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tDCS Modulation of Neurochemical and Cognitive Functions in Healthy and Depressive Individuals

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List of abbreviations

CT	Computed Tomography
DLPFC	Dorsolateral Prefrontal Cortex
E-field	Electric Field
FDR	False Discovery Rate
GABA	Gamma-Aminobutyric Acid
Glx	Combined Measurement of Glutamate and Glutamine
Glu	Glutamate
HiWi	Hilfswissenschaftler (Student Assistant)
LCModel	Linear Combination Model
lDLPFC	Left Dorsolateral Prefrontal Cortex
LMM	Linear Mixed Models
LMU	Ludwig-Maximilians-Universität
MADRS	Montgomery-Åsberg Depression Rating Scale
MDD	Major Depressive Disorder
MEGA-PRESS	Mescher-Garwood Point-Resolved Spectroscopy
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NICUM	NeuroImaging Core Unit Munich
NAA	N-Acetyl Aspartate
NS	Not Significant
Ppm	Parts Per Million
RF	Radiofrequency
SCZ	Schizophrenia
SSRIs	Selective Serotonin Reuptake Inhibitors
STAI	State-Trait Anxiety Inventory
tDCS	Transcranial Direct Current Stimulation
TBI	Traumatic Brain Injury
vmPFC	Ventromedial Prefrontal Cortex

List of publications

Subject of this PhD thesis

Vural, G., Soldini, A., Padberg, F., Karslı, B., Zinchenko, A., Goerigk, S., ... & Keeser, D. (2024). Exploring the Effects of Prefrontal Transcranial Direct Current Stimulation on Brain Metabolites: A Concurrent tDCS-MRS Study. Human Brain Mapping, 45(18), e70097. https://doi.org/10.1002/hbm.70097

Soldini, A., Vogelmann, U., Aust, S., Goerigk, S., Plewnia, C., Fallgatter, A., Normann, C., Frase, L., Zwanzger, P., Kammer, T., Schönfeldt-Lecuona, C., **Vural, G**., Bajbouj, M., Padberg, F., & Burkhardt, G. (2024). Neurocognitive function as outcome and predictor for prefrontal transcranial direct current stimulation in major depressive disorder: an analysis from the DepressionDC trial. European Archives of Psychiatry and Clinical Neuroscience, 1-10. https://doi.org/10.1007/s00406-024-01759-2

Others

Kapetaniou, G. E., **Vural, G**., & Soutschek, A. (2025). Frontoparietal theta stimulation causally links working memory with impulsive decision making. Cortex. https://doi.org/10.1016/j.cortex.2025.02.012

Vural, G., Katruss, N., & Soutschek, A. (2024). Pre-supplementary motor area strengthens reward sensitivity in intertemporal choice. NeuroImage, 299, 120838. https://doi.org/10.1016/j.neuroimage.2024.120838

Chen, S., Shi, Z., **Vural, G**., Müller, H. J., & Geyer, T. (2023). Statistical context learn-ing in tactile search: Crossmodally redundant, visuo-tactile contexts fail to enhance contextual cueing. Frontiers in Cognition, 2, 1124286. https://doi.org/10.3389/fcogn.2023.1124286

Mastropasqua, A., **Vural, G**., & Taylor, P. C. (2022). Elements of exogenous attentional cueing preserved during optokinetic motion of the visual scene. European Journal of Neuroscience, 55(3), 746-761. https://doi.org/10.1111/ejn.15582

Your contribution to the publications

1.1 Contribution to paper I

The PhD candidate played a key role in designing the methodological framework for Study 1. In the early stages of the project, she participated in numerous meetings to conceptualize the study design, discuss MRI voxel localization , optimize MRI parameters, formulate hypotheses, and establish a project timeline. These contributions played a pivotal role in ensuring the project's scientific robustness.

The candidate gained practical expertise in transcranial Direct Current Stimulation (tDCS) application under supervision of Daniel Keeser. Using the 10-20 EEG system, she accurately placed the anodal and cathodal electrodes on saline-soaked sponges outside and inside the MRI. Her prior experience with this method during her master's program and several other side projects as a student assistant (HiWi) provided a strong technical foundation. Additionally, she received training in domains such as manual shimming and Magnetic Resonance Spectroscopy (MRS) voxel placement under the guidance of Eva Mezger and Antonia Susnjar.

To fulfill the study's technical requirements,, the candidate completed an MRI safety course and obtained the necessary MRI scanning hours to become a certified MRI key user. She was also an active member of the NeuroImaging Core Unit Munich (NICUM), contributing to the execution of several neuroimaging studies with advanced set-ups.

The candidate was responsible for participant recruitment and communication. The study collaborated with the Ludwig-Maximilians-Universität (LMU) Biobank to enable further genetic analyses of the participants. She coordinated participant screening by managing eligibility questionnaires and scheduling testing dates, including initial Biobank sessions. She prepared participants for the MRI sessions by positioning the head and placing electrodes inside the MR-scanner, including the complete device set-up to ensure accurate data acquisition.

During experimental sessions, the candidate independently conducted MRS voxel placement at the left DLPFC and spectroscopy scans, along with acquiring structural MRI scans. She managed participant payments and administered additional questionnaires necessary for subsequent descriptive statistical analyses. Following data collection, the candidate took a central role in data analysis. She collaborated with various institutions to ensure comprehensive and accurate statistical evaluations. Specifically, she worked with the

Psychology Department at Ludwig-Maximilians-Universität (LMU) Munich and the Radiology Department at Harvard Medical School. Artyom Zinchenko and Alexander Soutschek provided consultation on statistical analyses, ensuring robust data interpretation.

The candidate conducted the MRS and structural data analyses, including electric field (E-field) modeling and cluster analyses. The MRS data analysis, including Osprey, Linear Combination Model (LCModel), and MATLAB tools, was conducted in collaboration with Antonia Susnjar, who provided essential guidance throughout the process. As the corresponding author of the project's primary manuscript, the candidate's responsibilities included writing the manuscript, preparing it for publication, and drafting the rebuttal letter to address reviewers' feedback during the peer-review process.

The PhD project was presented at several national and international conferences, where the candidate both prepared the posters and presented them. Along with these presentations, she introduced herself, built collaborations (e.g., with Antonia Susnjar), and enhanced her academic visibility. These conferences included:

- June 2023: 21st Turkish Neuroscience Congress
- February 2023: 5th International Brain Stimulation Conference
- August 2022: Magnetic Resonance Spectroscopy Workshop 2022

Through her involvement in this project, the candidate demonstrated comprehensive research competencies across all stages, including study design, data acquisition, analysis, advanced statistics, presentations, and manuscript preparation for journal submission, resulting in a published scientific contribution in an excellent peer-reviewed neuroimaging journal.

The candidate shares first authorship with Aldo Soldini. Mr. Soldini was responsible for assististed with the initial first part of the data collection phase process, including participant registration and blood sample collection. Additionally, he contributed to the review and editing of the manuscript draft, as well as supporting the data curation process. He attended weekly lab meeting and contributed the project discussions. Finally, both the candidate and Mr. Soldini presented the poster at the Magnetic Resonance Spectroscopy Workshop in 2022. This collaboration increased the quality of the research, with both authors making substantial contributions in their respective areas of expertise.

1.2 Contribution to paper II

The candidate contributed as a co-author to the second manuscript, which focused examined the impact of bifrontal tDCS on cognitive domains in major depressive disorder (MDD) and whether baseline cognition could serve as a predictor of treatment efficacy. Her involvement included participating in regular lab meetings where relevant topics were discussed in detail. She provided critical feedback on the study design, ensuring methodological rigor, and contributed to shaping the overall research direction. These meetings fostered a collaborative environment, facilitating the exchange of ideas and guaranteeing alignment with the project's objectives.

