. (2019). Reductions in GABA following a tDCS-language intervention for primary progressive aphasia. *Neurobiology of Aging*, *79*, 75–82.

Hasler, G., Van Der Veen, J. W., Tumonis, T., Meyers, N., Shen, J., & Drevets, W. C. (2007). Reduced Prefrontal Glutamate/Glutamine and-Aminobutyric Acid Levels in Major Depression Determined Using Proton Magnetic Resonance Spectroscopy. In *Arch Gen Psychiatry* (Vol. 64).

Heimrath, K., Brechmann, A., Blobel-Lüer, R., Stadler, J., Budinger, E., & Zaehle, T. (2020). Transcranial direct current stimulation (tDCS) over the auditory cortex modulates GABA and glutamate: a 7 T MR-spectroscopy study. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-77111-0

Holdcroft, A. (2007). Gender bias in research: how does it affect evidence based medicine? *Journal of the Royal Society of Medicine*, *100*. http://www.eoc.org.uk/

Hone-Blanchet, A., Edden, R. A., & Fecteau, S. (2016). Online Effects of Transcranial Direct Current Stimulation in Real Time on Human Prefrontal and Striatal Metabolites. *Biological Psychiatry*, *80*(6), 432–438. https://doi.org/10.1016/j.biopsych.2015.11.008

Huang, Y. J., Lane, H. Y., & Lin, C. H. (2017). New Treatment Strategies of Depression: Based on Mechanisms Related to Neuroplasticity. *Neural Plasticity*, *2017*. https://doi.org/10.1155/2017/4605971

Hunter, M. A., Coffman, B. A., Gasparovic, C., Calhoun, V. D., Trumbo, M. C., & Clark, V. P. (2015). Baseline effects of transcranial direct current stimulation on glutamatergic neurotransmission and large-scale network connectivity. *Brain Research*, *1594*, 92–107. https://doi.org/10.1016/j.brainres.2014.09.066

Hyeon Seo, & Sung Chan Jun. (2018). Relation between the electric field and activation of cortical neurons in transcranial electrical stimulation. *Brain Stimulation*.

Isitan, C., Yan, Q., Spencer, D. D., & Alkawadri, R. (2020). Brief history of electrical cortical stimulation: a journey in time from Volta to Penfield. *Epilepsy Research*, *166*, 106363.

Jog, M. V., Wang, D. J. J., & Narr, K. L. (2019). A review of transcranial direct current stimulation (tDCS) for the individualized treatment of depressive symptoms. In *Personalized Medicine in Psychiatry* (Vols. 17–18, pp. 17–22). Elsevier Inc. https://doi.org/10.1016/j.pmip.2019.03.001

Kim, J. H., Kim, D. W., Chang, W. H., Kim, Y. H., Kim, K., & Im, C. H. (2014). Inconsistent outcomes of transcranial direct current stimulation may originate from anatomical differences among individuals: Electric field simulation using individual MRI data. *Neuroscience Letters*, *564*, 6–10. https://doi.org/10.1016/j.neulet.2014.01.054

Knechtel, L., Schall, U., Cooper, G., Ramadan, S., Stanwell, P., Jolly, T., & Thienel, R. (2014). Transcranial direct current stimulation of prefrontal cortex: An auditory event-related potential and proton magnetic resonance spectroscopy study. *Neurology Psychiatry and Brain Research*, *20*(4), 96–101. https://doi.org/10.1016/j.npbr.2014.06.001

Koenigs, M., & Grafman, J. (2009). The functional neuroanatomy of depression: Distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behavioural Brain Research*, *201*(2), 239–243. https://doi.org/10.1016/j.bbr.2009.03.004

Koolschijn, R. S., Emir, U. E., Pantelides, A. C., Nili, H., Behrens, T. E. J., & Barron, H. C. (2019). The hippocampus and neocortical inhibitory engrams protect against memory interference. *Neuron*, *101*(3), 528–541.

Kumar, A. S., Khanra, S., Goyal, N., Dharani, R., & Roy, C. (2022). Adjunctive High-Definition Transcranial Direct Current Stimulation in Brain Glutamate-Glutamine and γ-Aminobutyric Acid, Withdrawal and Craving During Early Abstinence Among Patients With Opioid Use Disorder on Buprenorphine-Naloxone: A Proton Magnetic Resonance Spectroscopy–Based Pilot Study. *The Journal of ECT*, *38*(2). https://journals.lww.com/ectjournal/fulltext/2022/06000/adjunctive_high_definition_transcranial_direct.11.aspx

Kumpf, U., Palm, U., Eder, J., Ezim, H., Stadler, M., Burkhardt, G., Dechantsreiter, E., & Padberg, F. (2023). TDCS at home for depressive disorders: an updated systematic review and lessons learned from a prematurely terminated randomized controlled pilot study. *European Archives of Psychiatry and Clinical Neuroscience*. https://doi.org/10.1007/s00406-023-01620-y

Laakso, I., Mikkonen, M., Koyama, S., Hirata, A., & Tanaka, S. (2019). Can electric fields explain inter- individual variability in transcranial direct current stimulation of the motor cortex ? *Scientific Reports*, *December 2018*, 1–10. https://doi.org/10.1038/s41598-018-37226-x

Laakso, I., Tanaka, S., Mikkonen, M., Koyama, S., Sadato, N., & Hirata, A. (2016). NeuroImage Electric fi elds of motor and frontal tDCS in a standard brain space : A computer simulation study. *NeuroImage*, *137*, 140–151. https://doi.org/10.1016/j.neuroimage.2016.05.032

Lee, J., Yoon, Y. B., Wijtenburg, S. A., Rowland, L. M., Chen, H., Gaston, F. E., Song, I. C., Cho, K. I. K., Kim, M., Lee, T. Y., & Kwon, J. S. (2018). Lower glutamate level in temporo-parietal junction may predict a better response to tDCS in schizophrenia. *Schizophrenia Research*, 1–2. https://doi.org/10.1016/j.schres.2018.05.032

Lefaucheur, J.-P., Antal, A., Ayache, S. S., Benninger, D. H., Brunelin, J., Cogiamanian, F., Cotelli, M., De Ridder, D., Ferrucci, R., & Langguth, B. (2017). Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clinical Neurophysiology*, *128*(1), 56–92.

Liebetanz, D., Fregni, F., Monte-Silva, K. K., Oliveira, M. B., Amâncio-dos-Santos, Â., Nitsche, M. A., & Guedes, R. C. A. (2006). After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. *Neuroscience Letters*, *398*(1–2), 85–90.

Liu, A., Vöröslakos, M., Kronberg, G., Henin, S., Krause, M. R., Huang, Y., Opitz, A., Mehta, A., Pack, C. C., Krekelberg, B., Berényi, A., Parra, L. C., Melloni, L., Devinsky, O., & Buzsáki, G. (2018). Immediate neurophysiological effects of transcranial electrical stimulation. In *Nature Communications* (Vol. 9, Issue 1). Nature Publishing Group. https://doi.org/10.1038/s41467-018-07233-7

Liu, B., Liu, J., Wang, M., Zhang, Y., & Li, L. (2017). From Serotonin to Neuroplasticity: Evolvement of Theories for Major Depressive Disorder. *Front Cell Neurosci*, *11*, 305. https://doi.org/10.3389/fncel.2017.00305

Loo, C. K., Husain, M. M., McDonald, W. M., Aaronson, S., O'Reardon, J. P., Alonzo, A., Weickert, C. S., Martin, D. M., McClintock, S. M., Mohan, A., Lisanby, S. H., Krystal, A. D., Peterchev, A. V., Davis, W., Sklar, J., Weickert, C. S., Colagiuri, B., & Galvez, V. (2018). International randomized-controlled trial of transcranial Direct Current Stimulation in depression. *Brain Stimulation*, *11*(1), 125–133. https://doi.org/10.1016/j.brs.2017.10.011

Luborzewski, A., Schubert, F., Seifert, F., Danker-Hopfe, H., Brakemeier, E. L., Schlattmann, P., Anghelescu, I., Colla, M., & Bajbouj, M. (2007). Metabolic alterations in the dorsolateral prefrontal cortex after treatment with high-frequency repetitive transcranial magnetic stimulation in patients with unipolar major depression. *Journal of Psychiatric Research*, *41*(7), 606–615. https://doi.org/10.1016/j.jpsychires.2006.02.003

Luykx, J. J., Laban, K. G., van den Heuvel, M. P., Boks, M. P. M., Mandl, R. C. W., Kahn, R. S., & Bakker, S. C. (2012). Region and state specific glutamate downregulation in major depressive disorder: A meta-analysis of 1H-MRS findings. *Neuroscience and Biobehavioral Reviews*, *36*(1), 198–205. https://doi.org/10.1016/j.neubiorev.2011.05.014

MacKenbach, C., Tian, R., & Yang, Y. (2020). Effects of Electrode Configurations and Injected Current Intensity on the Electrical Field of Transcranial Direct Current Stimulation: A Simulation Study. *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*, *2020-July*, 3517–3520. https://doi.org/10.1109/EMBC44109.2020.9176686

Mackin, R. S., Tosun, D., Mueller, S. G., Lee, J.-Y., Insel, P., Schuff, N., Truran-Sacrey, D., Arean, P., Nelson, J. C., & Weiner, M. W. (2013). Patterns of reduced cortical thickness in late-life depression and relationship to psychotherapeutic response. *The American Journal of Geriatric Psychiatry*, *21*(8), 794–802.

McGirr, A., Berlim, M. T., Bond, D. J., Fleck, M. P., Yatham, L. N., & Lam, R. W. (2015). A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of ketamine in the rapid treatment of major depressive episodes. *Psychological Medicine*, *45*(4), 693–704. https://doi.org/10.1017/S0033291714001603

Medeiros, L. F., de Souza, I. C. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., Fregni, F., Caumo, W., & Torres, I. L. S. (2012). Neurobiological effects of transcranial direct current stimulation: A review. *Frontiers in Psychiatry*, *3*(DEC). https://doi.org/10.3389/fpsyt.2012.00110

Miller, E. M., Pomerleau, F., Huettl, P., Gerhardt, G. a, & Glaser, P. E. a. (2014). Aberrant glutamate signaling in the prefrontal cortex and striatum of the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder. *Psychopharmacology*, *231*(15), 3019–3029. https://doi.org/10.1007/s00213-014-3479-4

Mizutani-Tiebel, Y., Takahashi, S., Karali, T., Mezger, E., Bulubas, L., Papazova, I., Dechantsreiter, E., Stoecklein, S., Papazov, B., Thielscher, A., Padberg, F., & Keeser, D. (2022). Differences in electric field strength between clinical and non-clinical populations induced by prefrontal tDCS: A cross-diagnostic, individual MRI-based modeling study. *NeuroImage: Clinical*, *34*. https://doi.org/10.1016/j.nicl.2022.103011

Moffa, A. H., Brunoni, A. R., Nikolin, S., & Loo, C. K. (2018). Transcranial direct current stimulation in psychiatric disorders: a comprehensive review. *Psychiatric Clinics*, *41*(3), 447–463.

Moffa, A. H., Martin, D., Alonzo, A., Bennabi, D., Blumberger, D. M., Benseñor, I. M., Daskalakis, Z., Fregni, F., Haffen, E., Lisanby, S. H., Padberg, F., Palm, U., Razza, L. B., Sampaio-Jr, B., Loo, C., & Brunoni, A. R. (2020). Efficacy and acceptability of transcranial direct current stimulation (tDCS) for major depressive disorder: An individual patient data meta-analysis. In *Progress in Neuro-Psychopharmacology and Biological Psychiatry* (Vol. 99). Elsevier Inc. https://doi.org/10.1016/j.pnpbp.2019.109836

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2013). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2012.04.011

Moriguchi, S., Takamiya, A., Noda, Y., Horita, N., Wada, M., Tsugawa, S., Plitman, E., Sano, Y., Tarumi, R., ElSalhy, M., Katayama, N., Ogyu, K., Miyazaki, T., Kishimoto, T., Graff-Guerrero, A., Meyer, J. H., Blumberger, D. M., Daskalakis, Z. J., Mimura, M., & Nakajima, S. (2019). Glutamatergic neurometabolite levels in major depressive disorder: a systematic review and meta-analysis of proton magnetic resonance spectroscopy studies. In *Molecular Psychiatry* (Vol. 24, Issue 7, pp. 952–964). Nature Publishing Group. https://doi.org/10.1038/s41380-018-0252-9

Mutz, J., Edgcumbe, D. R., Brunoni, A. R., & Fu, C. H. Y. (2018). *Title: Efficacy and acceptability of non-invasive brain stimulation for the treatment of adult 1 unipolar and bipolar depression: A systematic review and meta-analysis of randomised sham-2 controlled trials 3 4*. https://doi.org/10.1101/287656

Mutz, J., Vipulananthan, V., Carter, B., Hurlemann, R., Fu, C. H. Y., & Young, A. H. (2019). Comparative efficacy and acceptability of non-surgical brain stimulation for the acute treatment of major depressive episodes in adults: systematic review and network meta-analysis. *BMJ (Online)*, *364*. https://doi.org/10.1136/bmj.l1079

Nandi, T., Puonti, O., Clarke, W. T., Nettekoven, C., Barron, H. C., Kolasinski, J., Hanayik, T., Hinson, E. L., Berrington, A., Bachtiar, V., Johnstone, A., Winkler, A. M., Thielscher, A., Johansen-Berg, H., & Stagg, C. J. (2022). tDCS induced GABA change is associated with the simulated electric field in M1, an effect mediated by grey matter volume in the MRS voxel. *Brain Stimulation*, *15*(5), 1153–1162. https://doi.org/10.1016/j.brs.2022.07.049

Nissen, C., Holz, J., Blechert, J., Feige, B., Riemann, D., Voderholzer, U., & Normann, C. (2010). Learning as a model for neural plasticity in major depression. *Biological Psychiatry*, *68*(6), 544–552.

Nitsche, M. A., & Paulus, W. (2001). *Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans*. 39–40. https://doi.org/10.1212/WNL.57.10.1899

Nwaroh, C., Giuffre, A., Cole, L., Bell, T., Kirton, A., & Harris, A. (2019). *Effects of Transcranial Direct Current Stimulation on GABA and Glutamate in Children: A Pilot Study*. https://doi.org/10.1101/759290

Onarheim, B. (2023). *EU regulators grossly misrepresent non-invasive brain stimulation*. Neuromodec.Org.

Opitz, A., Paulus, W., Will, S., Antunes, A., & Thielscher, A. (2015). Determinants of the electric field during transcranial direct current stimulation. *NeuroImage*, *109*, 140–150. https://doi.org/10.1016/j.neuroimage.2015.01.033

O'Shea, J., Boudrias, M. H., Stagg, C. J., Bachtiar, V., Kischka, U., Blicher, J. U., & Johansen-Berg, H. (2014). Predicting behavioural response to TDCS in chronic motor stroke. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2013.05.096

Palm, U., Hasan, A., Strube, W., & Padberg, F. (2016). tDCS for the treatment of depression: a comprehensive review. *Eur Arch Psychiatry Clin Neurosci*, *266*(8), 681–694. https://doi.org/10.1007/s00406-016-0674-9

Patel, H. J., Romanzetti, S., Pellicano, A., Nitsche, M. A., Reetz, K., & Binkofski, F. (2019). Proton Magnetic Resonance Spectroscopy of the motor cortex reveals long term GABA change following anodal Transcranial Direct Current Stimulation. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-39262-7

Pelletier, S. J., & Cicchetti, F. (2015). *Cellular and Molecular Mechanisms of Action of Transcranial Direct Current Stimulation : Evidence from In Vitro and In Vivo Models*. 1–13. https://doi.org/10.1093/ijnp/pyu047

Persson, J., Wall, A., Weis, J., Gingnell, M., Antoni, G., Lubberink, M., & Bodén, R. (2021). Inhibitory and excitatory neurotransmitter systems in depressed and healthy: A positron emission tomography and magnetic resonance spectroscopy study. *Psychiatry Research - Neuroimaging*, *315*. https://doi.org/10.1016/j.pscychresns.2021.111327

Pohl, H., Wyss, P., Sandor, P. S., Schoenen, J., Luechinger, R., O'Gorman, R., Riederer, F., Gantenbein, A. R., & Michels, L. (2023). The longitudinal influence of tDCS on occipital GABA and glutamate/glutamine levels in episodic migraineurs. *Journal of Neuroscience Research*, *101*(6), 815–825.