In addition to these collaborative discussions, the candidate actively participated in the manuscript preparation process. She was responsible for reviewing the manuscript drafts, providing constructive feedback and suggesting revisions to enhance clarity and scientific accuracy. Furthermore, she assisted in editing specific sections of the manuscript, improving the overall coherence and flow. A key objective of this study was to gain further experience with the same tDCS electrode configuration used in Study 1, specifically to investigate its effects on cognition as a potential transdiagnostic marker in a clinical sample. This experience broadened the candidate’s understanding of tDCS application in diverse populations and research contexts.

2. Introduction

2.1 The Role of DLPFC in Cognition and Psychiatric Disorders

The dorsolateral prefrontal cortex (DLPFC) plays an important role in high-level cognitive functions, including executive processing (Panikratova et al., 2020; Sylvester et al., 2003), decision-making (Mohr et al., 2010; Philiastides et al., 2011), problem-solving (Barbey & Barsalou, 2009; Juliyanto et al., 2021), and working memory (Barbey et al., 2013; Blumenfeld & Ranganath, 2006). It is also a key region in psychiatric research due to its fundamental role in the top-down modulation of emotions and behavior (Sallet et al., 2013). Abnormalities in this region have been associated with impulsivity (Sala et al., 2011), emotional dysregulation (Salehinejad et al., 2017), and various neuropsychiatric disorders (Grimm et al., 2008; Ye et al., 2012; Zugman et al., 2013). Among these disorders, major depressive disorder (MDD) and schizophrenia (SCZ) exhibit profound structural and functional abnormalities in the DLPFC (Zhang et al., 2020).

MDD, one of the most prevalent psychiatric disorders, is characterized by depressed mood, feelings of worthlessness, and a loss of energy and interest (American Psychiatric Association, 2013). These symptoms significantly impair daily functioning, contributing to a high societal burden and increased healthcare costs (Greenberg et al., 2015; Welch et al., 2009). Although various treatment options, including selective serotonin reuptake inhibitors (SSRIs) and psychotherapy, are available, a substantial subset of patients remain resistant to pharmacological interventions (Gaynes et al., 2012; Rush et al., 2006). This highlights the need for alternative therapeutic approaches that can effectively reduce symptoms in individuals who do not respond to conventional treatments.

One promising alternative involves non-invasive brain stimulation techniques, such as transcranial direct current stimulation (tDCS), which modulates DLPFC activity to reduce symptom severity (Li et al., 2022; Zheng et al., 2024). Understanding the function of the DLPFC has traditionally relied on studies of impairment, such as research on traumatic brain injuries (TBI) and focal lesions (Barbey et al., 2012; Cazalis et al., 2006), where damage to this region reveals its critical role in cognition and behavior. However, it has also recently been possible to investigate the DLPFC by directly manipulating its activity using tDCS. This technique has been preferred due to its practical and ethical reasons for exploring brain function in healthy and patient populations, allowing researchers to examine more causal relationships between DLPFC activity and cognitive processes. Given its crucial role in

psychiatric disorders and cognitive functions, targeting the DLPFC with tDCS not only enhances our understanding of its functions but also but also offers new possibilities for symptom management.

2.2 tDCS: Mechanisms and Evolution

tDCS is a non-invasive method to target specific brain areas with electrical stimulation. The use of electricity for medical purposes dates back centuries, with early examples including the use of torpedo fish, which naturally produce electricity, to reduce epileptic seizures (Harris, 1908). Natural electricity from animals was used for centuries until 1660, when Otto von Guericke invented the first machine to generate electricity. Later, Giovanni Aldini began using electrical currents in clinical trials for therapeutic purposes (Comroe & Dripps, 1976). tDCS lost its popularity in the 1970s due to the rise of pharmacological treatments for psychiatric disorders (Dubljević et al., 2014). However, it gained attention again after Nitsche and Paulus reintroduced tDCS and demonstrated that tDCS can create excitatory and inhibitory effects on neurons (Nitsche & Paulus, 2000), marking a significant turning point in research.

tDCS delivers a low current (typically 1-2 mA) through the scalp using at least two electrodes. The anode promotes depolarization (making neurons more likely to fire) of the resting membrane potentials, while the cathode induces hyperpolarization (reducing neuronal excitability) (Brunoni et al., 2012). After passing through the scalp and skull, the remaining current modulates cell membrane polarization and adjusts the spike timing of neurons receiving suprathreshold inputs rather than directly triggering action potentials (Anastassiou et al., 2011; Ruffini et al., 2013). This process may also influence neurotransmitter release (Rohan et al., 2015), though the precise mechanisms remain incompletely understood.

tDCS is widely used due to its low intensity and minimal side effects (Bikson et al., 2016; Chhatbar et al., 2017). Although various studies have documented its effects on cognitive functions and clinical conditions, contradictory findings highlight the need for more research to understand how tDCS works (Berryhill & Martin, 2018; Horvath et al., 2015; Jacobson et al., 2012; Narmashiri & Akbari, 2023). Combining tDCS with other techniques as a multimodal approach may help explore its effects on brain metabolism and better understand its mechanisms in healthy brains (Saiote et al., 2013). This knowledge could facilitate translating tDCS applications to clinical populations where they are most needed. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), in particular, offer a

unique opportunity to directly assess stimulation-induced changes at both neural and metabolic levels, making them strong candidates for investigating tDCS mechanisms.

2.3 MRI and MRS: History, Principles, and Applications

MRI is one of the most commonly used brain imaging techniques for investigating the structural and functional aspects of the brain. The history of MRI is marked by several groundbreaking discoveries. In 1938, Isidor Isaac Rabi demonstrated that the magnetic moments of atoms and molecules interact with magnetic fields in specific ways, laying the theoretical foundation for magnetic resonance (Rabi et al., 1938). Later, in 1971, Raymond Damadian discovered that cancerous tissues exhibit distinct magnetic resonance properties compared to healthy tissues, highlighting the potential of MRI as a clinical diagnostic tool (Damadian, 1971). Building on these findings, Paul Lauterbur (1973) created the first two-dimensional images using magnetic resonance, demonstrating the practical imaging capabilities of the technique (Lauterbur, 1973). The image quality and acquisition speed were further enhanced with Fourier transformation, enabling shorter imaging times and more detailed visualizations (Edelman, 2014).

Today, MRI is a non-invasive imaging technique that operates without ionizing radiation, making it a safer alternative to computed tomography (CT) (Brenner & Hall, 2007). It is also capable of facilitating the investigation of brain metabolites in specific regions of interest. This is called MRS and provides valuable insights into brain function and pathology. Proton magnetic resonance spectroscopy (^{1}H-MRS) is the most widely used MRS modality due to its high sensitivity in detecting a broad range of metabolites, including glutamate (Glu) and gamma-aminobutyric acid (GABA) (Govindaraju et al., 2000).

MRS is based on the principle that atomic nuclei, particularly those with a non-zero spin, such as hydrogen, exhibit distinct responses to radiofrequency (RF) pulses in a strong magnetic field. These responses vary depending on the molecular environment surrounding the nuclei, as the local electronic shielding alters the effective magnetic field experienced by each nucleus. This phenomenon, known as chemical shift, allows for the differentiation of nuclei within various chemical compounds (Cecil, 2013). MRS can measure these shifts, providing insights into the relative concentrations of specific metabolites within tissues. Specific metabolites are represented as peaks on an MRS graph where signal intensity corresponds to metabolite concentration, and the x-axis represents the signal frequency. The frequency is expressed in parts per million (ppm), a scale normalized to account for differences

in the magnetic field strength of the spectrometer, allowing for standardized comparison of chemical shifts (Cecil, 2013).