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, *30*(6), 672–679. https://doi.org/10.1002/mrm.1910300604

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., & Bikson, M. (2013). Cellular effects of acute direct current stimulation: Somatic and synaptic terminal effects. *Journal of Physiology*, *591*(10), 2563–2578. https://doi.org/10.1113/jphysiol.2012.247171

Rajkowska, G., Miguel-Hidalgo, J. J., Dubey, P., Stockmeier, C. A., & Krishnan, K. R. R. (2005). Prominent reduction in pyramidal neurons density in the orbitofrontal cortex of elderly depressed patients. *Biological Psychiatry*, *58*(4), 297–306.

Rango, M., Cogiamanian, F., Marceglia, S., Barberis, B., Arighi, A., Biondetti, P., & Priori, A. (2008). Myoinositol content in the human brain is modified by transcranial direct current stimulation in a matter of minutes: A 1H-MRS study. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine*, *60*(4), 782–789.

Razza, L. B., De Smet, S., Moffa, A., Sudbrack-Oliveira, P., Vanderhasselt, M. A., & Brunoni, A. R. (2021). Follow-up effects of transcranial direct current stimulation (tDCS) for the major depressive episode: A systematic review and meta-analysis. In *Psychiatry Research* (Vol. 302). Elsevier Ireland Ltd. https://doi.org/10.1016/j.psychres.2021.114024

Razza, L. B., Palumbo, P., Moffa, A. H., Carvalho, A. F., Solmi, M., Loo, C. K., & Brunoni, A. R. (2020). A systematic review and meta-analysis on the effects of transcranial direct current stimulation in depressive episodes. *Depression and Anxiety*, *37*(7), 594–608.

Razza, L. B., Wischnewski, M., Suen, P., De Smet, S., da Silva, P. H. R., Catoira, B., Brunoni, A. R., & Vanderhasselt, M.-A. (2023). A meta-analysis and electric field modeling to understand the antidepressant effects of tDCS. *Brazilian Journal of Psychiatry*. https://doi.org/10.47626/1516-4446-2023-3116

Reato, D., Rahman, A., Bikson, M., & Parra, L. C. (2013). Effects of weak transcranial alternating current stimulation on brain activity-a review of known mechanisms from

animal studies. In *Frontiers in Human Neuroscience* (Issue OCT). Frontiers Media S. A. https://doi.org/10.3389/fnhum.2013.00687

Reidler, J. S., Mendonca, M. E., Santana, M. B., Wang, X., Lenkinski, R., Motta, A. F., Marchand, S., Latif, L., & Fregni, F. (2012). Effects of motor cortex modulation and descending inhibitory systems on pain thresholds in healthy subjects. *The Journal of Pain*, *13*(5), 450–458.

Robitaille, P., & Abduljalil, A. (1998). Human magnetic resonance imaging at 8 T. *NMR in …*, *11*(6), 263–265. http://www.ncbi.nlm.nih.gov/pubmed/9802467%5Cnhttp://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1492(199810)11:6%3C263::AID-NBM549%3E3.0.CO;2-0/full

Sallet, J., Mars, R. B., Noonan, M. P., Neubert, F. X., Jbabdi, S., O'Reilly, J. X., Filippini, N., Thomas, A. G., & Rushworth, M. F. (2013a). The organization of dorsal frontal cortex in humans and macaques. *The Journal of Neuroscience*, *33*(30), 12255–12274. https://doi.org/10.1523/JNEUROSCI.5108-12.2013

Sallet, J., Mars, R. B., Noonan, M. P., Neubert, F.-X., Jbabdi, S., O'Reilly, J. X., Filippini, N., Thomas, A. G., & Rushworth, M. F. (2013b). The Organization of Dorsal Frontal Cortex in Humans and Macaques. *Journal of Neuroscience*, *33*(30), 12255–12274. https://doi.org/10.1523/JNEUROSCI.5108-12.2013

Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, *62*(1), 63–77. https://doi.org/10.1016/j.neuropharm.2011.07.036

Sarawagi, A., Soni, N. D., & Patel, A. B. (2021). Glutamate and GABA Homeostasis and Neurometabolism in Major Depressive Disorder. In *Frontiers in Psychiatry* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fpsyt.2021.637863

Sarmiento, C. I., San-Juan, D., & Prasath, V. B. S. (2016). Letter to the Editor: Brief history of transcranial direct current stimulation (tDCS): from electric fishes to microcontrollers. *Psychological Medicine*, *46*(15), 3259–3261. https://doi.org/DOI: 10.1017/S0033291716001926

Saturnino, G. B., Puonti, O., Nielsen, J. D., Antonenko, D., Madsen, K. H., & Thielscher, A. (2019). SimNIBS 2.1: a comprehensive pipeline for individualized electric field modelling for transcranial brain stimulation. *Brain and Human Body Modeling: Computational Human Modeling at EMBC 2018*, 3–25.

Schild, H. (2005). Klinische hochfeld-MRT. *RoFo Fortschritte Auf Dem Gebiet Der Rontgenstrahlen Und Der Bildgebenden Verfahren*, *177*(5), 621–631. https://doi.org/10.1055/s-2005-858048

Shinde, A., Nagarajan, R., Enes Gunduz, M., Visintainer, P., & Schlaug, G. (2023). *Assessing the Dose-Dependent Effects of tDCS on Neurometabolites using Magnetic Resonance Spectroscopy*. https://doi.org/10.1101/2023.06.13.544864

Siegel, G. J., Brady, S. T., Albers, R. W., Agranoff, B. W., & Katzmnn, R. (2012). *Basic Neurochemistry* (8th editio). American Society for Neurochemistry. Published by Elsevier Inc.

Simis, M., Reidler, J. S., Duarte Macea, D., Moreno Duarte, I., Wang, X., Lenkinski, R., Petrozza, J. C., & Fregni, F. (2015). Investigation of central nervous system dysfunction in chronic pelvic pain using magnetic resonance spectroscopy and noninvasive brain stimulation. *Pain Practice*, *15*(5), 423–432.

Smith, G. S., Oeltzschner, G., Gould, N. F., Leoutsakos, J. M. S., Nassery, N., Joo, J. H., Kraut, M. A., Edden, R. A. E., Barker, P. B., Wijtenburg, S. A., Rowland, L. M., & Workman, C. I. (2021). Neurotransmitters and Neurometabolites in Late-Life

Depression: A Preliminary Magnetic Resonance Spectroscopy Study at 7T. *Journal of Affective Disorders*, *279*, 417–425. https://doi.org/10.1016/j.jad.2020.10.011

Stagg, C. J. (2014). Magnetic Resonance Spectroscopy as a tool to study the role of GABA in motor-cortical plasticity. *NeuroImage*, *86*, 19–27. https://doi.org/10.1016/j.neuroimage.2013.01.009

Stagg, C. J., Bachtiar, V., O'shea, J., Allman, C., Bosnell, R. A., Kischka, U., Matthews, P. M., & Johansen-Berg, H. (2011). Cortical activation changes underlying stimulation-induced behavioural gains in chronic stroke. *Brain*, *135*(1), 276–284.

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., Morris, P. G., Matthews, P. M., & Johansen-Berg, H. (2009). Polarity-Sensitive Modulation of Cortical Neurotransmitters by Transcranial Stimulation. *Journal of Neuroscience*, *29*(16), 5202–5206. https://doi.org/10.1523/JNEUROSCI.4432-08.2009

Stagg, C. J., Jayaram, G., Pastor, D., Kincses, Z. T., Matthews, P. M., & Johansen-Berg, H. (2011). Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning. *Neuropsychologia*, *49*(5), 800–804. https://doi.org/10.1016/j.neuropsychologia.2011.02.009

Tadayonnejad, R., Deshpande, R., Ajilore, O., Moody, T., Morfini, F., Ly, R., O'Neill, J., & Feusner, J. D. (2018). Pregenual Anterior Cingulate Dysfunction Associated with Depression in OCD: An Integrated Multimodal fMRI/ 1 H MRS Study. *Neuropsychopharmacology*, *43*(5), 1146–1155. https://doi.org/10.1038/npp.2017.249

Talsma, L. J., Broekhuizen, J. A., Huisman, J., & Slagter, H. A. (2018). No evidence that baseline prefrontal cortical excitability (3T-MRS) predicts the effects of prefrontal tDCS on WM performance. *Frontiers in Neuroscience*, *12*(JUL), 1–13. https://doi.org/10.3389/fnins.2018.00481

Tanaka, T., Isomura, Y., Kobayashi, K., Hanakawa, T., Tanaka, S., & Honda, M. (2020). Electrophysiological effects of transcranial direct current stimulation on neural activity in the rat motor cortex. *Frontiers in Neuroscience*, *14*, 495.

Tremblay, S., Beaulé, V., Proulx, S., & Lafleur, L.-P. (2014). The Use of Magnetic Resonance Spectroscopy as a Tool for the Measurement of Bi-hemispheric Transcranial Electric Stimulation Effects on Primary Motor Cortex Metabolism. *Journal of Visualized Experiments*, *93*(e51631), 1–20.

van der Graaf, M. (2010). In vivo magnetic resonance spectroscopy: basic methodology and clinical applications. In *European Biophysics Journal* (Vol. 39, Issue 4, pp. 527–540). https://doi.org/10.1007/s00249-009-0517-y

Wilke, S., List, J., Mekle, R., Lindenberg, R., Bukowski, M., Ott, S., Schubert, F., Ittermann, B., & Flöel, A. (2016). No Effect of Anodal Transcranial Direct Current Stimulation on Gamma-Aminobutyric Acid Levels in Patients with Recurrent Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *33*, neu.2016.4399. https://doi.org/10.1089/neu.2016.4399

Wischnewski, M., Mantell, K. E., & Opitz, A. (2021). Identifying regions in prefrontal cortex related to working memory improvement: A novel meta-analytic method using electric field modeling. *Neuroscience & Biobehavioral Reviews*, *130*, 147–161.

Zago, S., Priori, A., Ferrucci, R., & Lorusso, L. (2021). Historical aspects of transcranial electric stimulation. *Transcranial Direct Current Stimulation in Neuropsychiatric Disorders: Clinical Principles and Management*, 3–19.

Zhang, R., Lam, C. L. M., Peng, X., Zhang, D., Zhang, C., Huang, R., & Lee, T. M. C. (2021). Efficacy and acceptability of transcranial direct current stimulation for treating depression: A meta-analysis of randomized controlled trials. In *Neuroscience and*

Biobehavioral Reviews (Vol. 126, pp. 481–490). Elsevier Ltd. https://doi.org/10.1016/j.neubiorev.2021.03.026

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11. Publications

11.1. Paper 1 - Effects of bifrontal transcranial direct current stimulation on brain glutamate levels and resting state connectivity: multimodal MRI data for the cathodal stimulation site

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ORIGINAL PAPER

Effects of bifrontal transcranial direct current stimulation on brain glutamate levels and resting state connectivity: multimodal MRI data for the cathodal stimulation site

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Abstract
Transcranial direct current stimulation (tDCS) over prefrontal cortex (PFC) regions is currently proposed as therapeutic intervention for major depression and other psychiatric disorders. The in-depth mechanistic understanding of this bipolar and non-focal stimulation technique is still incomplete. In a pilot study, we investigated the effects of bifrontal stimulation on brain metabolite levels and resting state connectivity under the cathode using multiparametric MRI techniques and computational tDCS modeling. Within a double-blind cross-over design, 20 subjects (12 women, 23.7 ± 2 years) were randomized to active tDCS with standard bifrontal montage with the anode over the left dorsolateral prefrontal cortex (DLPFC) and the cathode over the right DLPFC. Magnetic resonance spectroscopy (MRS) was acquired before, during, and after prefrontal tDCS to quantify glutamate (Glu), Glu + glutamine (Glx) and gamma aminobutyric acid (GABA) concentration in these areas. Resting-state functional connectivity MRI (rsfcMRI) was acquired before and after the stimulation. The individual distribution of tDCS induced electric fields (efields) within the MRS voxel was computationally modelled using SimNIBS 2.0. There were no significant changes of Glu, Glx and GABA levels across conditions but marked differences in the course of Glu levels between female and male participants were observed. Further investigation yielded a significantly stronger Glu reduction after active compared to sham stimulation in female participants, but not in male participants. For rsfcMRI neither significant changes nor correlations with MRS data were observed. Exploratory analyses of the effect of efield intensity distribution on Glu changes showed distinct effects in different efield groups. Our findings are limited by the small sample size, but correspond to previously published results of cathodal tDCS. Future studies should address gender and efield intensity as moderators of tDCS induced effects.

Keywords Magnetic resonance spectroscopy · Functional magnetic resonance imaging (fMRI) · Electrical field modelling · Glutamate · GABA · Transcranial direct current stimulation (tDCS)

Introduction

Due to its safe and cost-effective profile, transcranial direct current stimulation (tDCS) of prefrontal cortex (PFC) regions represents a promising therapeutic approach in major depression (MD) and other psychiatric disorders [1–5]. The technique is based on the application of a weak direct current flowing between bipolar electrodes positioned over the head with an intensity of 1–2 mA for 5–30 min, for one or several days [6–8].

However, there is still an ongoing debate on the basic mechanisms of tDCS, such as the direction of its effects in terms of polarity, intensity, session duration and individual

Eva Mezger, Frank Padberg and Daniel Keeser equally contributed to this work.

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neuroanatomy [9–12]. Promising studies had shown that tDCS effects over the motor cortex were polarity-dependent, shifting neuronal resting membrane potentials either toward depolarization (close to the anode) or hyperpolarization (close to the cathode) [13–15]. Moreover, the orientation of neuronal layers [16, 17], anatomical differences between individuals, and the variability of brain states [18] may influence tDCS effects, as may current intensity and the precise electrode position, size and orientation [19–24].

When applying tDCS in different brain regions in healthy subjects various effects have been described, including changes in brain networks, assessed by resting-state functional connectivity magnetic resonance imaging (rsfcMRI) [25, 26], cognitive performance, measured via working memory tasks [27–31], and changes in brain metabolite and neurotransmitter levels, investigated via ^{1}H-magnetic resonance spectroscopy (MRS) [28, 32–34]. In recent studies, rsfcMRI and computational modeling of the electrical field (efield) induced by tDCS in the brain have been included as additional tools to enhance the explanatory power, demonstrating an association of functional brain connectivity and/or efield strength with physiological changes [35–37].

Previous MRS studies mainly investigated tDCS over motor cortex regions [32, 34, 37–39], and very few MRS studies focused on tDCS of prefrontal regions [40, 41]. Bifrontal tDCS (anode: right dorsolateral PFC (right DLPFC; F4), cathode: left DLPFC (F3)) in gambling disorder increased GABA levels under the anode in the right DLPFC during stimulation. Another bifrontal tDCS montage (anode: F3, cathode: F4) in healthy participants increased prefrontal N-acetyl-aspartate (NAA) and striatal glutamate + glutamine (Glx) levels during and after stimulation. Combining on- and offline protocols for tDCS and MRS (i.e., MRS before, during and after stimulation) allows measuring dynamic effects of bifrontal tDCS. Similarly, adding another functional MR-based modality to MRS (i.e., rsfcMRI) can increase the explanatory power of these results as shown in a recent study [37]. In this pilot study, we investigated the effects of bifrontal tDCS on Glu, Glx and GABA levels in an MRS voxel close to the cathode over the right DLPFC before, during and after tDCS, expecting stimulation induced changes in metabolite concentration. In addition, we explored the impact of gender, efield distribution within the MRS voxel as well as rsfcMRI connectivity.

Materials and methods

All subjects participated in a sham-controlled combined tDCS-MRS protocol and received active and sham tDCS in a double-blind cross-over design with randomized order.

The study was approved by the local ethics committee (Faculty of Medicine, Ludwig Maximilian University Munich, Munich, Germany). All participants provided written informed consent, and received financial compensation for participation.

Participants

Twenty out of 25 recruited subjects (12 women [23.6 ± 2.0 years]/8 men [24.1 ± 2.0 years]; mean age of 23.7 ± 2.0 years) were analyzed. Five participants were excluded due to poor MRS data quality. Recruitment was performed via social networks (facebook.com) and postings at the University hospital. An online questionnaire was sent for screening to assess exclusion criteria, such as drug abuse, other psychiatric or neurological diseases and MRI contraindications (e.g., metals in/on the body, claustrophobia, pacemakers). In addition, a telephone interview was conducted to screen participants for psychiatric and neurological disorders, use of psychotropic medications, and unstable or severe physical health conditions. Participants were asked to abstain from alcohol the day before the measurement and to avoid caffeine on the day of the measurement. All participants were right-handed, as assessed by the Edinburgh Handedness Inventory [42].

Experimental design

All participants underwent two combined tDCS-MRI sessions (approximately 2 h each) at the same time of the day with a minimum interval of 2 weeks between both sessions to avoid carry-over effects. Before and after tDCS-MRI measurements, positive and negative affect were assessed using the PANAS trait and state questionnaire (Positive And Negative Affect Schedule; [43]).

The study design is shown in Fig. 1; before stimulation, structural MRI scans (T1- and T2-weighted isotropic 3D sequences), an MRS sequence and a rsfcMRI sequence were acquired. Two separate MRS sequences were measured during stimulation, initiated after 15 s of tDCS, to compare early and late periods of tDCS. After stimulation, another set of MRS and rsfcMRI sequences was acquired. A total of four MRS acquisitions named baseline, during1, during2 and post were conducted. Baseline MRS was recorded before rsfcMRI to exclude possible effects of echo planar imaging (EPI) sequences on MRS [44]. Only after measuring the first 10 subjects in our study, we noticed that the MRS ROI was placed according to the neurological instead of the radiological convention. Thus, the respective MRS ROI was erroneously placed underneath the cathode. However, expecting effects of bifrontal tDCS in proximity to both electrodes we continued our tDCS-MRS protocol with the MRS ROI positioned over the right DLPFC. Such effects under both electrodes with an increase of Glu under the anode and a reduction under the cathode has been previously been

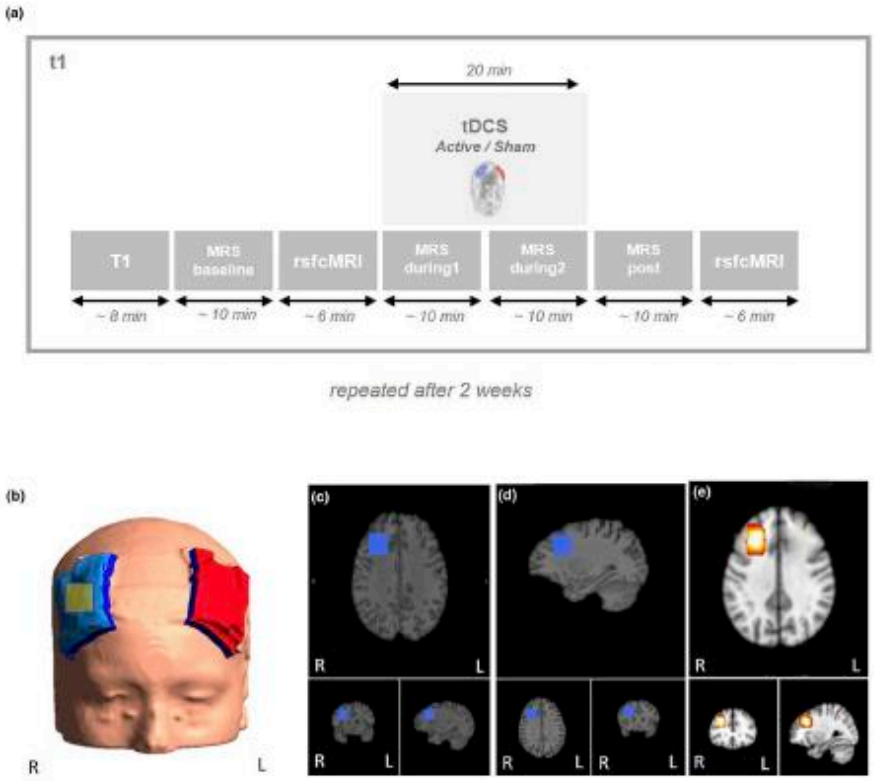

Fig. 1 Study protocol. **a** Four 10-min intervals of MRS were measured before (baseline), during (during1, during2) and after (post) tDCS. MRS during1 was started 15 s after the beginning of tDCS. **b** Electrode positioning with the anode over the left DLPFC and the cathode over the right DLPFC. MRS region of interest (ROI) was placed under the cathodal electrode in the right DLPFC (yellow box). **c** Example of the MRS ROI in a male participant. **d** Example of the MRS ROI in a female participant. **e** Combined ROI of all participants (male and female); ROIs projected onto the MNI152 standard template

demonstrated [32, 37, 45]. Future studies need to investigate further MRS ROI positions ideally applying multi-voxel MRS for localizing tDCS effects on metabolites.

Transcranial direct current stimulation

TDCS was administered using an MR-certified Eldith stimulator MR (neuroConn, Ilmenau, Germany) via two saline-soaked surface sponge electrodes (5×7 cm^2) placed over F3 (anode) and F4 (cathode; according to the international 10–20 system) corresponding to the left and right DLPFC. Active tDCS was administered in the scanner for 20 min at 2 mA intensity. Sham tDCS followed the built-in placebo-mode that limits stimulation to the 15 s ramp-up/ramp-down periods to mimic the somatosensory artefacts of active tDCS (skin warming, tingling). Blinding was assessed after every single session using a standardized questionnaire [46].

Magnetic resonance imaging/magnetic resonance spectroscopy

All MRI scans were conducted on a 3 Tesla MRI scanner (Magnetom Skyra, Siemens Healthineers, Erlangen, Germany). A T1-weighted 3D structural magnetization-prepared rapid gradient-echo (MPRAGE) sequence with 176 layers of slices, slice thickness 0.8 mm^3 isotropic voxels in sagittal orientation, repetition time (TR) = 1900 ms, echo time (TE) = 2.2 ms, flip angle (FA) of 9° and field of view (FoV) of 200×200 mm; and a T2-weighted 3D SPACE sequence with 160 slices, slice thickness 1.0 mm^3 isotropic voxels, TR = 5000 ms, TE = 386 ms and FoV of 256×256 mm were acquired. Single-voxel spectroscopy with a MEGA PRESS sequence [https://www.cmrr.umn.edu/spectro/] [47] (TR = 2000 ms, TE = 68 ms, spectral bandwidth = 2000 Hz, 144 averages and editing pulses applied to the GABA spins at 1.9 ppm for refocusing only the GABA spins for the ON-signal, and at 7.5 ppm that do not affect any GABA spins for the OFF-signal) was acquired. As the GABA signal acquired at 68 ms is roughly 50% macromolecule, we refer to GABA as GABA + in the following sections. Voxel placement was performed by experienced MRS operators on the individual 3D-reconstructed T1-weighted images using the superior frontal sulcus, the lateral fissure, and the genu of the corpus callosum as anatomical landmarks (see supplemental information, Fig. 4).

For quantification of GABA + and Glx concentrations, the open source software Gannet 3.0 (http://www.gabamrs.com) was used, while for the Glu quantification off-spectra of the MEGA-PRESS sequence were analyzed in LCModel (Linear Combination Model, Version 2.1-1A; [48]. Results are

presented in ratios to creatine (for more detailed information of processing steps please see supplemental information section 3.2).

Data with standard deviations (Cramér-Rao lower bounds)> 20% estimated by the LCModel and Gannet 3.0 were considered as poor quality and excluded from further analysis (five out of twenty-five). Tissue segmentation in the ROIs was performed using FSL FAST [49] to estimate the content of cerebrospinal fluid (CSF), grey matter (GM), and white matter (WM). The metabolite concentrations were corrected for partial CSF volume in the ROI [50].

Resting state functional MRI connectivity

Sixteen out of twenty datasets were analyzed (4 datasets were excluded due to failed data processing). An EPI sequence with the following parameters was acquired: TR = 2000 ms; TE = 30 ms; flip FA = 80°; spatial resolution, 3 × 3 x 3 mm^3; imaging matrix, 64 × 64; FoV = 192 × 192 mm^2; number of slices 36; number of volumes, 250. The individual high-resolution MPRAGE data served as anatomical reference.

Pre-processing of the data was conducted using FSL 5.0.10 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), AFNI (https://afni.nimh.nih.gov/) version 18 and in-house scripts (Karali et al. [51, 52]; https://zenodo.org/record/3530897#.XfdzSWRKhPZ). For detailed information about pre- and post-processing of the data please see supplemental information, page 2 and 3.

Computational modeling of electrical fields

Eighteen out of twenty datasets were analyzed (7 men, two datasets were excluded due to missing T2-weighted datasets). SimNIBS 2.0 (Stimulation in Non Invasive Brain Stimulation, https://www.simnibs.org/; Thielscher et al. [53]) was used to model the distribution and intensity of the efield. To generate the head models, T1- and T2-weighted MR images were fed into the 'mri2mesh' function of SimNIBS, that employs FreeSurfer and FSL functions to automatically segment the MR images into five tissue types (white matter, grey matter, skin, skull and cerebrospinal fluid) and subsequently creates individual tetrahedral head meshes from the segmentations [54–56]. Efield simulations are based on the Finite Element Method (FEM).

For twelve out of eighteen participants (4 men, six datasets were excluded due to failing transformation into volumetric space), the simulated data of the norm of the electric field was transformed into MNI standard volumetric space using a customized python script based on FSL and the following GitHub resource: https://github.com/ncullen93/mesh2nifti to extract the number of activated voxels thresholded at 0.3 in GM only, as a measure for efield strength.

Exploratively, we investigated the relationship between Glu changes and individual efield strength within the MRS ROI by dividing the sample of twelve participants into a "small" ($n = 5$) and a "large" ($n = 7$) efield group. The cut-off value defined to separate the two groups was the mean value of activated voxels of all subjects (6000 activated voxels). We hypothesize that a larger number of activated efield voxels in the volumetric space reflects a stronger potential response to electrical stimulation as indicated by the results of the simulation.

Exact number of data sets for each analysis is shown in the study flow chart in the supplemental information, Fig. 2.

Statistical analysis

Statistical analyses were conducted using R (R Development Core Team, 2008, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, https://www.r-project.org/). We performed linear mixed effects models (LMM) for repeated measurements to investigate differences in metabolite concentration change between the active and sham group incorporating four different time points (baseline, during1, during2 and post). Measurements were considered as nested within subjects. To control for gender effects, gender was included as a covariate to the LMM. Inter-individual differences in metabolite concentration at baseline were accounted for by including a random intercept term to the model. To account for subject-specific change rates, we tested if the inclusion of a random slope term would significantly improve the model fit. Nested models were compared using χ^2-likelihood-ratio tests. Effect sizes reflecting the between-group differences in metabolite concentration change over time were calculated (Cohen's d). Post-hoc analyses were planned (e.g., multiple comparisons between time points) if factors reached significance in the original LMM (two-sided $p < 0.05$).

Open science

All raw data and scripts will be available via OSF: https://osf.io/qgs57/.

Results

Behavioural data

PANAS scores before and after the stimulation were evaluated showing differences of the effect of time and PANAS scores for active and sham stimulation ($p = 0.037$). For both conditions PANAS scores were higher before ($mean_{active} = 16.00 \pm 9.6$; $mean_{sham} = 14.95 \pm 7.1$) compared

to after ($mean_{active} = 13.25 \pm 8$; $mean_{sham} = 12.15 \pm 8.6$) the stimulation. For more detailed information on behavioral data please see supplemental information page 5 and 6.

tDCS effects on metabolite concentrations

We investigated changes of Glu, Glx and GABA + concentration over time in the two conditions (active, sham; see Fig. 2). Including a random slope term did not significantly improve model fit for all outcomes (Glu: $\chi^2 = 0.03$, $p = 0.98$; Glx: $\chi^2 = 1.14$, $p = 0.57$; GABA +: $\chi^2 = 0.00$, $p = 1$). Hence, a random intercept fixed slope solution was selected.

To control effects of Cr changes on Glu, GABA or Glx related effects, we also analyzed the NAA/Cr ratio. However, we did not detect any significant effects for time ($F(1, 140) = 0.166$, $p = 0.685$), condition ($F(1, 140) = 0.065$, $p = 0.799$) or the time*condition interaction ($F(1, 140) = 0.037$, $p = 0.848$).

Effects of tDCS on Glu concentrations in the DLPFC

While no significant effects were found for the factors time and condition, we observed a trend for the factor condition ($F_{(1, 140)} = 3.01$, $p = 0.085$) and a trend for time*condition ($F_{(1, 140)} = 2.88$, $p = 0.092$). Descriptive statistics showed a marked difference in male and female participants (Fig. 3). Therefore, we decided to additionally investigate how tDCS-induced changes differed with regard to gender as a model factor. No significant effects were found for the factors time ($F_{(1, 140)} = 2.67$, $p = 0.102$) and time*condition ($F_{(1, 140)} = 1.48$, $p = 0.226$) in the full sample (Fig. 2); however, the three-way interaction with gender indicated significant differences in tDCS-induced change of metabolite concentrations between male and female subjects ($F_{(1, 140)} = 2.04$, $p = 0.017$). Female subjects showed a significant reduction in Glu concentration in the active compared to the sham condition ($\beta = 0.03$ [0.01–0.05], $t_{(140)} = 2.87$, $p = 0.004$, $d = 1.29$ [0.41–2.17]), while male subjects did not ($\beta = -0.01$ [−0.03 to 0.02], $t_{(140)} = 0.78$, $p = 0.440$, $d = 0.33$ [− 0.50 to 1.16]) (see Fig. 3 and supplemental information Table 1). To determine at which time point tDCS-induced reduction in Glu (i.e., time*condition interactions) was at its strongest, Bonferroni-corrected LMM models were fit by consecutively including the next latest time point from baseline revealing a significant interaction for Glu change between baseline and the "during 2" time point ($\beta = -0.03$ [− 0.05 to − 0.02], $t_{(12)} = -4.56$, $p = 0.004$, $d = 1.50$ [0.86–2.15]) (see supplemental information, Table 2).