2.4 Investigating the Effects of Bifrontal tDCS: Neurochemical, Cognitive, and Clinical Perspectives

MRS provides insights into the neurobiological mechanisms underlying tDCS effects by quantifying neurotransmitter levels before, after, and even during stimulation. Bifrontal tDCS, which targets the DLPFC, is a commonly used montage due to its ability to modulate activity bilaterally, making it an effective approach in both cognitive and clinical research (Brunoni et al., 2014; Fecteau et al., 2007; Ferrucci et al., 2009). This dual-hemisphere stimulation is particularly advantageous for addressing functional impairments observed in psychiatric conditions while facilitating investigations into cognitive functions. Several studies have attempted to investigate the metabolic effects of tDCS on the prefrontal cortex using MRS. However, the findings remain inconsistent (see Table 1), which may be attributed to variations in stimulation intensities, durations, experimental protocols, sample sizes, and the specific brain metabolites measured. These discrepancies highlight the need for more standardized and refined methodologies to better understand the neurochemical impact of tDCS.

Table 1

Summary of Bifrontal tDCS Studies Utilizing MRS

Study	Total n	Stimulation A=anodal; C=cathodal	Intensity duration	Baseline	MRS regions	Time points	GABA	Glx	Glu	NAA
Present study	40	A: lDLPFC C: rDLPFC	2mA 20m	corrected	lDLPFC	pre-during1-during2-post	NS	lDLPFC	NS	NS
(Mezger et al., 2021)	20	A: lDLPFC C: r DLPFC	2mA 20m	corrected	rDLPFC	pre-during1-during2-post	NS	NS	only females	-
(Hone-Blanchet et al., 2016)	17	A: lDLPFC C: rDLPFC	1mA 30m	no baseline	lDLPFC and left striatum	during-post	NS	left striatum	-	lDLPFC
(Dickler et al., 2018)	16	A: rDLPFC C: lDLPFC	1mA 30m	no baseline	rDLPFC and right striatum	during	right DLPFC	NS	-	NS
(Mugnol-Ugarte et al., 2022)	41*	A: vmPFC. C: rDLPFC	2mA 20m	not corrected	vmPFC and DLPFC	pre-post	vmPFC & right DLPFC	vmPFC & right DLPFC	-	-
(Bunai et al., 2021)	17	A: lDLPFC. C: rDLPFC	2mA 13m (x2)	no info	lDLPFC and bilateral striata	post	striatum DLPFC	-	-	NS (GABA:NAA ratio)
(Habich et al., 2020)	55**	A: lDLPFC C: right supraorbital area	1mA 20m	not corrected	lDLPFC	pre-after	NS	NS	-	-

Note. Parametric and technical details of the studies which combine bifrontal tDCS with MRS. This table was adapted from the supplemental material of (Vural et al., 2024).

* Between group design

** 33 were healthy young participants

"-" not measured

NS: not significant

While neurochemical assessments such as MRS provide valuable insights into the underlying mechanisms of stimulation, cognitive and clinical measures remain essential for evaluating the efficacy and therapeutic potential of tDCS, particularly in patient populations. To address this, the present thesis includes two complementary investigations aimed at understanding the impact of bifrontal tDCS in both healthy individuals and clinical populations: (1) to investigate the changes in brain metabolites induced by bifrontal tDCS in healthy individuals (Study 1) and (2) to examine the effects of bifrontal tDCS on symptom severity and cognitive performance in patients with SSRI-resistant MDD, as well as to assess the potential role of baseline cognitive performance as a predictor of tDCS treatment response (Study 2). Both studies employed 2 mA bifrontal stimulation, with the anode positioned over the left DLPFC (F3) and the cathode over the right DLPFC (F4). The protocols were sham-controlled and double-blinded including ramp-up and ramp-down phases to maintain blinding.

3. Experimental Studies

3.1 Study 1: tDCS and MRS in Healthy Participants

3.1.1 Aim and Hypothesis

The first project investigates the impact of tDCS on brain metabolites in healthy individuals, providing insights into how stimulation influences metabolic processes. By examining the tDCS effect in the healthy brain, this study contributes to the understanding of stimulation-induced metabolic changes, which may help to translate these findings to clinical populations.

Due to the differential methodological approaches in the existing literature, the first study aimed to investigate tDCS-induced changes in brain metabolites using a well-controlled design that addresses key methodological gaps. Accordingly, baseline metabolite levels were measured to account for individual differences, and post-tDCS assessments were conducted to evaluate the after-effects of stimulation. By employing a placebo-controlled, double-blind, crossover methodology and one of the largest sample sizes in this field, this study aimed to address discrepancies in previous findings. Key metabolites, including GABA, Glu, Glx, and NAA, were measured via single-voxel ^{1}H-MRS, targeting the left DLPFC, providing an overview of neurometabolic changes induced by tDCS.

We hypothesized that a single session of bifrontal tDCS leads to significant alterations in these brain metabolites, measured from the anodal side (left DLPFC), during and/or after active stimulation compared to sham stimulation. These neurometabolic changes are expected to provide valuable insights into the mechanisms of tDCS in the healthy brain.

3.1.2 Methods

A total of 41 participants were scanned using a 3T Siemens Prisma scanner. Each participant completed two sessions - one with active stimulation and one with sham stimulation, separated by one week to minimize potential carryover effects.

tDCS was delivered using a NeuroConn single-channel device. The stimulation targeted the left DLPFC with a 2 mA bifrontal montage, where the anode was positioned over F3 and the cathode over F4. MRS data were acquired using a 2.2 cm^3 single-voxel placement in the left DLPFC. Spectroscopy data were analyzed using LCModel, and Mescher-Garwood

Point-Resolved Spectroscopy (MEGA-PRESS) sequences were used to acquire GABA metabolite concentrations, which were further processed with Gannet. Metabolite concentrations were measured at four time points: before, during the first half (during1), and the second half (during2) of the stimulation and after the stimulation. Each acquisition lasted 10 minutes per time point, resulting in a total scanning time of 40 minutes.

3.1.3 Results

Two main analyses and one exploratory analysis were conducted to investigate the tDCS-induced metabolite effects. Linear mixed models (LMMs), implemented in R Studio, were used to analyze the data while controlling for sex and age for each brain metabolite. P-values were corrected for multiple comparisons.

A significant difference in Glx levels was observed between the active and sham stimulation sessions during the second half of stimulation and the post-stimulation period (during2 stimulation: $p_{\text{Bonferroni}} = .049$; post-stimulation: $p_{\text{Bonferroni}} = .01$). Specifically, Glx levels were significantly higher in the active stimulation session compared to the sham session. No significant differences were found in other measured metabolites (GABA, Glu, or NAA) across time points.

E-field analyses at the 75^{th} percentile were conducted to examine individual neuroanatomical differences and assess the relationship between electric field intensities and Glx concentration levels. The analysis revealed no significant correlation between E-field intensities and Glx levels.

Finally, an exploratory cluster analysis was performed to examine the distribution of responders and non-responders to the stimulation, specifically for the Glx metabolite. The results identified three distinct groups based on stimulation response in Glx levels.

These findings demonstrate that tDCS can selectively modulate brain metabolite levels, specifically increasing Glx during active stimulation. This effect persisted after stimulation, highlighting the after-effects of tDCS. These results support the potential of tDCS as a tool for neuromodulation. Further implications and interpretations will be discussed in the Conclusion/Discussion section.