Effects of tDCS on GABA + and Glx concentrations in the DLPFC

A significant effect of *gender* on GABA + concentration ($F_{(1, 140)} = 6.26$, $p = 0.014$) was detected; however, no

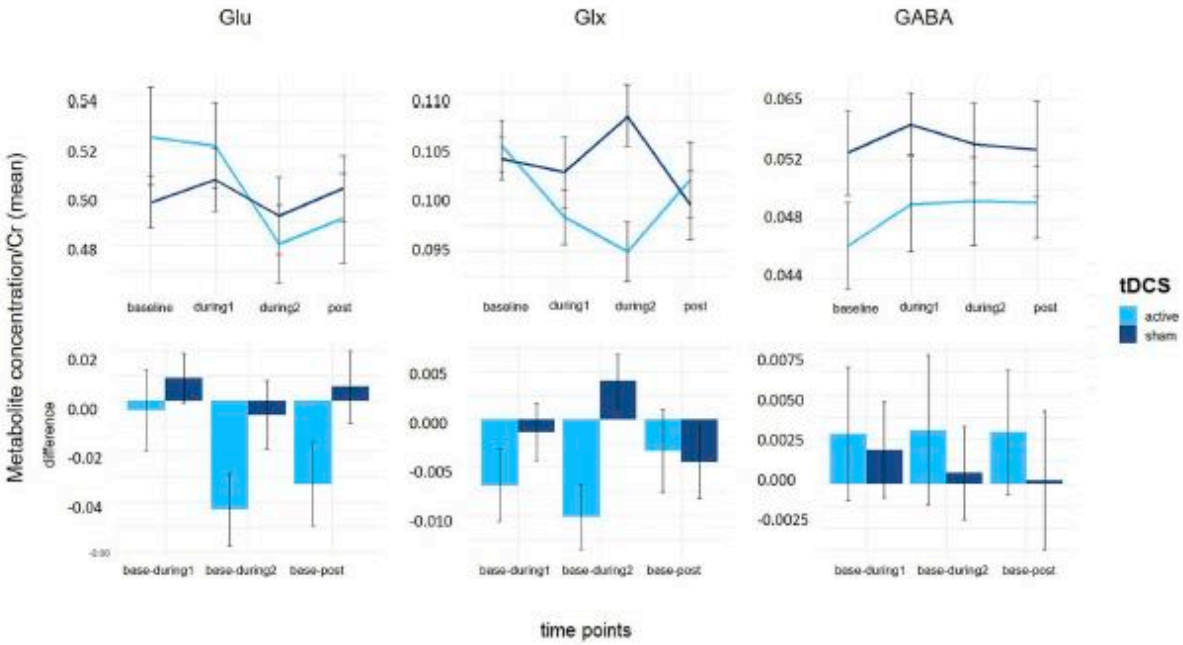

Fig. 2 Glu, Glx and GABA + values from baseline to post stimulation of active and sham tDCS showing a significant reduction of Glu concentrations during active stimulation (see supplemental information, Table 1) and difference plots of metabolite changes to baseline concentrations. Error bars represent standard error of the mean (SEM). Glu, glutamate; Glx, glutamate & glutamine; GABA+, gamma aminobutyric acid (+ macromolecules)

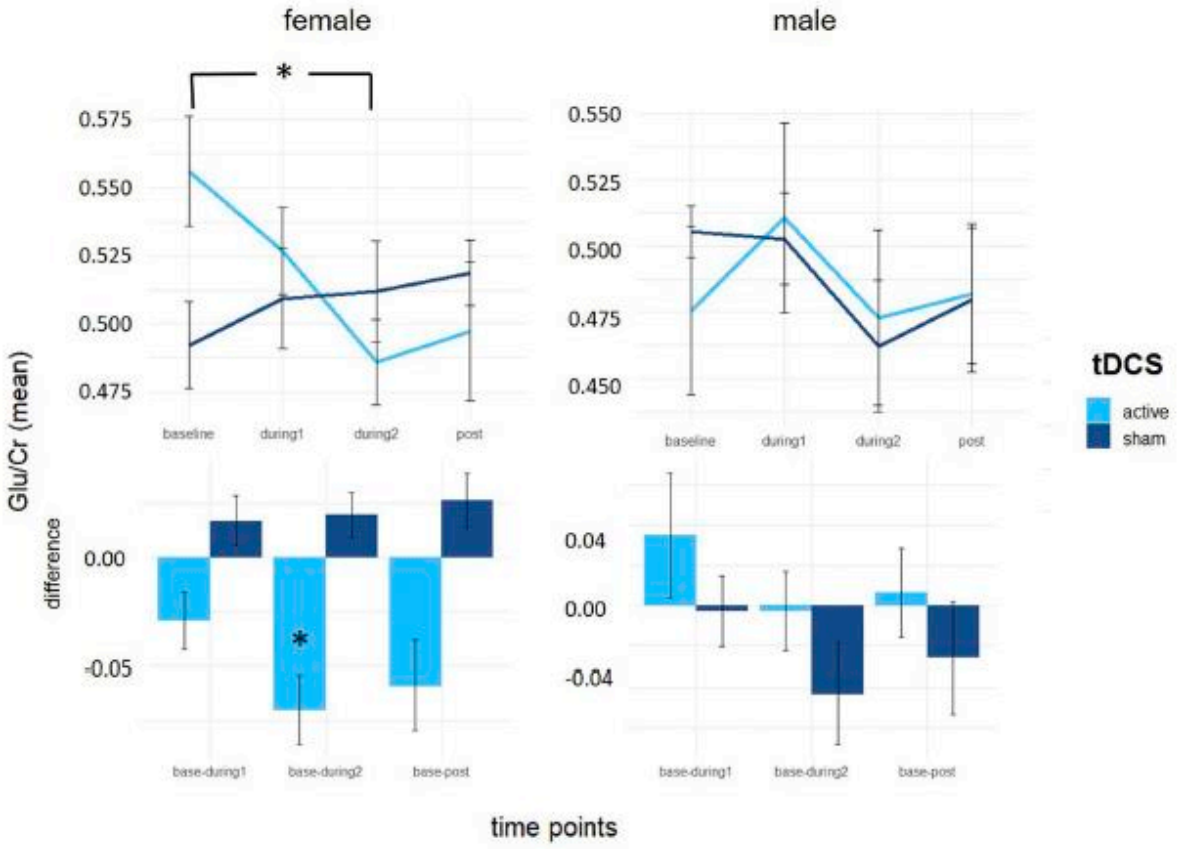

Fig. 3 Glu concentration significantly decreased following bifrontal stimulation under the cathode in female but not in male participants. Error bars represent standard error of the mean (SEM); $*p < 0.05$. Glu, glutamate

other significant interactions for the factors time (GABA: $F_{(1, 140)} = 0.28$, $p = 0.600$; Glx: $F_{(1, 140)} = 1.57$, $p = 0.213$), condition (GABA: $F_{(1, 140)} = 2.78$, $p = 0.100$; Glx: $F_{(1, 140)} = 0.21$, $p = 0.645$) and time*condition (GABA: $F_{(1, 140)} = 0.38$, $p = 0.540$; Glx: $F_{(1, 140)} = 0.09$, $p = 0.764$) were observed, neither for GABA + nor for Glx, even if examining gender separately (see supplemental information, Table 1).

Effects of tDCS on resting-state functional connectivity

After active tDCS, rsfcMRI connectivity between the individual MRS ROI and whole brain showed an increase within the subgenual/subcallosal cortex (at trend level; cluster-corrected at 20 voxels, cluster: $x = 2$; $y = 28$; $z = -22$ (21 voxel); log-p value = 1.0; FDR-corrected, see supplemental information Fig. 3). More detailed results are presented in supplemental information, page 8 and 9.

Relationship between tDCS induced efields and brain metabolite changes

The individually modelled tDCS induced efields showed a high inter-individual variability of their distribution and peak intensities with efields widely spreading between the electrodes within the medial prefrontal cortex (see supplemental information, Fig. 1). Female participants showed significantly more activated voxels (mean = 7620, sd = 1676) compared to men (mean = 3141, sd = 1968) within the MRS ROI ($t_{(3)} = 3.54$, $p = 0.038$). We investigated whether the sex specific differences in tDCS induced Glu concentrations could also be found in subgroups of small and large efield. Therefore, we fit the same model with efield as grouping factor instead of gender. Based on the number of activated voxels of the transformed efield data into volumetric space, we defined groups of "small" and "large" efield. The "large" efield group revealed a trend towards stronger Glu reduction in the active compared to the sham condition ($\beta = 0.02$ [0.00–0.05], $t_{(84)} = 1.69$, $p = 0.096$, $d = 1.23$ [− 0.18 to 2.44]),

while the "small" efield group did not ($\beta = -0.01$ [− 0.04 to 0.02], $t_{(84)} = -0.51$, $p = 0.613$, $d = 0.29$ [− 0.84 to 1.43]) (see supplemental information, Table 3).

Discussion

Our pilot study investigated the effects of a bifrontal tDCS protocol on Glu, Glx and GABA + levels close to the cathode over the right DLPFC. This tDCS protocol uses a standard montage which is commonly applied for the treatment of MD in clinical trials [57–60]. A reduction of Glu levels was observed for the active tDCS condition in a gender-dependent manner; however, no significant effects were found for Glx and GABA + concentration. For rsfcMRI, neither significant changes nor correlations with MRS data were observed except a trend (FDR-corrected, $p < 0.1$) for increased connectivity from the MRS-ROI to the subgenual/subcallosal cortex after active stimulation. Based on a computational model and individual MRI data, efields induced by tDCS were calculated to approximate the real efield distribution as a potential key parameter of individual tDCS dosing. Therefore, the study also aims to conceptually test a comprehensive multimodal neuroimaging approach (i.e., MRS, rsfcMRI and structural MRI based efield modeling), which to our knowledge has previously not been reported for prefrontal tDCS.

Prior studies combining tDCS and MRS have rather focused on M1 and SM1 and only very limited data are available for PFC regions. For motor and sensorimotor regions, a reduction of Glu levels was observed with several montages (i.e., cathode: left M1, anode: contralateral supraorbital ridge or cathode: left SM1, anode: right supraorbital region) [32, 37]. Thus, our results are in line with these previous findings supporting the central hypothesis of divergent effects of tDCS underneath cathode and anode, i.e., an inhibitory or excitatory action, respectively. Accordingly, increased Glu levels were detected in the right intraparietal sulcus after tDCS with the anode over the parietal cortex [45]. Opposite effects of tDCS on GABA levels were observed in prior studies, i.e., a reduction of GABA levels was detected in M1 (anode: left M1, cathode: contralateral supraorbital ridge) [32, 61, 62] and the occipital lobe (anode: occipital-temporal lobe, cathode: contralateral supraorbital ridge) [63].

Very few MRS studies to date have investigated the effects of prefrontal tDCS on brain metabolites. In the left DLPFC, NAA and striatal Glx levels were found to increase during tDCS with the anode over the left DLPFC (cathode over right DLPFC [40]) as well as GABA + concentrations during tDCS with the cathode over this region (anode over right DLPFC) [41]. The current study did not show such a modulation of GABA + levels. However, this negative finding should be interpreted with caution due to the limited sample size and a potentially large beta error.

Changes of Glu levels during and after tDCS as observed in the current study are hypothesized to emerge from a direct effect on neural firing rates and NMDA receptor dependent, long-lasting synaptic potentiation in animal models [64, 65]. However, metabolic changes in distinct MRS ROIs may also be induced transsynaptically through other brain regions functionally connected to the ROI, e.g., tDCS of the DLPFC may modulate metabolite concentrations in medial prefrontal regions [66]. In addition, it is not clear how PFC and motor regions actually differ in their functional response to tDCS with respect to Glu and GABA + levels, since both macro- and microconnectivity as well as regional neurotransmission differ largely across brain regions. In this pilot project, we observed Glu changes during, but not after tDCS as shown in previous studies [29, 34, 37, 38, 61, 67].

The gender-dependent Glu reduction in our study may be discussed in the light of gender-specific differences in metabolite levels (mainly Glu, Glx, GABA + and NAA) as previously reported; however, findings in prior studies were not fully consistent [68–70]. Numerous factors may theoretically contribute to a gender-dependence of tDCS effects on MRS measures, e.g., differences in brain metabolism and structure or hormonal status [71–73]. Though we found significant differences between male and female participants in their response to prefrontal tDCS, we have to consider that this effect may as well be due to the responder vs non-responder distribution in this small sample. Previous studies showed marked inter-individual differences between subjects in terms of their response to tDCS [25, 77]. This is an important factor and should be addressed in future studies by including additional measures (e.g., behavioral or neurophysiological information) which allows to classify responders vs non-responders. Moreover, future studies should survey gender-specific parameters to systematically investigate the role of these factors.

Having observed a marked difference in gender-specific efield intensities, we were interested in the question whether effects of tDCS may be related to individual efield intensities as shown in a previous study [37]. We observed similar differences in Glu concentrations between participants with "small" and "large" efields as defined by below or above the mean value of activated voxels for all subjects. Although these results are preliminary, the relationship between efield intensity and tDCS effects on metabolite concentrations may be relevant and should be further investigated and may be an avenue for establishing dose–response relationships for tDCS. The inter-individual variation of efields beyond the MRS ROI converges with previous evidence of a marked inter-individual variability in terms of efield intensities and their distribution [74, 75], and raises the question at which brain regions bifrontal

montages actually exert their effects. In contrast to our study, Antonenko et al. [37] investigated normal components of efield strength (i.e., calculation of the efield including information about the efield entering or leaving the surface which is only available in SimNIBS 2.1) to address polarity effects of the stimulation, showing peaks of efield intensities at the stimulation site which may provide a superior approach for analyzing target specificity.

Offline rsfcMRI showed an increased network connectivity at a trend level (FDR-corrected, $p<0.1$) from the right DLPFC ROI to the midline/right subgenual region, underscoring the importance of connectivity between both regions for network effects of prefrontal tDCS [2, 25, 76]. However, this was not associated with changes in Glu concentrations. We did not find differences between active and sham tDCS for within-ROI connectivity or ICA networks, though other studies showed this effect [18, 25, 37, 77]. The negative result may be explained by the small sample size and future studies with larger samples should address this issue again. Despite its relevance as a conceptual pilot project, our study has obvious limitations that need to be considered when interpreting the data. As said, the sample size is critically low, which is even more problematic at the subgroup level (defined by gender or efield parameters); however, it is comparable with sample sizes in previous tDCS-MRS studies (e.g., $N=17$ in Hone-Blanchet et al. [40], $N=12$ in Bachtiar et al. [38], $N=20$ in Dwyer et al. [78], $N=24$ in Antonenko et al. [37]). Thus, larger trials are clearly missing in the field. Another issue is that study protocols are critically diverse hampering a direct comparison of our results with previous findings by the large variation in tDCS and imaging methods including different on- and offline designs. In contrast to offline tDCS, MRS protocols, which were applied in the majority of studies [32, 35, 40, 45, 61, 63], combined on- and offline protocols as used here could be very informative regarding dynamic changes of brain metabolites, but were used in very few studies [38, 40, 61]. There is also a marked heterogeneity of tDCS targets and parameters (i.e., stimulation intensity and duration). Stimulation intensity varied between 1 and 2 mA and duration between 10 and 30 min in earlier MRS studies [14, 32, 35, 40, 45, 61, 63]. Here, we applied 2 mA intensity with a bifrontal montage (anode F3, cathode F4), since such protocols were used in previous studies in MDD and schizophrenia [2, 8, 60, 79, 80].

A specific restriction in using MRS for experimental research on tDCS is the key limitation of single voxel MRS, which does not allow to investigate tDCS effects for several regions in parallel. This is particularly critical in bipolar tDCS montages where already two regions are of main interest, and neither electrode can be a priori defined as inactive or reference. As a solution, multi-voxel MRS should be established in future tDCS studies to measure stimulation effects across several brain regions at the same time [81–83].

A final limitation is the investigation of only one stimulation montage, in which specific questions such as the relevance of electrode positions or current directions cannot be addressed [18, 84]. MRS data for ROIs close to anodal [14, 85] as well as cathodal cortical targets [27, 32] are available, and differences in baseline metabolite concentrations are still in the range of known variability [86–88].

Conclusion

To the best of our knowledge, this is the first study investigating prefrontal tDCS in a combined on– and offline approach with the anode over the left DLPFC and the cathode over the right DLPFC using multimodal neuroimaging including MRS and MRI based efield modeling. Our main focus was feasibility and we observed that a standard bifrontal tDCS montage (anode—F3, cathode—F4), as is common in therapeutic trials, led to a reduction of Glu levels in the MRS voxel close to the cathode in female but not in male participants. Computational modelling of tDCS-induced efields based on individual MRI data shows a large inter-individual variation in efield intensity distribution, and preliminary evidence suggests that effects on Glu levels may vary with efield strength. As a conclusion, we support the idea to further develop the combined approach using MRS (ideally multi-voxel MRS), rsfcMRI and individual MRI based efield modeling for investigating the effects of current tDCS protocols on brain metabolites [37].

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Compliance with ethical standards

Conflict of interest ARB was recipient of a CAPES/Alexander von Humboldt fellowship award for experienced researchers and received speakers' fee from Neurocademy GmBH (Munich, Germany). ARB is sponsored by the Brazilian National Scientific Fund (CNPq-1B) and the Academic Productivity Program (PIPA-A) from the Faculdade de Medicina da UNiversidade de São Paulo. ARB is also medical advisor of Flow Neuroscience and has a small equity of Flow. The work of L.B. 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. AT was supported by the Lundbeck foundation (Grant no. R244-2017-196), and the Novo Nordisk foundation (Grant no. NNF14OC0011413). 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

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References

1. Brunoni AR, Shiozawa P, Truong D, Javitt DC (2015) Understanding tDCS effects in schizophrenia : a systematic review of clinical data and an integrated computation modeling analysis Understanding tDCS effects in schizophrenia : a systematic review of clinical data and an integrated computation modeling analysis. Expert Rev Med Devices 2015:383–394. https://doi.org/10.1586/17434440.2014.911082
2. Palm U, Keeser D, Hasan A, Kupka MJ, Blautzik J, Sarubin N et al (2016) Prefrontal transcranial direct current stimulation for treatment of schizophrenia with predominant negative symptoms: a double-blind. Sham-controlled proof-of-concept study. Schizophr Bull 42:1253–1261. https://doi.org/10.1093/schbul/sbw041
3. Lee J, Yoon YB, Wijtenburg SA, Rowland LM, Chen H, Gaston FE et al (2018) Lower glutamate level in temporo-parietal junction may predict a better response to tDCS in schizophrenia. Schizophr Res 2018:1–2. https://doi.org/10.1016/j.schres.2018.05.032
4. Agarwal SM, Shivakumar V, Bose A, Subramaniam A, Nawani H, Chhabra H et al (2013) Transcranial direct current stimulation in schizophrenia. Clin Psychopharmacol Neurosci 11:118
5. Palm U, Hasan A, Keeser D, Falkai P, Padberg F (2013) Transcranial random noise stimulation for the treatment of negative symptoms in schizophrenia. Schizophr Res 146:372–373. https://doi.org/10.1016/j.schres.2013.03.003
6. Medeiros LF, de Souza ICC, Vidor LP, de Souza A, Deitos A, Volz MS et al (2012) Neurobiological effects of transcranial direct current stimulation: a review. Front Psychiatry 2012:3. https://doi.org/10.3389/fpsyt.2012.00110
7. Kalu UG, Sexton CE, Loo CK, Ebmeier KP (2012) Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. Psychol Med. https://doi.org/10.1017/S0033291711003059
8. Brunoni AR, Ferrucci R, Fregni F, Boggio PS, Priori A (2012) Transcranial direct current stimulation for the treatment of major depressive disorder: a summary of preclinical, clinical and translational findings. Prog Neuro-Psychopharmacology Biol Psychiatry 39:9–16. https://doi.org/10.1016/j.pnpbp.2012.05.016
9. Vöröslakos M, Takeuchi Y, Brinyiczki K, Zombori T, Oliva A, Fernández-Ruiz A et al (2018) Direct effects of transcranial electric stimulation on brain circuits in rats and humans. Nat Commun 2018:9. https://doi.org/10.1038/s41467-018-02928-3
10. Bestmann S, Walsh V (2017) Transcranial electrical stimulation. Curr Biol 27:R1258–R1262. https://doi.org/10.1016/j.cub.2017.11.001
11. Bulubas L, Padberg F, Bueno PV, Duran F, Busatto G, Amaro E et al (2019) Antidepressant effects of tDCS are associated with prefrontal gray matter volumes at baseline: evidence from the ELECT-TDCS trial. Brain Stimul 2019:1–8. https://doi.org/10.1016/j.brs.2019.05.006
12. Filmer HL, Ehrhardt SE, Shaw TB, Mattingley JB, Dux PE (2019) The efficacy of transcranial direct current stimulation to prefrontal areas is related to underlying cortical morphology. Neuroimage 196:41–48. https://doi.org/10.1016/j.neuroimage.2019.04.026
13. Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527(Pt 3):633–639
14. Stagg CJ, Nitsche MA (2011) Physiological basis of transcranial direct current stimulation. Neuroscientist 17:37–53. https://doi.org/10.1177/1073858410386614
15. Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001:39–40. https://doi.org/10.1212/wnl.57.10.1899
16. Rahman A, Reato D, Arlotti M, Gasca F, Datta A, Parra LC et al (2013) Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. J Physiol 591:2563–2578. https://doi.org/10.1113/jphysiol.2012.247171
17. Kabakov AY, Muller PA, Pascual-Leone A, Jensen FE, Rotenberg A (2012) Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. J Neurophysiol 107:1881–1889. https://doi.org/10.1152/jn.00715.2011
18. Wörsching J, Padberg F, Ertl-Wagner B, Kumpf U, Kirsch B, Keeser D (2016) Imaging transcranial direct current stimulation (tDCS) of prefrontal cortex—correlation or causality in stimulation-mediated effects? Neurosci Biobehav Rev 69:333–356
19. Nitsche M, Doemkes S (2007) Shaping the effects of transcranial direct current stimulation of the human motor cortex. J Neurophysiol 2007:3109–3117. https://doi.org/10.1152/jn.01312.2006
20. Opitz A, Paulus W, Will S, Antunes A, Thielscher A (2015) Determinants of the electric field during transcranial direct current stimulation. Neuroimage 109:140–150. https://doi.org/10.1016/j.neuroimage.2015.01.033
21. Charvet LE, Kasschau M, Datta A, Knotkova H, Stevens MC, Alonzo A et al (2015) Remotely-supervised transcranial direct current stimulation (tDCS) for clinical trials: guidelines for technology and protocols. Front Syst Neurosci 9:1–13. https://doi.org/10.3389/fnsys.2015.00026
22. Bikson M, Datta A, Rahman A, Scaturro J (2010) Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. Clin Neurol 6:1976–1978. https://doi.org/10.1021/nn300902w.release
23. Purpura DP, McMurtry JG (1965) Intracellular activities and evoked potential changes during polarization of motor cortex. J Neurophysiol 28:166–185
24. Opitz A, Yeagle E, Thielscher A, Schroeder C, Mehta AD, Milham MP (2018) On the importance of precise electrode placement for targeted transcranial electric stimulation. Neuroimage 181:560–567. https://doi.org/10.1016/j.neuroimage.2018.07.027
25. Keeser D, Meindl T, Bor J, Palm U, Pogarell O, Mulert C et al (2011) Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. J Neurosci 31:15284–15293. https://doi.org/10.1523/JNEUROSCI.0542-11.2011
26. Park C-H, Chang WH, Park J-Y, Shin Y-I, Kim ST, Kim Y-H (2013) Transcranial direct current stimulation increases resting state interhemispheric connectivity. Neurosci Lett 539:7–10. https://doi.org/10.1016/j.neulet.2013.01.047

27. O'Shea J, Boudrias MH, Stagg CJ, Bachtiar V, Kischka U, Blicher JU et al (2014) Predicting behavioural response to TDCS in chronic motor stroke. Neuroimage. https://doi.org/10.1016/j.neuroimage.2013.05.096
28. Stagg CJ, Johansen-Berg H, Reis J, Byblow WD, Sehm B (2013) Human neuroscience Studying the effects of transcranial direct-current stimulation in stroke recovery using magnetic resonance imaging. Front Human Neurosci. https://doi.org/10.3389/fnhum.2013.00857
29. Kim S, Stephenson MC, Morris PG, Jackson SR (2014) TDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: a 7T magnetic resonance spectroscopy study. Neuroimage. https://doi.org/10.1016/j.neuroimage.2014.05.070
30. Andrews SC, Hoy KE, Enticott PG, Daskalakis ZJ, Fitzgerald PB (2011) Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. Brain Stimul 4:84–89. https://doi.org/10.1016/j.brs.2010.06.004
31. Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E et al (2005) Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 166:23–30. https://doi.org/10.1007/s00221-005-2334-6
32. Stagg CJ, Best JG, Stephenson MC, O'Shea J, Wylezinska M, Kincses ZT et al (2009) Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. J Neurosci 29:5202–5206. https://doi.org/10.1523/JNEUROSCI.4432-08.2009
33. Stagg CJ, Bachtiar V, Johansen-Berg H (2011) The role of GABA in human motor learning. Curr Biol 21:480–484. https://doi.org/10.1016/j.cub.2011.01.069
34. Antonenko D, Schubert F, Bohm F, Ittermann B, Aydin S, Hayek D et al (2017) tDCS-induced modulation of GABA levels and resting-state functional connectivity in older adults. J Neurosci 2017:0079-17. https://doi.org/10.1523/jneurosci.0079-17.2017
35. Stagg CJ, Bachtiar V, Amadi U, Gudberg CA, Ilie AS, Sampaio-Baptista C et al (2014) Local GABA concentration is related to network-level resting functional connectivity. Elife 2014:1–9. https://doi.org/10.7554/eLife.01465
36. Hunter MA, Coffman BA, Gasparovic C, Calhoun VD, Trumbo MC, Clark VP (2015) Baseline effects of transcranial direct current stimulation on glutamatergic neurotransmission and large-scale network connectivity. Brain Res 33:395–401. https://doi.org/10.1038/nbt.3121.ChIP-nexus
37. Antonenko D, Thielscher A, Bicalho G (2019) Brain Stimulation Towards precise brain stimulation : is electric field simulation related to neuromodulation ? Brain Stimul. https://doi.org/10.1016/j.brs.2019.03.072
38. Bachtiar V, Johnstone A, Berrington A, Lemke C, Johansen-Berg H, Emir U et al (2018) Modulating regional motor cortical excitability with noninvasive brain stimulation results in neurochemical changes in bilateral motor cortices. J Neurosci 38:7327–7336. https://doi.org/10.1523/JNEUROSCI.2853-17.2018
39. Kim JH, Kim DW, Chang WH, Kim YH, Kim K, Im CH (2014) Inconsistent outcomes of transcranial direct current stimulation may originate from anatomical differences among individuals: electric field simulation using individual MRI data. Neurosci Lett 564:6–10. https://doi.org/10.1016/j.neulet.2014.01.054
40. Hone-Blanchet A, Edden RA, Fecteau S (2016) Online effects of transcranial direct current stimulation in real time on human prefrontal and striatal metabolites. Biol Psychiatry 80:432–438. https://doi.org/10.1016/j.biopsych.2015.11.008
41. Dickler M, Lenglos C, Renauld E, Ferland F, Edden RA, Leblond J et al (2018) Online effects of transcranial direct current stimulation on prefrontal metabolites in gambling disorder. Neuropharmacology 131:51–57. https://doi.org/10.1016/j.neuropharm.2017.12.002
42. Oldfield RC (1971) the assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:1–17
43. Watson D, Clark LA (1999) The PANAS-X: manual for the positive and negative affect schedule—expanded form. Iowa Res Online 277:1–27. https://doi.org/10.1111/j.1742-4658.2010.07754.x
44. Lange T, Zaitsev M, Buechert M (2011) Correction of frequency drifts induced by gradient heating in 1H spectra using interleaved reference spectroscopy. J Magn Reson Imaging 33:748–754. https://doi.org/10.1002/jmri.22471
45. Clark VP, Coffman BA, Trumbo MC, Gasparovic C (2011) Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: a 1H magnetic resonance spectroscopy study. Neurosci Lett 500:67–71. https://doi.org/10.1016/j.neulet.2011.05.244
46. Palm U, Feichtner KB, Hasan A, Gauglitz G, Langguth B, Nitsche MA et al (2014) The role of contact media at the skin-electrode interface during transcranial direct current stimulation (tDCS). Brain Stimul 7:762–764. https://doi.org/10.1016/j.brs.2014.06.006
47. Mullins PG, McGonigle DJ, O'Gorman RL, Puts NA, Evans CJ, Edden RA (2014) Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. Neuroimage 1:43–52. https://doi.org/10.1021/nl061786n.Core-Shell
48. Provencher SW (1993) Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 30:672–679. https://doi.org/10.1002/mrm.1910300604
49. Zhang Y, Brady M, Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 20:45–57
50. Quadrelli S, Mountford C, Ramadan S (2016) Hitchhiker's guide to voxel segmentation for partial volume correction of in vivo magnetic resonance spectroscopy. Magn Reson Insights 9:MRI-S32903
51. Karali T, Kirsch V, Goerigk S, Ertl-Wagner B, Padberg FKD (2019) NAMNIs: neuromodulation and multimodal neuroimaging scripts. In: 25th Human Brain Mapping Congress, Rome, Italy, p 1
52. Karali T, Kirsch V, Padberg F, Ertl-Wagner BKD (2017) LMU scripts: ready-made HCP-applicaple pipeline for structural and functonal data analysis. In: 23th Human Brain Mapping Congress, Vancouver, Canada, p 1
53. Thielscher A, Antunes A, Saturnino GB (2015) Field modeling for transcranial magnetic stimulation: a useful tool to understand the physiological effects of TMS? Proc Annu Int Conf IEEE Eng Med Biol Soc EMBS 2015:222–225. https://doi.org/10.1109/embc.2015.7318340
54. Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis: I. Segmentation and surface reconstruction. Neuroimage 9:179–194
55. Fischl B (2012) FreeSurfer. Neuroimage 62:774–781
56. Windhoff M, Opitz A, Thielscher A (2013) Electric field calculations in brain stimulation based on finite elements: an optimized processing pipeline for the generation and usage of accurate individual head models. Hum Brain Mapp 34:923–935. https://doi.org/10.1002/hbm.21479
57. Blumberger D, Tran L, Fitzgerald P, Hoy KB, Daskalakis ZJ (2012) A randomized double-blind sham-controlled study of transcranial direct current stimulation for treatment-resistant major depression. Front Psychiatry 3:74
58. Brunoni AR, Valiengo L, Baccaro A, Zanão TA, de Oliveira JF, Goulart A et al (2013) The sertraline vs electrical current therapy

for treating depression clinical study. JAMA Psychiatry 70:383. https://doi.org/10.1001/2013.jamapsychiatry.32
59. Brunoni AR, Zanao TA, Vanderhasselt M, Valiengo L, de Oliveira JF, Boggio PS et al (2014) Enhancement of affective processing induced by bifrontal transcranial direct current stimulation in patients with major depression. Neuromodul Technol Neural Interface 17:138–142
60. Padberg F, Kumpf U, Mansmann U, Palm U, Plewnia C, Langguth B et al (2017) Prefrontal transcranial direct current stimulation (tDCS) as treatment for major depression: study design and methodology of a multicenter triple blind randomized placebo controlled trial (DepressionDC). Eur Arch Psychiatry Clin Neurosci. https://doi.org/10.1007/s00406-017-0769-y
61. Bachtiar V, Near J, Johansen-Berg H, Stagg CJ (2015) Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation. Elife 4:1–9. https://doi.org/10.7554/eLife.08789
62. Stagg CJ, Jayaram G, Pastor D, Kincses ZT, Matthews PM, Johansen-Berg H (2011) Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning. Neuropsychologia 49:800–804. https://doi.org/10.1016/j.neuropsychologia.2011.02.009
63. Barron HC, Vogels TP, Emir UE, Makin TR, O'Shea J, Clare S et al (2016) Unmasking latent inhibitory connections in human cortex to reveal dormant cortical memories. Neuron 90:191–203. https://doi.org/10.1016/j.neuron.2016.02.031
64. Andrew RB, Guillaume L, Hayley B, Andrew M, Stavros Z, Eberhard EF (2019) Cortical network mechanisms of anodal and cathodal transcranial direct current stimulation in awake primates. BioRxiv 1028725:1–34
65. Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG et al (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 66:198–204. https://doi.org/10.1016/j.neuron.2010.03.035
66. Menon V (2015) Large-scale functional brain organization, vol 2. Elsevier Inc. https://doi.org/10.1016/b978-0-12-397025-1.00024-5
67. Hunter MA, Coffman BA, Gasparovic C, Calhoun VD, Trumbo MC, Clark VP (2015) Baseline effects of transcranial direct current stimulation on glutamatergic neurotransmission and large-scale network connectivity. Brain Res 1594:92–107. https://doi.org/10.1016/j.brainres.2014.09.066
68. Grachev ID, Apkarian AV (2000) Chemical heterogeneity of the living human brain: a proton MR spectroscopy study on the effects of sex, age, and brain region. Neuroimage 11:554–563. https://doi.org/10.1006/nimg.2000.0557
69. Maudsley AA, Domenig C, Govind V, Darkazanli A, Studholme C, Arheart K et al (2009) Mapping of brain metabolite distributions by volumetric proton MR spectroscopic imaging (MRSI). Magn Reson Med 61:548–559. https://doi.org/10.1002/mrm.21875
70. Gorman RL, Michels L, Edden RA, Murdoch JB, Martin E (2011) In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. J Magn Reson Imaging 33:1262–1267. https://doi.org/10.1002/jmri.22520
71. Epperson CN, Haga K, Mason GF, Sellers E, Gueorguieva R, Zhang W et al (2002) Cortical γ-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: a proton magnetic resonance spectroscopy study. Arch Gen Psychiatry 59:851–858
72. Harada M, Kubo H, Nose A, Nishitani H, Matsuda T (2011) Measurement of variation in the human cerebral GABA level by in vivo MEGA-editing proton MR spectroscopy using a clinical 3 T instrument and its dependence on brain region and the female menstrual cycle. Hum Brain Mapp 32:828–833. https://doi.org/10.1002/hbm.21086
73. Russell MJ, Goodman TA, Visse JM, Beckett L, Russell MJ (2017) Sex and electrode configuration in transcranial electrical stimulation. Front Psychiatry 8:1–9. https://doi.org/10.3389/fpsyt.2017.00147
74. Seibt O, Brunoni AR, Huang Y, Bikson M (2015) The pursuit of DLPFC: non-neuronavigated methods to target the left dorsolateral pre-frontal cortex with symmetric bicephalic transcranial direct current stimulation (tDCS). Brain Stimul Basic Transl Clin Res Neuromodulation 8:590–602. https://doi.org/10.1016/j.brs.2015.01.401
75. De Witte S, Klooster D, Dedoncker J, Duprat R, Remue J, Baeken C (2018) Left prefrontal neuronavigated electrode localization in tDCS: 10–20 EEG system versus MRI-guided neuronavigation. Psychiatry Res Neuroimaging 274:1–6. https://doi.org/10.1016/j.pscychresns.2018.02.001
76. Abend R, Sarel R, Gonen T, Jalon I, Vaisvaser S, Bar-Haim Y et al (2018) Modulating emotional experience using electrical stimulation of the medial-prefrontal cortex: a preliminary tDCS-fMRI Study. Neuromodul Technol Neural Interface 22:884–893
77. Peña-Gómez C, Sala-Lonch R, Junqué C, Clemente I, Vidal D, Bargalló N et al (2012) Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI. Brain Stimul 5:252–263. https://doi.org/10.1016/j.brs.2011.08.006.Pe
78. Dwyer GE, Craven AR, Hirnstein M, Kompus K, Assmus J, Ersland L et al (2019) No effects of anodal tDCS on local GABA and GLx levels in the left posterior superior temporal gyrus. Front Neurol 10:1–10. https://doi.org/10.3389/fneur.2018.01145
79. Brunoni AR, Nitsche MA, Bolognini N, Bikson M, Wagner T, Merabet L et al (2013) Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stimul 5:175–195. https://doi.org/10.1016/j.brs.2011.03.002.Clinical
80. Dondé C, Amad A, Nieto I, Brunoni AR, Neufeld NH, Bellivier F et al (2017) Transcranial direct-current stimulation (tDCS) for bipolar depression: a systematic review and meta-analysis. Prog Neuro-Psychopharmacol Biol Psychiatry 78:123–131. https://doi.org/10.1016/j.pnpbp.2017.05.021
81. Spurny B, Seiger R, Moser P, Vanicek T, Reed MB, Heckova E et al (2020) Hippocampal GABA levels correlate with retrieval performance in an associative learning paradigm. Neuroimage 204:116244. https://doi.org/10.1016/j.neuroimage.2019.116244
82. Spurny B, Heckova E, Seiger R, Moser P, Klöbl M, Vanicek T et al (2019) Automated ROI-based labeling for multi-voxel magnetic resonance spectroscopy data using FreeSurfer. Front Mol Neurosci 12:28
83. Parmar H, Lim TCC, Yin H, Chua V, Khin L-W, Raidy T et al (2005) Multi-voxel MR spectroscopic imaging of the brain: utility in clinical setting-initial results. Eur J Radiol 55:401–408. https://doi.org/10.1016/j.ejrad.2005.01.002
84. Wörsching J, Padberg F, Goerigk S, Heinz I, Bauer C, Plewnia C et al (2018) Testing assumptions on prefrontal transcranial direct current stimulation: comparison of electrode montages using multimodal fMRI. Brain Stimul 11:998–1007. https://doi.org/10.1016/j.brs.2018.05.001
85. Knechtel L, Schall U, Cooper G, Ramadan S, Stanwell P, Jolly T et al (2014) Transcranial direct current stimulation of prefrontal cortex: an auditory event-related potential and proton magnetic resonance spectroscopy study. Neurol Psychiatry Brain Res 20:96–101. https://doi.org/10.1016/j.npbr.2014.06.001
86. Kirov II, George IC, Jayawickrama N, Babb JS, Perry NN, Gonen O (2012) Longitudinal inter- and intra-individual human brain metabolic quantification over 3 years with proton MR spectroscopy at 3 T. Magn Reson Med 67:27–33. https://doi.org/10.1002/mrm.23001