3.2 Study 2: tDCS Effect on Symptom Severity in MDD

3.2.1 Aim and Hypothesis

The second project extends this work by assessing the effects of tDCS on cognitive and clinical symptoms in MDD, a group characterized by cognitive dysfunction and neurometabolic imbalances. We hypothesized that repeated sessions of bifrontal tDCS would result in significant improvements in cognitive performance across multiple domains, such as working memory, attention, and executive functions in patients with SSRI-resistant MDD. Furthermore, we proposed that reductions in symptom severity, as assessed by the Montgomery-Åsberg Depression Rating Scale (MADRS), would indicate greater clinical improvement in the active tDCS group compared to the sham tDCS group.

3.2.2 Methods

The second study (DepressionDC trial) aimed to investigate changes in symptom severity and cognitive performance before and after 24 sessions of bifrontal tDCS (2 mA, 30 minutes per session) in MDD patients. This triple-blind, sham-controlled study assessed symptom severity using the MADRS and the State-Trait Anxiety Inventory (STAI). Cognitive performance was evaluated across multiple domains using the validated EmoCogMeter test battery, including memory span, working memory, selective attention, sustained attention, executive function, and processing speed. Assessments were conducted at baseline, after treatment (week 6), and at a 6-month follow-up. This comprehensive assessment allowed for a detailed evaluation of both clinical symptoms and cognitive functioning, offering insights into the therapeutic potential of tDCS in treatment-resistant depression.

3.2.3 Results

LMM was used to perform the analyses on the effect of the active stimulation on cognitive performance via R by controlling sex, age and MADRS scores from the baseline level. False discovery rate (FDR) correction was applied for multiple comparisons. Baseline characteristics between the groups were compared using Pearson’s chi-square tests and Wilcoxon rank-sum tests.

The investigated neurocognitive functions did not show any significant differences between active and sham tDCS over the 6-month study period. Moreover, baseline cognitive scores did not predict treatment response. The results indicate that the investigated

neurocognitive domains do not predict the tDCS response in the MDD group. Additionally, baseline cognitive performance did not significantly influence treatment outcomes.

4. Conclusion/Discussion

This thesis incorporates two interrelated studies investigating the neurometabolic effects of tDCS and its potential clinical applications. By examining how tDCS influences brain metabolites in healthy individuals (Study 1), we aim to inform the development of personalized tDCS treatment strategies for MDD. These studies seek to bridge the gap between basic research and clinical practice, exploring the potential effect of tDCS both as a cognitive enhancement tool and as a treatment option for psychiatric conditions.

The significant Glx change observed in Study 1, but the absence of changes in other metabolites, highlights the complexity of interpreting the metabolic effects of tDCS. Glx represents the combined measurement of Glu and Gln. While Glu is primarily associated with neuronal activity, astrocytes reuptake Glu from the synaptic cleft and convert it into Gln. This process suggests that tDCS may also influence astrocytic functions and that Glx changes reflect both neuronal and astrocytic processes, suggesting a broader metabolic effect. The rapid astrocytic reuptake of Glu, combined with the inherent limitations of 3T MRI scanners in detecting subtle metabolite changes, may explain the absence of significant alterations in Glu levels. While GABA and NAA levels also remained unchanged, the significant modulation of Glx indicates a localized effect of anodal stimulation on the left DLPFC.

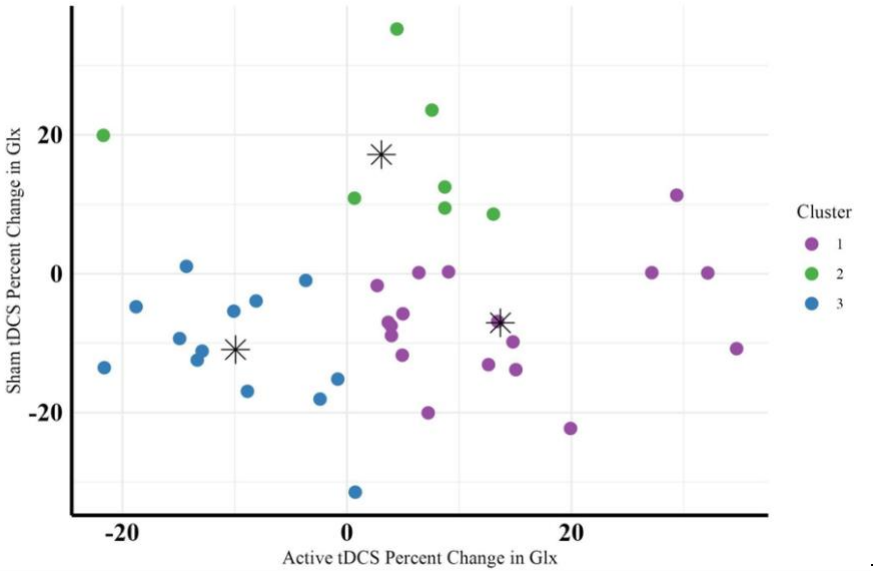
Contrary to our expectations, the Study 2 found that tDCS did not result in significant improvements in either symptom severity or cognitive test scores. These results suggest that while tDCS may influence neurometabolic processes in healthy individuals, its clinical and cognitive benefits in SSRI-resistant MDD require further investigations such as different protocols with higher current intensities or longer stimulation times. The possible explanations for the findings will be discussed in the following section.

5. Future Directions

Our cluster analyses in Study 1 demonstrated distinct subgroups of responders and non-responders based on Glx metabolic levels, highlighting the heterogeneity in neurometabolic responses to stimulation (Figure 1). Future research should investigate how factors such as scalp thickness and hormonal levels contribute to this variability, particularly in clinical populations where comorbidities and treatment histories introduce additional layers of complexity (Mastria et al., 2021; Vergallito et al., 2022).

Figure 1

K-means Clustering of Percent Changes in Glx



Note. Scatter plot visualizing the outcomes of k-means clustering, showing percentage changes in Glx responses to active and sham stimulation grouped by the derived clusters. Each point represents a participant's response, with asterisks indicating the centroids of the clusters. This figure was adapted from the supplemental material of (Vural et al., 2024).

The limited effectiveness of tDCS observed in Study 2 can be attributed, in part, to the specific characteristics of the participant sample. These individuals were classified as medication-resistant, a condition often associated with a more severe and treatment-refractory form of depression. As highlighted in a meta-analysis of 342 studies (Meron et al., 2015) and a comprehensive review (Palm et al., 2016), tDCS effects are generally diminished in this

population. This resistance likely stems from the complex neurobiological alterations that accompany long-term medication use and the more entrenched nature of the depressive symptoms. Therefore, relying solely on standalone tDCS may be insufficient for achieving significant clinical improvements in medication-resistant depression. Future research should explore adjunctive strategies, such as combining tDCS with pharmacological agents. Specifically, pairing tDCS with medications that modulate the glutamatergic or dopaminergic systems, as suggested by previous studies (Brunoni et al., 2014; Loo et al., 2012), could potentially synergize and enhance treatment efficacy in this challenging patient group.