87. Schirmer T, Auer DP (2000) On the reliability of quantitative clinical magnetic resonance spectroscopy of the human brain—Schirmer—2000—NMR in Biomedicine—Wiley Online Library. NMR Biomed 2000:28–36
88. Bogner W, Gruber S, Doelken M, Stadlbauer A, Ganslandt O, Boettcher U et al (2010) In vivo quantification of intracerebral GABA by single-voxel 1H-MRS—how reproducible are the results? Eur J Radiol 73:526–531. https://doi.org/10.1016/j.ejrad.2009.01.014

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11.2. Paper 2 - Association between tDCS computational modeling and clinical outcomes in depression: data from the ELECT-TDCS trial

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ORIGINAL PAPER

Association between tDCS computational modeling and clinical outcomes in depression: data from the ELECT-TDCS trial

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Abstract
Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation intervention investigated for the treatment of depression. Clinical results have been heterogeneous, partly due to the variability of electric field (EF) strength in the brain owing to interindividual differences in head anatomy. Therefore, we investigated whether EF strength was correlated with behavioral changes in 16 depressed patients using simulated electric fields in real patient data from a controlled clinical trial. We hypothesized that EF strength in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC), brain regions implicated in depression pathophysiology, would be associated with changes in depression, mood and anxiety scores. SimNIBS were used to simulate individual electric fields based on the MRI structural T1-weighted brain scans of depressed subjects. Linear regression models showed, at the end of the acute treatment phase, that simulated EF strength was inversely associated with negative affect in the bilateral ACC (left: $\beta = -160.463$, CI [−291.541, −29.385], $p = 0.021$; right: $\beta = -189.194$, CI [−289.479, −88.910], $p = 0.001$) and DLPFC (left: $\beta = -93.210$, CI [−154.960, −31.461], $p = 0.006$; right: $\beta = -82.564$, CI [−142.867, −22.262], $p = 0.011$) and with depression scores in the left ACC ($\beta = -156.91$, CI [−298.51, −15.30], $p = 0.033$). No association between positive affect or anxiety scores, and simulated EF strength in the investigated brain regions was found. To conclude, our findings show preliminary evidence that EF strength simulations might be associated with further behavioral changes in depressed patients, unveiling a potential mechanism of action for tDCS. Further studies should investigate whether individualization of EF strength in key brain regions impact clinical response.

Keywords Transcranial direct current stimulation · Electric field modeling · tDCS modeling · Major depressive disorder · Depression · SimNIBS · General linear models

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Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation intervention that applies direct electric currents to modulate cortical excitability according to the parameters of stimulation [1]. The technique has been used in several neuropsychiatric conditions, most notably in major depressive disorder (MDD) [2, 3], in which electrodes are usually positioned over the dorsolateral prefrontal cortex (DLPFC), which tends to be hypoactive in depression [4, 5].

The clinical efficacy of tDCS for treatment of MDD has been investigated in several randomized clinical trials [6–8]. Although meta-analyses have shown active tDCS to be superior to sham for response, remission and depression improvement [3, 9], clinical results have been moderate and heterogeneous [3, 7, 10]. Recently, it has been hypothesized [11] that variations in the electrical current on the targeted brain region might account for such heterogeneity [12–15]. Although invasive estimation of such currents is not feasible in most cases, recent technical developments permit, using brain imaging, the simulation of the strength and spatial distribution of the electrical current injected into the brain, as well as to assess individual differences in strength and distribution of such currents owing to one's head and brain anatomy [16]. Neurophysiological studies have shown that tDCS-induced electric fields (EFs) in the brain of healthy volunteers correlate with changes in gamma-aminobutyric acid (GABA), measured by magnetic resonance spectroscopy [17], and with the resting motor threshold, measured by transcranial magnetic stimulation [18]. Nonetheless, individual EFs have not been systematically investigated as a predictor of tDCS clinical effects.

Given these initial results, we hypothesized that tDCS-induced EF strength (measured in Vm^{-1} and representing the intensity of the electric field distributed over a given anatomical region) in brain regions of interest (ROIs) would be associated with behavioral changes in depressed patients within a controlled clinical trial previously performed by our group [7]. The selected ROIs were the DLPFC and the anterior cingulate cortex (ACC) bilaterally, since these regions are structurally implicated in MDD [19–21] and are targeted by brain stimulation interventions [22, 23]. For instance, a recent meta-analysis quantifying structural brain changes associated with MDD showed that gray matter reduction in the ACC to be one of the most robust findings between studies [19]. Additionally, clinical improvement has been associated with increase in gray matter volume of the DLPFC [24], and connectivity between these two regions predicted antidepressant response to rTMS [25]. Our primary outcome variable was changes in the Hamilton depression rating scale (HDRS-17) [26]. As secondary outcomes, we explored changes in affective scores (indexed by the Positive and Negative Affect Scale (PANAS) [27]) and in trait or state anxiety (measured by the State–Trait Anxiety Inventory (STAI) [28]), as previous studies have shown that prefrontal tDCS modulates affective and anxiety processing [29–31].

Methods

Study design

Our Escitalopram versus Electrical Current Therapy for Treating Depression Clinical Study (ELECT-TDCS) trial, a non-inferiority triple-arm study, randomized patients into three groups: sham-tDCS/placebo-pill (placebo group), sham-tDCS/escitalopram (escitalopram group) and active-tDCS/placebo-pill (tDCS group) [32]. The original study compared 22, 2-mA, 30-min tDCS sessions (1 × 1 tDCS-CT, SoterixMedical, New York, NY) applied in a 10-week period, with 15 sessions applied consecutively once a day (expect for weekends), and 7 more sessions applied once per week until the study endpoint at week 10, to a first-line antidepressant treatment (escitalopram 20 mg/day), and found it to be superior to placebo and not non-inferior to escitalopram [7]. Our study was registered in ClinicalTrials.gov (NCT01894815).

Participants

We recruited patients aged 18–75 years who were diagnosed with major depressive disorder during an acute depressive episode per DSM-5 criteria (Diagnostic and Statistical Manual of Mental Disorders, 5th edition). The main inclusion criteria were: (1) ≥ 17 points on HDRS-17; (2) baseline low risk of suicide; (3) at least 8 years of schooling; (4) and adherence to study protocol. Exclusion criteria were other neuropsychiatric disorders (except for anxiety disorders as a comorbidity), pregnancy, specific contraindications to tDCS (e.g., cranial plates), current or previous escitalopram use, and previous or concomitant participation in other tDCS trials. Patients under antidepressant drug therapy underwent drug washout. Benzodiazepines were allowed up to 20 mg/day diazepam-equivalent.

In this ancillary study, all participants received active tDCS according to the protocol described above. As they were part of a placebo-controlled study, these patients also received placebo pill, as they were not aware to which study group they were assigned to.

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 $1 \times 1 \times 1$ 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.

tDCS modeling

SimNIBS (v3.1, Danish Research Centre for Magnetic Resonance, Copenhagen, Denmark) [33] was used for tDCS modeling. It is a free and open-source software package for the simulation of tDCS-induced electric field in the individual brain. It allows for realistic calculations using the finite element method (FEM), and integrates free software for neuroimaging, computer graphics and FEM calculations into one coherent pipeline. TDCS modeling was done using T1-weighted anatomical images of each subject to reconstruct a high-resolution head model of that individual using the SimNIBS pipeline. We manually verified each segmentation to check for possible errors in the established boundaries between tissues, and no subjects were excluded in the process. The estimated EF distribution in one's brain is obtained by placing simulated electrodes on the head model and setting simulated electric current intensity according to the stimulation protocol used in the clinical trial.

Parameters of the tDCS modeling were set according to the ELECT-TDCS protocol [32]: current intensity was set to 2 mA and electrode sizes of 5×5 cm. For electrode positioning, we simulated the F5 and F6 areas, according to the EEG 10–20 system, for the anode and cathode, respectively, targeting the left and right DLPFC. The DLPFC has a complex cortical structure that is highly variable between individuals and, despite its widespread use as a target, there remains a lack of consensus for how this region should be best localised. Although in the original study we used the Omni-Lateral Electrode (OLE) system, the F5-F6 positioning was employed since it can be directly implemented in SimNIBS and considering that the simulated EF strength in the brain for both montages is similar [5].

Electric field values

According to our hypotheses, a ROI-based approach was used to define the DLPFC and ACC, brain areas in which simulated EF strength was evaluated. To define the DLPFC, we used the Sallet et al. atlas [34], which provides a parcellation of the dorsal frontal cortex based on functional and tractography data in a cross-species comparison of both humans and primates, and divides it into 10 subregions (clusters) also identified by their corresponding Brodmann areas (BAs). This approach was used in a previous study by our group correlating structural DLPFC changes with tDCS antidepressant response [15]. This atlas was chosen as it allows to identify ROIs in the proximity of the DLPFC area, while incorporating anatomical and functional data. The Sallet et al. atlas also incorporates motor and premotor areas, but they were not included in our analysis as they were not part of our hypotheses. We defined the DLPFC by Sallet et al. clusters 3, 4, 5, 6, 7, 8 and 10, which correspond to Brodmann Areas (BAs) 8, 9, 10 and 46. For the ACC ROI, we used the parcellation of the Brainnetome atlas [35], a whole-brain, multimodal parcellation atlas based on structural magnetic resonance imaging (MRI), diffusion tensor imaging and resting-state fMRI connectivity.

As significant effects in these hypothesis-driven regions were observed for HDRS-17 and PANAS, and as performed in our previous study [15], we analyzed subregions of the DLPFC and the ACC in an exploratory manner so as to identify subregions driving these effects. In the DLPFC, we investigated 7 clusters according to Sallet et al.: cluster 3 (corresponds to BA 9), cluster 4 (BA 10), cluster 5 (BA 9/46D), cluster 6 (BA 9/46V), cluster 7 (BA 46), cluster 8 (BA 8A) and cluster 10 (BA 8B). In the ACC, we further explored the subgenual ACC (sgACC) and pregenual ACC (pgACC) using the "A32sg" and "A32p" ROIs from the Brainnetome atlas, because of their particular roles in predicting antidepressant response specifically in the rTMS literature [36–38].

Statistical analysis

We used Python 3.7.0 [39], Spyder 3.3.6 and the StatsModels library [40] to perform a regression analysis to explore in which brain regions simulated EF strength was associated with depression improvement. Statistical results were considered significant under a p threshold of 0.05.

EF strength was obtained as the average EF strength within the ROI (Emean), calculated by summing the simulated EF in each voxel and dividing it by the number of voxels. We used linear regression models, adjusted for gender and age, with changes in the HDRS-17, STAI, and PANAS scales as dependent variables and Emean at the ROIs as independent variables. We evaluated whether simulated EF would be correlated with changes immediately after the end of the acute treatment phase (i.e., 15 sessions) and at study endpoint (i.e., week 10). These two time frames were also used in main and ancillary analyses of ELECT-TDCS [7, 15, 41, 42] and reflect timepoints in which acute and long-lasting tDCS effects are usually observed [3]. These

analyses were not corrected for multiple comparisons since they were hypothesis driven. Also, five patients did not complete the study, and models for study endpoint include only trial completers.

Additionally, we investigated the subregions of the DLPFC and ACC in an exploratory manner using the same models, which produced another 9 models for each hemisphere per outcome. For this exploratory analysis, the correction for multiple comparisons was done using the Bonferroni correction for each outcome individually, in both hemispheres. For each outcome variable, a total number of 18 tests were performed (9 subregions—7 in the DLPFC and 2 in the ACC—in two hemispheres); therefore, the correction was performed using a threshold $\alpha = 0.05/18$.

Results

Out of the original sample, only 68 patients received MRI at baseline. The most important reasons for the absence collection of MRI were (1) the delayed start of the MRI collection that initiated only after 30% of the sample had already been recruited, (2) patient refusal, and (3) lack of MRI slots available. Other reasons included MRI contraindications and technical reasons. Moreover, MRI scans of 15 patients were excluded after an initial quality check (absence of T1 anatomical sequences, abnormal anatomical findings, and poor quality due to head motion). The remaining 53 scans were divided into three groups: active tDCS (16 patients), escitalopram (16 patients) and placebo (21 patients). Here, we performed simulations in the 16 patients who had undergone tDCS. Their characteristics are shown in Table 1, and the individual simulated EF strength distribution in Fig. 1. It can be visually depicted that such distribution is notably different between participants (Table 2).

Table 1 Patient group characteristics

	tDCS
Gender (male/female)	7/9
Age (mean ± SD), years	42.8 ± 10.9
HDRS[a]	
Baseline	21.6 ± 3.9
Week 3	14.3 ± 6.1
Week 10	13.8 ± 10.1
PA	
Baseline	17.1 ± 6.3
Week 3	21.5 ± 9.1
Week 10	25.0 ± 11.4
NA	
Baseline	29.6 ± 9.3
Week 3	26.4 ± 11.4
Week 10	21.8 ± 10.1
STAI—state	
Baseline	54.6 ± 10.1
Week 3	51.6 ± 13.4
Week 10	48.3 ± 16.4
STAI—trait	
Baseline	65.6 ± 6.3
Week 3	60.6 ± 11.5
Week 10	54.3 ± 17.7
Emean (V/m)	
DFLP—left	0.317 ± 0.055
DLFPC—right	0.332 ± 0.059
ACC—left	0.153 ± 0.030
ACC—right	0.145 ± 0.030

Distribution of characteristics, clinical outcomes, and mean electric field strength of the four main analyzed regions. Values are displayed as mean ± standard deviation

HDRS Hamilton depression rating scale, *PA* positive affect, *NA* negative affect, *STAI* state–trait anxiety inventory, *Emean* Mean electric field strength inside ROI, *DLPFC* dorsolateral prefrontal cortex, *ACC* anterior cingulate cortex

[a]Scores on the 17-item Hamilton depression rating scale (0–52, the higher the more severely depressed)

Changes in depression scores

For the acute treatment phase, HDRS-17 change was significantly correlated with Emean in the left ACC ($\beta = -156.91$, CI [−298.51, −15.30], $p = 0.033$) (Table 3). No other significant correlation was found for this time frame or at study endpoint (Tables 2 and 3).

Changes in positive and negative affect

For the acute treatment phase, negative affect reduction was associated to Emean in the left DLPFC ($\beta = -93.21$, CI [−154.96, −31.46], $p = 0.006$) (Table 2), right DLPFC ($\beta = -82.56$, CI [−142.87, −22.62], $p = 0.011$) (Table 2), left ACC ($\beta = -160.46$, CI [−291.54, −29.38], $p = 0.021$) (Table 3), right ACC ($\beta = -189.19$, CI [−289.48, −88.91], $p = 0.001$) (Table 3) (Fig. 2). No significant correlation was found for the 10-week period (Tables 2 and 3).

No significant correlations were found between change in positive affect and EF strength (Table 2 and 3).

STAI improvement

No significant correlations were found between trait and state anxiety changes and simulated EF strength for any of the explored clusters in any time frame (Table 2 and 3).

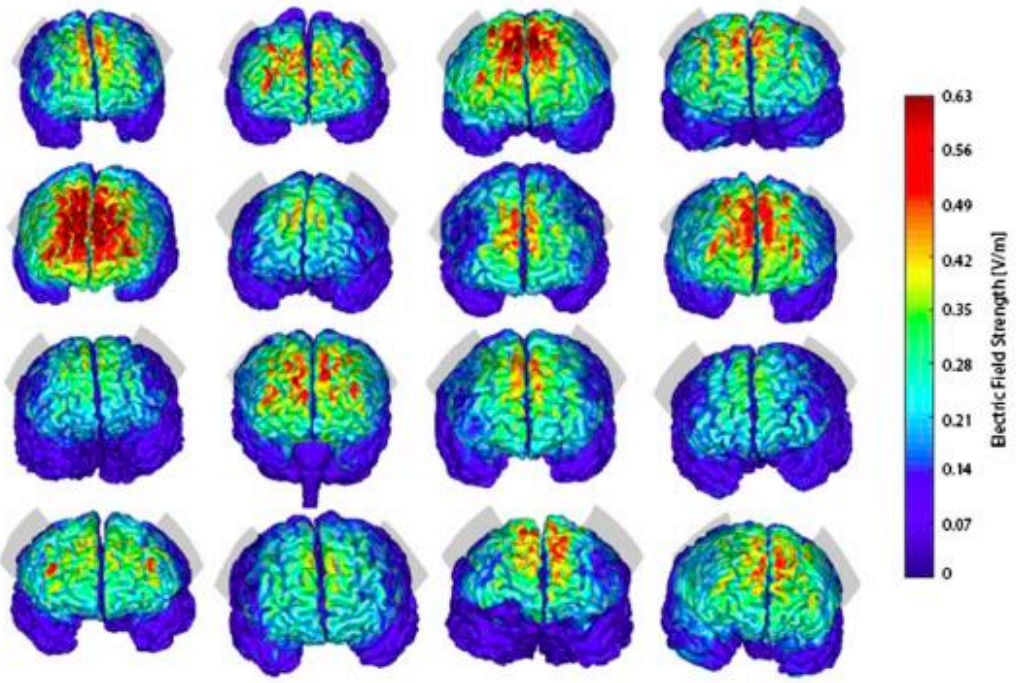

Fig. 1 Individual tDCS-induced simulated brain electrical field strength distribution. Illustrates the location of the stimulation electrodes (EEG-based F5-F6 location) and the distribution of the electric field on all 16 subjects of the study. Peak electric field strength (measured in V/m) occurs in intermediate regions between stimulation electrodes and is notably different between participants

Table 2 Linear models for the DLPFC

Linear models for DLPFC	Left hemisphere			Right hemisphere		
	Beta	95% CI	*p*	Beta	95% CI	*p*
HDRS (week 3)	− 48.25	[− 131.74, 35.25]	0.232	− 45.34	[− 123.32, 32.64]	0.229
HDRS (week 10)	24.23	[− 174.97, 223.42]	0.782	19.40	[− 169.51, 208.31]	0.815
PA (week 3)	42.50	[− 33.92, 118.91]	0.249	41.22	[− 29.86, 112.30]	0.230
PA (week 10)	− 43.92	[− 185.99, 98.15]	0.489	− 45.02	[− 178.65, 88.60]	0.452
NA (week 3)	**− 93.21**	**[− 154.96, − 31.46]**	**0.006**	**− 82.56**	**[− 142.87, − 22.62]**	**0.011**
NA (week 10)	17.51	[− 133.60, 168.63]	0.792	23.52	[− 118.78, 165.82]	0.708
STAI—state (week 3)	− 12.21	[− 112.61, 88.19]	0.795	2.73	[− 91.36, 96.83]	0.951
STAI—state (week 10)	43.78	[− 177.29, 264.86]	0.654	73.82	[− 128.24, 275.88]	0.416
STAI—trait (week 3)	− 3.72	[− 117.92, 110.48]	0.945	− 6.73	[− 113.40, 99.94]	0.893
STAI—trait (week 10)	30.84	[− 208.47, 270.14]	0.769	49.3	[− 174.47, 273.07]	0.618

Results of the linear models obtained for the dorsolateral prefrontal cortex in both brain hemispheres. Table values marked in bold indicate regions in which the correlation was found to be significant. Beta is the linear coefficient of the relation between mean electric field strength and negative affect change. 95% CI is the confidence interval for the linear coefficient with a 95% confidence level. *p* is the *p*-value of the linear model and was not corrected for multiple comparisons since these analyses were hypothesis driven

HDRS Hamilton depression rating scale, *PA* positive affect, *NA* negative affect, *STAI* state–trait anxiety inventory, *DLPFC* dorsolateral prefrontal cortex

Exploratory analysis in subregions of DLPFC and ACC

Post-acute treatment phase changes in negative affect were significantly associated with Emean after Bonferroni correction in right BA 9/46D ($\beta = -83.87$, CI [− 130.02, − 37.71], $p = 0.002$, $p_{corr} = 0.034$), right pgACC ($\beta = -159.92$, CI [− 245.51, − 74.32], $p = 0.002$, $p_{corr} = 0.028$), and left BA 9 ($\beta = -49.38$, CI [− 74.22, − 24.53], $p = 0.001$, $p_{corr} = 0.018$). No other significant

Table 3 Linear models for the ACC

Linear models for ACC	Left			Right		
	Beta	95% CI	*p*	Beta	95% CI	*p*
HDRS (week 3)	**− 156.91**	**[− 298.51, − 15.30]**	**0.033**	− 110.46	[− 257.21, 36.29]	0.127
HDRS (week 10)	− 55.89	[− 398.49, 286.72]	0.711	14.24	[− 338.41, 366.89]	0.927
PA (week 3)	101.91	[− 41.73, 245.54]	0.148	86.84	[− 50.67, 224.34]	0.194
PA (week 10)	− 1.61	[− 256.31, 253.08]	0.988	− 57.70	[− 312.12, 196.71]	0.608
NA (week 3)	**− 160.46**	**[− 291.54, − 29.38]**	**0.021**	**− 189.19**	**[− 289.48, − 88.91]**	**0.001**
NA (week 10)	− 1.18	[263.70, 261.35]	0.992	30.73	[− 235.43, 296.88]	0.792
STAI—state (week 3)	− 30.98	[− 225.64, 163.69]	0.735	− 74.34	[− 252.33, 103.66]	0.381
STAI—state (week 10)	10.11	[− 377.74, 397.95]	0.953	41.68	[− 351.86, 435.33]	0.809
STAI—trait (week 3)	− 54.18	[− 273.47, 165.11]	0.600	− 34.63	[− 242.26, 172.99]	0.723
STAI—trait (week 10)	− 63.78	[− 476.09, 348.55]	0.725	− 4.53	[− 428.75, 419.69]	0.980

Results of the linear models obtained for the anterior cingulate cortex in both brain hemispheres. Table values marked in bold indicate regions in which the correlation was found to be significant. Beta is the linear coefficient of the relation between mean electric field strength and negative affect change. 95% CI is the confidence interval for the linear coefficient with a 95% confidence level. *p* is the *p*-value of the linear model and was not corrected for multiple comparisons since these analyses were hypothesis driven

HDRS Hamilton depression rating scale, *PA* positive affect, *NA* negative affect, *STAI* state–trait anxiety inventory, *ACC* anterior cingulate cortex

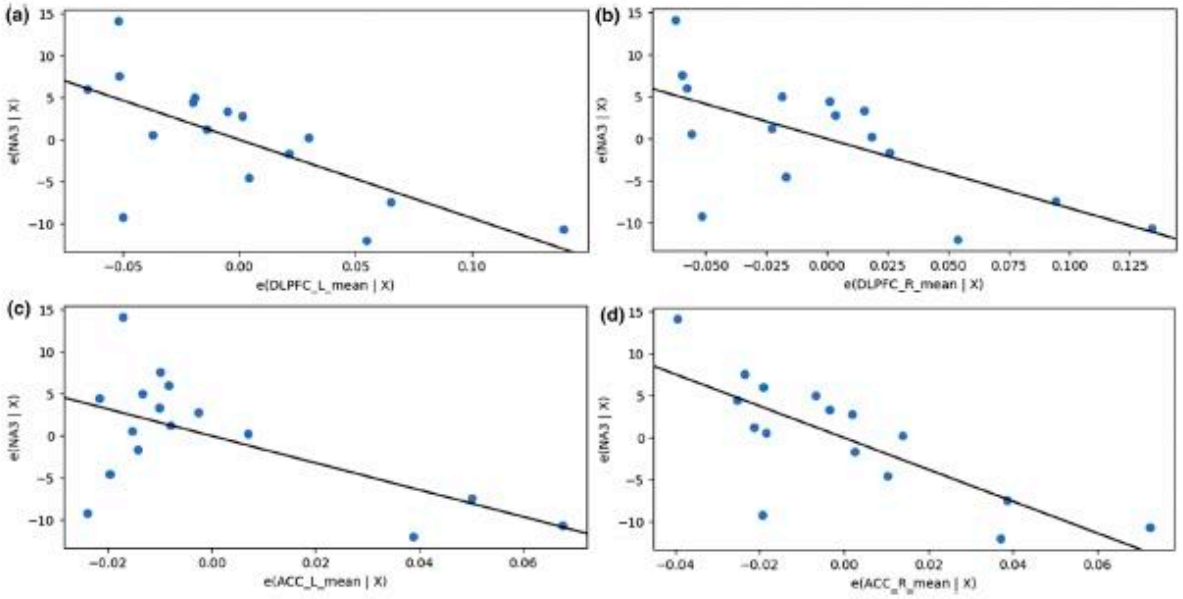

Fig. 2 Association between negative affect and EF strength for brain regions of interest. Partial regression plots of the mean electric field strength (measured in V/m) in hypothesis-defined regions of interest in relation to change in negative affect after acute treatment phase. A higher mean field value corresponds to a more pronounced decrease of negative affect as measured by the Positive and Negative Affect Scale (PANAS). **a**, **b**, **c** and **d** show the partial regression plots of the left dorsolateral prefrontal cortex, right dorsolateral prefrontal cortex, left anterior cingulate cortex and right anterior cingulate cortex, respectively. *DLPFC* dorsolateral prefrontal cortex, *ACC* anterior cingulate cortex, *NA* negative affect

associations were observed for other outcomes, regions, or timeframes (data not shown).

Discussion

In this study, we investigated the association between individual tDCS-induced simulated EF strength, using state-of-the-art computational simulation modeling approaches, and behavioral outcomes in depressed patients, based on our ELECT-TDCS trial. To the best of our knowledge, this is the first study correlating simulated EF strength with clinical outcomes in a depressed sample submitted to tDCS treatment.

Our findings showed that simulated EF strength in the left ACC was correlated with changes in HDRS-17. This is relevant since ACC acts as a bridge between attentional and emotional processing. In fact, alterations in its structure and function have been implicated in the pathophysiology of MDD. Depressed patients show decreased gray matter volume in the ACC [43], and, although our previous study did not find a significant correlation between baseline gray matter volume in the ACC and tDCS treatment response [15], it has been suggested that increases in ACC volume after repetitive transcranial magnetic stimulation (rTMS) and electroconvulsive therapy (ECT), other brain stimulation techniques, are correlated with its antidepressant effect [44, 45]. Additionally, connectivity between the DLPFC and ACC is altered in MDD [46], and changes in DLPFC-ACC connectivity are associated with rTMS efficacy [36, 47]. Therefore, our finding suggests that the ACC is implicated in prefrontal tDCS antidepressant effects. Future studies should investigate whether tDCS induces volumetric or connectivity changes in the ACC of depressed patients, and whether such changes are correlated with the EF strength in this brain region.

We also found that simulated EF strength in the bilateral ACC and DLPFC was inversely associated with negative affect, i.e., greater score reductions were associated with larger EF strengths in these regions. Since both brain regions are involved in implementing emotion regulation strategies and modulating activity in other emotion-encoding brain regions [48–52], it is possible that the applied EF contributed to increasing the functional coupling within this neural circuitry, facilitating emotional regulation of affect. In fact, this finding might be associated with the direct effects of tDCS over the PFC that regulates negative affect [53]. In previous studies using ELECT-TDCS data, we found that negative affect was the most important predictor associated with tDCS antidepressant response [54] and that the DLPFC volume predicted antidepressant response [15]. Moreover, several studies showed that prefrontal tDCS can enhance affective processing of emotionally loaded tasks [55–57]. Thus, our study confirms and expands previous evidence suggesting that changes in negative affect are implicated in tDCS antidepressant mechanisms of action. Further studies are necessary to explore this hypothesis and determine the specific mechanisms by which this is accomplished.

We found no correlation between trait or state anxiety and EF strength over the DLPFC and ACC. Although some studies suggested that tDCS can downregulate anxiety [31, 58], negative findings have been also reported. For instance, recent trials showed modest or null effects of prefrontal tDCS in ameliorating anxiety symptoms [59, 60]. In this context, other tDCS protocols that could be more effective in improving anxiety symptoms should be investigated [61]. In addition, tDCS effects on anxiety might be more effective when down-regulating stress-induced tasks [62, 63].

All the observed effects occurred immediately after the acute treatment phase (3 weeks of trial onset), but not at study endpoint (week 10). Interestingly, most studies have observed that tDCS effects are delayed, i.e., only differentiate from placebo after the acute treatment phase [7, 64, 65]. It is possible that other, non-specific factors (e.g., placebo effects, natural history of disease, regression to the mean) occurring between weeks 3 and 10 mitigated a possible association between simulated EF strength and our behavioral outcomes. Conversely, another possible explanation is that missing data from patients who did not complete the trial decreased the power of our analyses.