Previous research in our lab reported decreased Glu levels on the cathodal side in females under similar stimulation parameters (Mezger et al., 2021). While tDCS modulated Glu levels in Mezger et al.'s study and Glx levels in Study 1 of this thesis, it did not significantly alter cognitive scores or symptom severity in Study 2. This discrepancy highlights the variability in neurometabolic and cognitive responses to tDCS, suggesting that standardized stimulation protocols are not universally effective across populations. The complex neurophysiological underpinnings of depression explained through theories such as interhemispheric frontal imbalance (Hui et al., 2021), hypofrontality (Galynker et al., 1998), and limbic-cortical dysfunction (Mayberg, 1997) likely contribute to these heterogeneous responses. Given these complexities, refining stimulation parameters - such as increasing the number of sessions (Bennabi & Haffen, 2018) or personalizing current intensity and duration based on individual anatomy (Mosayebi-Samani et al., 2021), baseline cortical excitability (Filmer et al., 2019), hormonal status (Rudroff et al., 2020), and resting-state connectivity (Abellaneda-Pérez et al., 2020) is necessary to enhance cognitive and clinical outcomes (Esmaeilpour et al., 2018; Rudroff et al., 2020; Sabé et al., 2024). Furthermore, individual differences in gray matter density, previously linked to antidepressant response (Bulubas et al., 2019), should be explored as a potential biomarker for optimizing tDCS efficacy in neuropsychiatric disorders. Identifying such robust predictors across both neurometabolic and cognitive domains will be crucial to maximize the therapeutic potential of tDCS.

In conclusion, future research should adopt a precision-medicine approach to tDCS, integrating neurobiological and individual patient characteristics to enhance clinical efficacy while utilizing high-field MRI, such as 7T, to gain deeper insights into metabolic concentrations and neuromodulatory effects.

6. Paper I

Human Brain Mapping

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Exploring the Effects of Prefrontal Transcranial Direct Current Stimulation on Brain Metabolites: A Concurrent tDCS-MRS Study

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ABSTRACT

Transcranial Direct Current Stimulation (tDCS) is a non-invasive brain stimulation technique used to modulates cortical brain activity. However, its effects on brain metabolites within the dorsolateral prefrontal cortex (DLPFC), a crucial area targeted for brain stimulation in mental disorders, remain unclear. This study aimed to investigate whether prefrontal tDCS over the left and right DLPFC modulates levels of key metabolites, including gamma-aminobutyric acid (GABA), glutamate (Glu), glutamine/glutamate (Glx), *N*-acetylaspartate (NAA), near to the target region and to explore potential sex-specific effects on these metabolite concentrations. A total of 41 healthy individuals (19 female, M_age = 25 years, SD = 3.15) underwent either bifrontal active (2 mA for 20 min) or sham tDCS targeting the left (anode: F3) and right (cathode: F4) DLPFC within a 3 Tesla MRI scanner. Magnetic resonance spectroscopy (MRS) was used to monitor neurometabolic changes before, during, and after 40 min of tDCS, with measurements of two 10-min intervals during stimulation. A single voxel beneath F3 was used for metabolic quantification. Results showed a statistically significant increase in Glx levels under active tDCS compared to the sham condition, particularly during the second 10-min window and persisting into the post-stimulation phase. No significant changes were observed in other metabolites, but consistent sex differences were detected. Specifically, females showed lower levels of NAA and GABA under active tDCS compared to the sham condition, while no significant changes were observed in males. E-field modeling showed no significant differences in field magnitudes between sexes, and the magnitude of the e-fields did not correlate with changes in Glx levels between active and sham stimulation during the second interval or post-stimulation. This study demonstrates that a single session of prefrontal tDCS significantly elevates Glx levels in the left DLPFC, with effects persisting post-stimulation. However, the observed sex differences in the neurochemical response to tDCS were not linked to specific stimulation intervals or variations in e-field magnitudes, highlighting the complexity of tDCS effects and the need for personalized neuromodulation strategies.

Gizem Vural, Aldo Soldini, Antonia Šušnjar, and Daniel Keeser contributed equally to this work.

Summary

- Active tDCS is associated with an enhanced Glx concentration at the stimulation site (left DLPFC).
- Active tDCS, in comparison to sham, does not influence GABA, NAA, and Glu concentrations.
- Findings reveal sex-specific variations in GABA and NAA concentrations, underscoring the importance of investigating sex differences and their implications.

1 | Introduction

The prefrontal cortex, central to cognitive functions (Roberts, Robbins, and Weiskrantz 1998) and psychiatric disorders (Wible et al. 2001; Pizzagalli and Roberts 2022) plays a pivotal role in mental health. The dorsolateral prefrontal cortex (DLPFC), in particular, is a critical hub for major depressive disorder (MDD) and schizophrenia (SCZ), two of the most prevalent psychiatric disorders (Bunney 2000; Barch and Ceaser 2012; Wang et al. 2021; Zhang et al. 2022). Neurochemical and metabolic imbalances, the primary excitatory neurotransmitter glutamate (Glu) and inhibitory neurotransmitter gamma-aminobutyric acid (GABA) are also taken into account, have an effect in the pathophysiology of these conditions (Nakahara et al. 2022; Sarawagi, Soni, and Patel 2021).

Transcranial direct current stimulation (tDCS) emerges as an innovative method for modulating brain metabolites, utilizing a weak electrical current to influence membrane polarization and neurotransmitter release. Despite its promising potential, the molecular effects of tDCS remain incompletely understood. Employing tDCS alongside magnetic resonance spectroscopy (MRS) could shed light on the physiological impact of tDCS on metabolite concentrations.

Understanding the impact of tDCS on the healthy brain, using optimal parameters, is crucial for enhancing clinical outcomes and addressing inconsistent findings in the literature. To date, only a small number of studies have investigated the metabolic effects of prefrontal tDCS (Bunai et al. 2021; Dickler et al. 2018; Habich et al. 2020; Hone-Blanchet, Edden, and Fecteau 2016; Jeong et al. 2021; Mezger et al. 2021; Mugnol-Ugarte et al. 2022). Among these studies, findings have varied: one study reported an increase in prefrontal *N*-Acetyl Aspartate (NAA) following bifrontal tDCS (Hone-Blanchet, Edden, and Fecteau 2016), while another observed elevated prefrontal GABA levels (Dickler et al. 2018). A study with a methodology similar to the current work found a reduction in Glu specifically in female participants (Mezger et al. 2021), and another reported no significant effect of left DLPFC stimulation on GABA or glutamine/glutamate (Glx) concentrations (Habich et al. 2020). (Details of these studies are provided in Table S1).

The variability in findings may be attributed to differences in experimental designs, including the lack of baseline or post-stimulation measurements, variations in stimulation intensity (1–2 mA), duration (13–30 min) and sample size (12–33). Our study addresses these inconsistencies by employing a more detailed design using 2 mA intensity and a larger sample size, which improves the statistical power, reliability of the results and translation to clinical settings (Brunoni, Ferrucci, et al. 2012; Brunoni, Nitsche, et al. 2012; Padberg et al. 2017; Palm et al. 2016). By dividing the 20-min tDCS session into two 10-min intervals, we tracked metabolite changes throughout the stimulation period. Baseline measurements controlled for pre-existing differences, while post-stimulation measurements allowed for the observation of sustained effects.

The tDCS involved anodal stimulation over the left DLPFC and cathodal stimulation over the right DLPFC. We assessed the effects of active stimulation versus sham before, during, and after tDCS application. Employing in vivo MRS, we hypothesized that prefrontal tDCS would result in changes in neurotransmitter concentrations, specifically modifying levels of Glu, Glx, GABA and NAA during and/or after active stimulation compared to sham tDCS. Moreover, based on the findings of Mezger et al. (2021) regarding sex differences, we hypothesize that tDCS might have sex-specific effects on metabolite concentrations, with a particular focus on the potential differences between male and female participants. Given this approach, our study represents the most detailed and advanced investigation to date, utilizing online tDCS to provide real-time insights into these effects.

2 | Methods

2.1 | Participants

Previous studies exploring this topic have employed various sample sizes, reflecting the diversity in research designs and objectives. For this discussion, we focus only on studies with healthy controls, omitting combined samples that include elderly or patient populations. For instance, the most comparable study, Mezger et al. (2021) tested 20 participants, while Habich et al. (2020) included 33 healthy young individuals. Bunai et al. (2021) focused on 17 male participants, in their research. Hone-Blanchet, Edden, and Fecteau (2016) involved 17 participants after exclusions. Mugnol-Ugarte et al. (2022) also tested 41 participants, but these authors used a between-groups design, similar to Guan et al. (2020), who tested 12 individuals (50% controls).

The sample sizes across these studies ranged from as few as 12 to as many as 33 healthy individuals. A common thread among these studies is the absence of explicit sample size calculations. Among those that did report calculations, the reported effect sizes, Cohen's *d*, varied from 0.56 to 1.1 (Guan et al. 2020; Habich et al. 2020), reflecting the heterogeneity in anticipated effects across different studies. Given the inconsistencies and the wide range of effect sizes in the literature, we decided to opt for a weak-to-moderate effect size of 0.45 for Cohen's *d*. This decision was informed by the need to balance the detectability of effects and practical considerations of sample recruitment and management. Utilizing the G*Power calculator with parameters set for a two-tailed test, an effect size (*dz*) of 0.45, an alpha of 0.05, and a power of 80%, the recommended sample size for our study was determined to be *41 participants*. This choice aims to ensure robustness in detecting the intended effects while acknowledging the limitations and findings of preceding studies.

Therefore, 41 Healthy young adults (19 female) with a mean age of 25 years (SD = 3.15) were recruited via university-wide recruitment posts. The participants were then screened by physicians for eligibility and to obtain their informed consent. The exclusion criteria included substance abuse, a pre-existing psychiatric, neurological, endocrinological, auto-immune disorder or severe illness, as well as any contraindication to undergo magnetic resonance imaging (MRI), such as non-MRI-compatible metal implants (Winter et al. 2021), claustrophobia or pregnancy. Participants were instructed to abstain from consuming alcohol and caffeine on the day before each MRI session. The female participants recruited for this study were not in the menstruation phase during data acquisition. The study received approval from the Local Ethics Committee of the Faculty of Medicine at Ludwig Maximilian University of Munich, Germany. All participants voluntarily signed informed consent documents and were monetarily compensated for their contribution to the study.

2.2 | Experimental Design

The study protocol was divided into three separate visits. During the initial visit, participants were informed about the details of the study. They completed relevant questionnaires consisting of primary socio-demographic data, medical history, and psychometric self-report tests adapted to fit the Research Domain Criteria (RDoC) framework (Cuthbert and Insel 2013) (see Tables S3 and S4).

The study design followed a double-blind, sham-controlled crossover approach. Participants were randomly placed to start with either active or sham tDCS. A seven-day interval was maintained between consecutive MRI sessions to minimize the potential carryover effects of the stimulation. T1-weighted and T2-weighted anatomical sequences were recorded, and four spectroscopy sequences were conducted sequentially. The first sequence served as a baseline measurement before tDCS stimulation. The subsequent two sequences were performed during tDCS. The final spectroscopy sequence took place immediately after the stimulation.

2.3 | Transcranial Direct-Current Stimulation (tDCS)

MRI-compatible tDCS was set up using a neuroConn DC-Stimulator Plus device (neuroConn GmbH, Ilmenau, Germany). Each participant's head was assessed using a customized (electroencephalogram) EEG cap available in different sizes to accommodate individual head sizes with predefined markers on the cap that allow marking reference spots (Padberg et al. 2017) and measured according to the 10–20 international system (Jasper 1958). Two sponges ($7 \times 5\,cm^2$) were placed in the F3 (anode) and F4 (cathode) electrode positions, representing the left and right DLPFC, respectively. Sponges were soaked with isotonic saline solution and were secured using rubber bands (see Figure 1b). The active stimulation paradigm involved an electrical current of 2 mA applied over 20 min. A ramp-up/ramp-down sequence of 15 s was used in

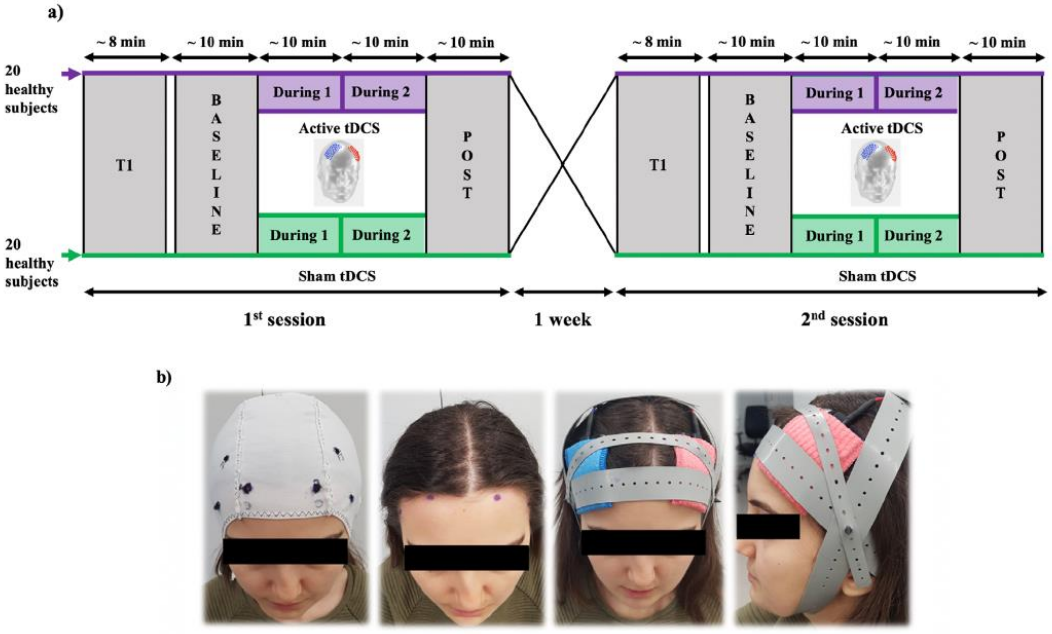

FIGURE 1 | Overview of the tDCS protocol. (a) Study Protocol: Assessment of 20-min, 2 mA active or sham prefrontal tDCS at four times, using an adapted MRS sequence. (b) tDCS Setup: Anodal electrode (red) positioned over F3 (left DLPFC) and cathodal electrode (blue) over F4 (right DLPFC), according to the 10–20 system EEG. Predefined cap spots mark F3 and F4 locations.

the sham protocol to induce tactile sensations. Impedance values separately for each condition are provided in Table S6.

2.4 | MRI Acquisition

A Siemens PRISMA 3T MRI scanner (Siemens Healthcare, Erlangen, Germany) was used to acquire MRS data, employing a 64-channel phased-array head coil. A structural T1 sequence was acquired for subsequent image processing to obtain segmented brain structures. The sequence utilized a three-dimensional magnetization-prepared rapid gradient echo (MPRAGE) with the following parameters: repetition time (TR) = 2300 ms, echo time (TE) = 2.98 ms, and field of view (FoV) = 256 mm × 240 mm. The in-plane resolution for the T1-weighted imaging was 1 mm × 0.938 mm, with a voxel size of 1 mm × 0.938 mm × 1 mm. Additionally, the parameters for the T2-weighted sequence included TR = 3200 ms, TE = 408 ms, and FoV = 230 × 230 mm. The in-plane resolution for the T2-weighted imaging was 0.898 mm × 0.898 mm, with a voxel size of 0.898 mm × 0.898 mm × 0.90 mm.