Our study has several limitations worth notice. First, our sample size is small as only a subsample of patients from the original study had MRI data collected. Therefore, some analyses might have been underpowered and our results should be primarily interpreted as hypothesis driven for future studies. Our limited sample size highlights the urgent need for larger tDCS depression studies performing baseline MRI measurements for replication of our findings. In addition, as 40 models were performed, at least 2 false positive findings might have emerged just by chance. Second, we are using simulated electric fields in reconstructed models of patient's heads. Although validated and considered state of the art [66, 67], they nonetheless represent an approximation of the "real" current distribution in the brain, which cannot be measured in a non-invasive manner. Third, the electrode positioning for the simulations on the models does not follow the exact correct location of the electrodes on the montage of the clinical trial (OLE system, which uses a 10 cm distance between electrodes), because of technical difficulties positioning virtual electrodes over simulated models' scalps using a 10 cm distance on irregular surfaces with distinct curvatures. Instead, the F5-F6 montage used in this study's simulations favors uniformity in the electrode positioning between subjects. Finally, the model is static,

i.e., it does not incorporate fluctuations in blood flow and changes in tissue conductivity that likely occur when tDCS is applied [68].

Whether further studies confirm that EF strength of certain brain regions correlates with clinical response, it would be possible to tailor individual tDCS montages and parameters to increase EF strength in such areas, theoretically improving clinical outcomes. This would represent an advancement towards individualizing tDCS parameters [69] whose parameters have been hitherto mostly fixed, not considering one's brain and skull anatomy.

Conclusion

We have investigated the association between simulated EF strength in brain regions implicated in depression pathophysiology and changes in behavioral outcomes in 16 depressed patients. We found that simulated EF strength presented a large variation in individual brains, even under the same parameters of stimulation. According to our hypotheses, associations were observed between simulated EF strength in the DLPFC and ACC and negative affect and depression scores. Nonetheless, the sample size was small and multiple tests were performed. Therefore, our findings should be regarded as exploratory. Notwithstanding, they show that EF strength might be associated with behavioral changes in clinical samples, suggesting a potential mechanism of action of tDCS antidepressant effects and fomenting further studies exploring whether tDCS interventions could be tailored to maximize EF strength in key brain regions to enhance clinical outcomes.

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Conflict of interest On behalf of all authors, the corresponding author states that there are no conflicts of interest to disclose.

References

1. Brunoni AR, Nitsche MA, Bolognini N et al (2012) Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stimul 5:175–195
2. Moffa AH, Brunoni AR, Nikolin S, Loo CK (2018) Transcranial direct current stimulation in psychiatric disorders: a comprehensive review. Psychiatr Clin North Am 41:447–463
3. Moffa AH, Martin D, Alonzo A, et al (2019) Efficacy and acceptability of transcranial direct current stimulation (tDCS) for major depressive disorder: an individual patient data meta-analysis. Progress in Neuro-Psychopharmacology and Biological Psychiatry pp 109836
4. Grimm S, Beck J, Schuepbach D et al (2008) Imbalance between left and right dorsolateral prefrontal cortex in major depression is linked to negative emotional judgment: an fMRI study in severe major depressive disorder. Biol Psychiatr 63:369–376
5. Seibt O, Brunoni AR, Huang Y, Bikson M (2015) The pursuit of DLPFC: non-neuronavigated methods to target the left dorsolateral pre-frontal cortex with symmetric bicephalic transcranial direct current stimulation (tDCS). Brain Stimul 8:590–602
6. Brunoni AR, Valiengo L, Baccaro A et al (2013) The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial. JAMA Psychiatr 70:383–391
7. Brunoni AR, Moffa AH, Sampaio-Junior B et al (2017) Trial of electrical direct-current therapy versus escitalopram for depression. N Engl J Med 376:2523–2533
8. Sampaio-Junior B, Tortella G, Borrione L et al (2018) Efficacy and safety of transcranial direct current stimulation as an add-on treatment for bipolar depression: a randomized clinical trial. JAMA Psychiatr 75:158–166
9. Brunoni AR, Moffa AH, Fregni F et al (2016) Transcranial direct current stimulation for acute major depressive episodes: meta-analysis of individual patient data. Br J Psychiatr 208:522
10. Loo CK, Husain MM, McDonald WM et al (2018) International randomized-controlled trial of transcranial direct current stimulation in depression. Brain Stimul 11:125–133
11. Lisanby SH (2017) Noninvasive brain stimulation for depression—the devil is in the dosing. N Engl J Med 376:2593–2594
12. Lang N, Nitsche MA, Dileone M et al (2011) Transcranial direct current stimulation effects on I-wave activity in humans. J Neurophysiol 105:2802–2810
13. Fresnoza S, Paulus W, Nitsche MA, Kuo M-F (2014) Nonlinear dose-dependent impact of D1 receptor activation on motor cortex plasticity in humans. J Neurosci 34:2744–2753
14. Datta A, Truong D, Minhas P et al (2012) Inter-individual variation during transcranial direct current stimulation and normalization of dose using MRI-derived computational models. Front Psychiatr 3:91
15. Bulubas L, Padberg F, Bueno PV et al (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
16. Opitz A, Paulus W, Will S et al (2015) Determinants of the electric field during transcranial direct current stimulation. Neuroimage 109:140–150
17. Antonenko D, Thielscher A, Saturnino GB et al (2019) Towards precise brain stimulation: is electric field simulation related to neuromodulation? Brain Stimul 12:1159–1168
18. Mikkonen M, Laakso I, Sumiya M et al (2018) TMS motor thresholds correlate with TDCS electric field strengths in hand motor area. Front Neurosci 12:426
19. Bora E, Fornito A, Pantelis C, Yücel M (2012) Gray matter abnormalities in major depressive disorder: a meta-analysis of voxel based morphometry studies. J Affect Disord 138:9–18
20. Lai C-H (2013) Gray matter volume in major depressive disorder: a meta-analysis of voxel-based morphometry studies. Psychiatr Res 211:37–46
21. Liao Y, Huang X, Wu Q et al (2013) Is depression a disconnection syndrome? Meta-analysis of diffusion tensor imaging studies in patients with MDD. J Psychiatr Neurosci 38:49–56
22. Brunoni AR, Sampaio-Junior B, Moffa AH et al (2019) Noninvasive brain stimulation in psychiatric disorders: a primer. Braz J Psychiatr 41:70–81
23. Baeken C, Brem A-K, Arns M et al (2019) Repetitive transcranial magnetic stimulation treatment for depressive disorders. Curr Opin Psychiatr 32:409
24. Smith R, Chen K, Baxter L et al (2013) Antidepressant effects of sertraline associated with volume increases in dorsolateral prefrontal cortex. J Affect Disord 146:414–419

25. Weigand A, Horn A, Caballero R et al (2018) Prospective validation that subgenual connectivity predicts antidepressant efficacy of transcranial magnetic stimulation sites. Biol Psychiatr 84:28–37
26. Hamilton M (1980) Rating depressive patients. J Clin Psychiatr 41:21–24
27. Watson D, Clark LA, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol 54:1063–1070
28. Spielberger CD, Gorsuch RL (1983) State-trait anxiety inventory for adults: manual, instrument, and scoring guide. Mind Garden Incorporated, Menlo Park
29. Brunoni AR, Zanao T, Vanderhasselt MA, et al (2013) Enhancement of affective processing induced by bi-frontal transcranial direct current stimulation in patients with major depression. Neuromodulation **[Epub–ahead of print]**
30. Moreno ML, Vanderhasselt M-A, Carvalho AF et al (2015) Effects of acute transcranial direct current stimulation in hot and cold working memory tasks in healthy and depressed subjects. Neurosci Lett 591:126–131
31. Ironside M, O'Shea J, Cowen PJ, Harmer CJ (2016) Frontal cortex stimulation reduces vigilance to threat: implications for the treatment of depression and anxiety. Biol Psychiatr 79:823–830
32. Brunoni AR, Sampaio-Junior B, Moffa AH et al (2015) The escitalopram versus electric current therapy for treating depression clinical study (ELECT-TDCS): rationale and study design of a non-inferiority, triple-arm, placebo-controlled clinical trial. Sao Paulo Med J 133:252–263
33. Thielscher A, Antunes A, Saturnino GB (2015) Field modeling for transcranial magnetic stimulation: a useful tool to understand the physiological effects of TMS? In: 2015 37th annual international conference of the IEEE engineering in medicine and biology society (EMBC). pp 222–225
34. Sallet J, Mars RB, Noonan MP et al (2013) The organization of dorsal frontal cortex in humans and macaques. J Neurosci 33:12255–12274
35. Fan L, Li H, Zhuo J et al (2016) The Human brainnetome atlas: a new brain atlas based on connectional architecture. Cereb Cortex 26:3508–3526
36. Fox MD, Buckner RL, White MP et al (2012) Efficacy of transcranial magnetic stimulation targets for depression is related to intrinsic functional connectivity with the subgenual cingulate. Biol Psychiatr 72:595–603
37. Li C-T, Wang S-J, Hirvonen J et al (2010) Antidepressant mechanism of add-on repetitive transcranial magnetic stimulation in medication-resistant depression using cerebral glucose metabolism. J Affect Disord 127:219–229
38. Downar J, Geraci J, Salomons TV et al (2014) Anhedonia and reward-circuit connectivity distinguish nonresponders from responders to dorsomedial prefrontal repetitive transcranial magnetic stimulation in major depression. Biol Psychiatr 76:176–185
39. Rossum G (1995) Python reference manual. CWI (Centre for Mathematics and Computer Science), Amsterdam, The Netherlands.
40. Seabold S, Perktold J (2010) Statsmodels: econometric and statistical modeling with python of the 9th Python in science conference
41. Brunoni AR, Padberg F, Vieira ELM et al (2018) Plasma biomarkers in a placebo-controlled trial comparing tDCS and escitalopram efficacy in major depression. Prog Neuropsychopharmacol Biol Psychiatr 86:211–217
42. Moreno ML, Goerigk SA, Bertola L et al (2020) Cognitive changes after tDCS and escitalopram treatment in major depressive disorder: results from the placebo-controlled ELECT-TDCS trial. J Affect Disord 263:344–352
43. Zhang F-F, Peng W, Sweeney JA et al (2018) Brain structure alterations in depression: psychoradiological evidence. CNS Neurosci Ther 24:994–1003
44. Lan MJ, Chhetry BT, Liston C et al (2016) Transcranial magnetic stimulation of left dorsolateral prefrontal cortex induces brain morphological changes in regions associated with a treatment resistant major depressive episode: an exploratory analysis. Brain Stimul 9:577–583
45. Yrondi A, Péran P, Sauvaget A et al (2018) Structural-functional brain changes in depressed patients during and after electroconvulsive therapy. Acta Neuropsychiatr 30:17–28
46. Iseger TA, van Bueren NER, Kenemans JL et al (2020) A frontal-vagal network theory for major depressive disorder: implications for optimizing neuromodulation techniques. Brain Stimul 13:1–9
47. Taylor SF, Ho SS, Abagis T et al (2018) Changes in brain connectivity during a sham-controlled, transcranial magnetic stimulation trial for depression. J Affect Disord 232:143–151
48. Rive MM, van Rooijen G, Veltman DJ et al (2013) Neural correlates of dysfunctional emotion regulation in major depressive disorder. A systematic review of neuroimaging studies. Neurosci Biobehav Rev 37:2529–2553
49. Greening SG, Osuch EA, Williamson PC, Mitchell DGV (2014) The neural correlates of regulating positive and negative emotions in medication-free major depression. Soc Cogn Affect Neurosci 9:628–637
50. Dixon ML, Thiruchselvam R, Todd R, Christoff K (2017) Emotion and the prefrontal cortex: an integrative review. Psychol Bull 143:1033–1081
51. Ochsner KN, Bunge SA, Gross JJ, Gabrieli JDE (2002) Rethinking feelings: an FMRI study of the cognitive regulation of emotion. J Cogn Neurosci 14:1215–1229
52. Chikazoe J, Lee DH, Kriegeskorte N, Anderson AK (2014) Population coding of affect across stimuli, modalities and individuals. Nat Neurosci 17:1114–1122
53. Heller AS, Johnstone T, Peterson MJ et al (2013) Increased prefrontal cortex activity during negative emotion regulation as a predictor of depression symptom severity trajectory over 6 months. JAMA Psychiatry 70:1181–1189
54. Kambeitz J, Goerigk S, Gattaz W et al (2020) Clinical patterns differentially predict response to transcranial direct current stimulation (tDCS) and escitalopram in major depression: a machine learning analysis of the ELECT-TDCS study. J Affect Disord 265:460
55. Wolkenstein L, Plewnia C (2012) Amelioration of cognitive control in depression by transcranial direct current stimulation. Biol Psychiatr. https://doi.org/10.1016/j.biopsych.2012.10.010
56. Vanderhasselt M-A, De Raedt R, Namur V et al (2016) Emotional reactivity to valence-loaded stimuli are related to treatment response of neurocognitive therapy. J Affect Disord 190:443–449
57. Sanchez-Lopez A, Vanderhasselt M-A, Allaert J et al (2018) Neurocognitive mechanisms behind emotional attention: Inverse effects of anodal tDCS over the left and right DLPFC on gaze disengagement from emotional faces. Cogn Affect Behav Neurosci 18:485–494
58. Ironside M, Browning M, Ansari TL et al (2019) Effect of prefrontal cortex stimulation on regulation of amygdala response to threat in individuals with trait anxiety: a randomized clinical trial. JAMA Psychiatr 76:71–78
59. de Lima AL, Braga FMA, da Costa RMM et al (2019) Transcranial direct current stimulation for the treatment of generalized anxiety disorder: a randomized clinical trial. J Affect Disord 259:31–37
60. D'Urso G, Dell'Osso B, Rossi R et al (2017) Clinical predictors of acute response to transcranial direct current stimulation (tDCS) in major depression. J Affect Disord 219:25–30

61. Sanchez A, Vanderhasselt M-A, Baeken C, De Raedt R (2016) Effects of tDCS over the right DLPFC on attentional disengagement from positive and negative faces: an eye-tracking study. Cogn Affect Behav Neurosci 16:1027–1038
62. Ankri YLE, Braw Y, Luboshits G, Meiron O (2020) The effects of stress and transcranial direct current stimulation (tDCS) on working memory: a randomized controlled trial. Cogn Affect Behav Neurosci. https://doi.org/10.3758/s13415-019-00755-7
63. Brunoni AR, Vanderhasselt M-A, Boggio PS et al (2013) Polarity- and valence-dependent effects of prefrontal transcranial direct current stimulation on heart rate variability and salivary cortisol. Psychoneuroendocrinology 38:58–66
64. da Valiengo L, Goerigk S, Gordon PC et al (2019) Efficacy and safety of transcranial direct current stimulation for treating negative symptoms in schizophrenia: a randomized clinical trial. JAMA Psychiatr. https://doi.org/10.1001/jamapsychiatry.2019.3199
65. Valiengo LC, Goulart AC, de Oliveira JF et al (2016) Transcranial direct current stimulation for the treatment of post-stroke depression: results from a randomised, sham-controlled, double-blinded trial. J Neurol Neurosurg Psychiatr. https://doi.org/10.1136/jnnp-2016-314075
66. Opitz A, Falchier A, Yan C-G et al (2016) Spatiotemporal structure of intracranial electric fields induced by transcranial electric stimulation in humans and nonhuman primates. Sci Rep 6:31236
67. Datta A, Krause MR, Pilly PK et al (2016) On comparing in vivo intracranial recordings in non-human primates to predictions of optimized transcranial electrical stimulation. Conf Proc IEEE Eng Med Biol Soc 2016:1774–1777
68. Bikson M, Brunoni AR, Charvet LE et al (2018) Rigor and reproducibility in research with transcranial electrical stimulation: an NIMH-sponsored workshop. Brain Stimul 11:465–480
69. Borrione et al (2020) Precision non-implantable neuromodulation therapies: a perspective for the depressed brain. Brazil J Psychiatr. https://doi.org/10.1590/1516-4446-2019-0741

12. Affidavit

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Dean's Office Medical Faculty
Faculty of Medicine

Affidavit

Erhardt, Eva Friederike

Surname, first name

I hereby declare, that the submitted thesis entitled

Effects of bifrontal transcranial direct current stimulation on brain metabolites and clinical outcome - Investigations using multimodal imaging methods

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the dissertation presented here has not been submitted in the same or similar form to any other institution for the purpose of obtaining an academic degree.

Munich, 24.09.2024
Place, Date

Eva Erhardt
Signature doctoral candidate