2.5 | MRS Acquisition

The MEGA PRESS sequence was employed with the following parameters: TR = 2000 ms, TE = 68 ms, spectral bandwidth = 2000 Hz, 144 averages, and editing pulses applied to the GABA spins. These editing pulses were explicitly targeted at 1.9 ppm to selectively refocus the GABA spins (ON-signal), while pulses at 7.5 ppm were used not to affect the GABA spins (OFF-signal). This approach incorporates macromolecular signals and is commonly called GABA+ (Mullins et al. 2014). After the metabolite spectra, unsuppressed water spectra are collected in a separate scan to enable concentration reference to tissue water using the same parameters, except for fewer averages (8).

Manual high-order shimming was employed to enhance the uniformity of the magnetic field, which is essential for the precision of MRS. This step addresses the critical influence of field homogeneity on acquisition sensitivity, water suppression efficacy, and spatial alignment accuracy, as highlighted by Juchem and De Graaf (2017). Furthermore, the procedure mitigates potential field drifts and artifacts induced by coil heating during consecutive sequence executions, which are known to compromise editing accuracy. Manual highorder shimming process resulted in a mean B0 value of 19.89 Hz (detailed in Table S6) aligning with the recommended specifications for Siemens 3T MRI scanners. To ensure the reliability and comparability of spectroscopy data, acquisition parameters were rigorously standardized across all sequences.

T1-weighted images were used for three-dimensional (3D) reconstruction, and a manually placed isotropic 2.2 cm^3 MRS single voxel was positioned in the left DLPFC of each participant. Anatomical landmarks, including the superior frontal sulcus, corpus callosum, and lateral fissure, were utilized for voxel placement, similar to the method described by Brambilla et al. 2005. We assessed voxel placement accuracy to ensure consistency between stimulation sessions (active vs. sham) and between participants (active first vs. sham first). Our analyses suggested that voxel placements were consistent across the conditions. Detailed statistical analyses, including within-subject and between-subject consistency measures, are provided in the Supporting Information (see Figure S5). The MEGA PRESS acquisition sequence assessed GABA and other brain metabolites from the selected voxel location (Mullins et al. 2014).

MRS data was acquired at three time intervals: before (baseline), during, and after stimulation (post). To assess the reliability of tDCS, two separate "during" MRS sequences ("during1" and "during2") were performed and analyzed independently. The "during1" was initiated 10 s after tDCS started, and both sequences were run continuously without interruption (see Figure 1a). Participants were instructed to minimize their movements to reduce motion artifacts during the one-hour-long MRS data acquisition. Further details on MRS acquisition parameters can be found in Table S2.

2.6 | MRS Analysis

The MRS data was exported as .rda files. Therefore, preprocessing was performed on the Siemens scanner, including coil combination, alignment of individual averages, and spectral averaging following Near et al. (2021).

LCModel (Linear Combination Model, Version 6.3-1R), a reliable and model-free method for analyzing brain metabolites, was employed (Provencher 1993, 2001). Basis sets for MEGA-PRESS off-spectrum (see Figure 2a) and MEGA-PRESS difference spectrum (see Figure 2b) were generated using density matrix simulations of the sequence by Dr. Jim Murdoch, incorporating published values for chemical shifts and J-couplings (Kaiser et al. 2008). The fitting range was set to 0.2–4 ppm. For in vivo data, eddy current correction and water scaling were applied within LCModel analysis. For further details on the GABA signal isolation using MEGA-PRESS editing, please see Figure S1.

We utilized Osprey (version 2.5.0; Oeltzschner et al. 2020) for voxel registration and tissue segmentation. The co-registration module of Osprey, integrated within SPM12 (Ashburner et al. 2014), enabled precise alignment of MRS voxels with T1-weighted MRI images, which was confirmed through visual inspection and Euclidean distance measurements between the spatial coordinates of the MRS voxel center and anatomical MRI landmarks (see Supporting Information, Validity of the Voxel Placement section). Subsequently, we applied a cerebrospinal fluid (CSF) correction factor for accurate metabolite concentration adjustments, enhancing the reliability of our neurochemical measurements. The concentrations of CSF-corrected Glu, Glx, GABA, and NAA were expressed in millimolar (mM).

2.7 | Computational Modeling of Electrical Fields

We used SimNIBS 4.0 software to calculate head models based on the T1- and T2-weighted MRI images and simulated these models. The process was automated with a Python script optimized for multi-core computing systems. The simulations were

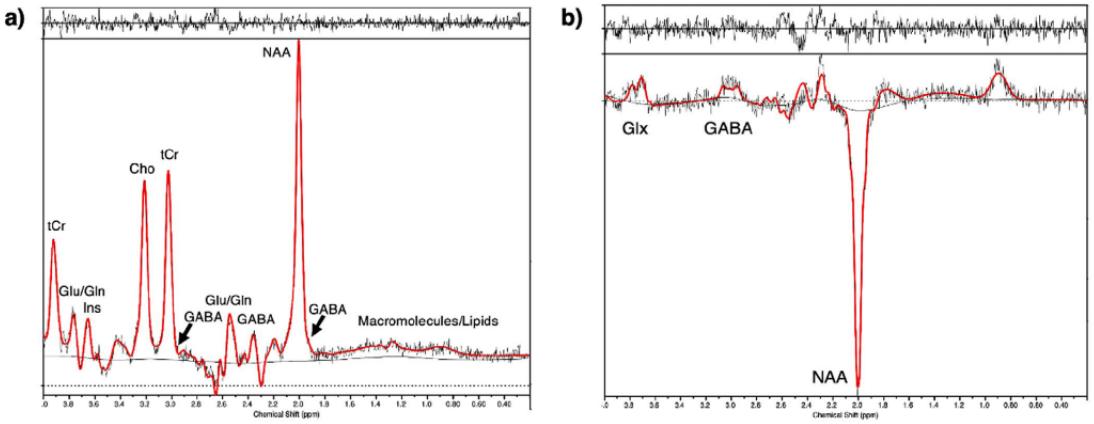

FIGURE 2 | Overview of spectroscopy analysis. (a) Example of an LCModel Output MR Spectrum: Display of metabolites, including Inositol (Ins), Total Creatine (tCr), Gamma-Aminobutyric Acid (GABA), N-acetylaspartic Acid (NAA), Choline (Cho), Glutamate/Glutamine (Glu/Gln). (b) Single Subject LCModel Output with MEGA-PRESS Spectra: Presentation of MEGA-PRESS spectra; the red line indicates the fit, and the black line shows the edited spectrum.

configured to compute and save the electric field magnitude (|E|) and vector (E). The resulting data were mapped to various anatomical and standardized spaces, including the subject's middle gray matter surface, FreeSurfer's FSAverage template, NIfTI volumes, and MNI space. For electrode configuration, two rectangular electrodes, each measuring 50 mm × 70 mm with a thickness of 9 mm, were positioned over the scalp locations F3 and F4. The orientation of each electrode was defined relative to the other, with the electrode over F3 oriented towards F4. A bipolar montage was established, with +2 mA applied to the electrode over F3 and −2 mA to the electrode over F4. After the simulation, the resulting electric field data were loaded, and the 75 percentile of the electric field magnitude was computed (see Figure 3 for electric field distribution variability and MRS ROI alignment).

2.8 | Statistical Analysis

Outlier identification was conducted in two phases. First, we employed the median absolute deviations method (MAD), using the "mad" function in R, classifying values exceeding a threshold of 2.5 as outliers in line with the methodologies of He et al. (2021) and Leys et al. (2013). Accordingly, outliers were identified in the metabolite data: one for Glx, six for Glu, seven for NAA, and five for GABA across various conditions. These outliers were marked as missing values separately for each metabolite dataset to prevent the loss of useful data. The "lme4" package was applied in linear mixed-effects models (LMMs) to manage the missing data. Second, the quality control of MRS data was assessed using criteria established by Wilson et al. (2019), defining good quality data as meeting specific thresholds: Cramér-Rao Lower Bound (CRLB) below 20%, a signal-to-noise ratio (SNR) exceeding 3, and water linewidths at half height (FWHM) below 0.1 ppm, as recommended in study of Lin et al. (2021) and detailed in the Supporting Information (see Table S5). Following these guidelines, the entire dataset of one subject was excluded from further Glu, NAA, and Glx analyses. The analysis, focusing on metabolite concentrations, was conducted with data from 40 participants (19 female, average age 25 years, range 19–35 years) who met our stringent quality criteria.

Statistical analyses were conducted using the R programming language (version 4.2.2). The study utilized alpha = 0.05 as the threshold for determining statistical significance. To investigate the effects of active versus sham stimulation on metabolite concentration over time, we employed LMM using the R package "lme4" (Bates et al. 2015). The LMMs included fixed effects for the stimulation (active vs. sham) and time (during1, during2, post). To control for potential confounding factors, sex, age, and condition order (sham-first vs. active-first) were included as covariates. Subjects were entered as random intercepts to account for inter-individual variations in baseline metabolite concentrations. To account for subject-specific differences in metabolite change rates, we introduced a random slope for time, which was retained if it significantly improved model fit as measured with the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) parameters, following our approach described in Mezger et al. (2021). The significance of model factors was determined with Type-III analyses of variance using the Satterthwaite method. Finally, Bonferroni-corrected post hoc analyses were conducted to follow up on the significant probe differences in each time point of the full-factorial LMM. The detailed statistical values are presented in a table in the Supporting Information in the manuscript (see Tables S7 and S8).

Following the identification of significant metabolite changes, we conducted two e-field analyses to investigate potential relationships between e-field magnitudes and metabolite changes, as well as sex differences in e-field distributions. First, we conducted Pearson correlation analyses between the 75th percentile e-field magnitudes and the differences in metabolite levels between active and sham conditions across different time points. Second, independent samples *t*-tests were used to compare e-field magnitudes between males and females.

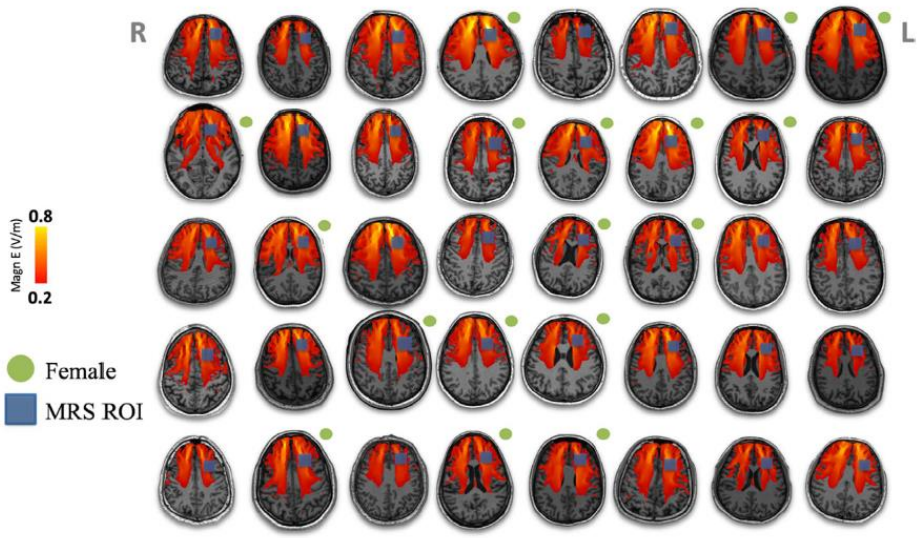

FIGURE 3 | Electric field distribution variability and MRS ROI alignment. Axial MRI slices from a cohort of healthy subjects, each of whom underwent computational modeling of tDCS using SimNIBS 4.0. The simulations were performed with a 2 mA current applied via electrodes positioned at the F3 and F4 locations on the scalp. The overlaid color maps depict the resulting electric field (E-field) distribution within the brain tissue, specifically illustrating the magnitude of the electric field (|E|) in volts per meter (V/m). The color scale indicated that the E-field distributions were thresholded from 0.2 to 0.8 V/m. Regions of lower E-field magnitudes are represented in red, while yellow denotes regions of higher E-field magnitudes. The variability in E-field distribution across different subjects is captured in these slices, emphasizing differences in individual neuroanatomy and their impact on field intensity. The blue rectangular overlay in each brain image marks the location of the MRS Region of Interest (ROI), centered on a 22 mm^2 area placed individually on the DLPFC region. Subjects identified as female are denoted by green dots adjacent to their respective brain slices. Right hemisphere (R) presented on the left side and the left hemisphere on the right side (radiological convention).

3 | Results

3.1 | Effects of tDCS on Glu, NAA, and GABA Concentrations in the DLPFC

We examined changes over time in Glu, Glx, NAA, and GABA concentrations in the active versus sham conditions (see Figure 4). Results of χ^2-likelihood-ratio tests to compare the fit of the nested models (i.e., with and without random slope term) did not show significant improvements in model fit for all outcomes (all $ps > 0.05$) thus, models with random intercepts and fixed slopes were used for further analysis. Neither the main effects of stimulation and time nor their 2-way interactions for Glu, NAA and GABA concentrations reached statistical significance for the outcomes (all $ps > 0.05$). Consequently, the data did not provide evidence to suggest that prefrontal tDCS impacted Glu, NAA, or GABA concentrations.

Concentrations of metabolites detectable with the basis set, including myo-inositol (mI), creatine and phosphocreatine (Cr_PCr), and glycerophosphocholine and phosphocholine (GPc_PCh), did not show significant changes following active compared to sham tDCS (see Figure S4).

3.2 | Effects of tDCS on Glx Concentrations in the DLPFC

There was a significant main effect of stimulation ($F_{(1, 193.18)} = 6.51$, $p = 0.011$) and an interaction effect between stimulation and time ($F_{(2, 188.96)} = 3.10$, $p = 0.047$; see Figure 4b). Bonferroni-corrected post hoc analyses revealed significant differences between active and sham stimulation for the "during2" $t(190) = 1.973$, $p_{Bonferroni} = 0.049$ and "post" $t(190) = 2.930$, $p_{Bonferroni} = 0.01$ measurements. Note that the post-stimulation effect would remain significant even when we Bonferroni correct for the number of investigated metabolites ($p = 0.04$). During the active stimulation at the "during2" time point, Glx concentration increased significantly compared to the sham condition (active = 0.10, SE = 0.59, sham = −0.19, SE = 0.70), $\beta = -0.37$, 95% CI [−0.57, −0.01]. This increase was sustained in the post-stimulation phase (active = 0.16, SE = 0.76, sham = −0.27, SE = 0.71), $\beta = -0.51$, 95% CI [−0.76, −0.12]. Thus, DLPFC-targeted tDCS increased prefrontal Glx concentrations. To further explore individual differences in response to stimulation, a cluster analysis was conducted to identify participants driving the interaction effects observed in Glx metabolite concentrations (see Supporting Information and Figure S6 for details).

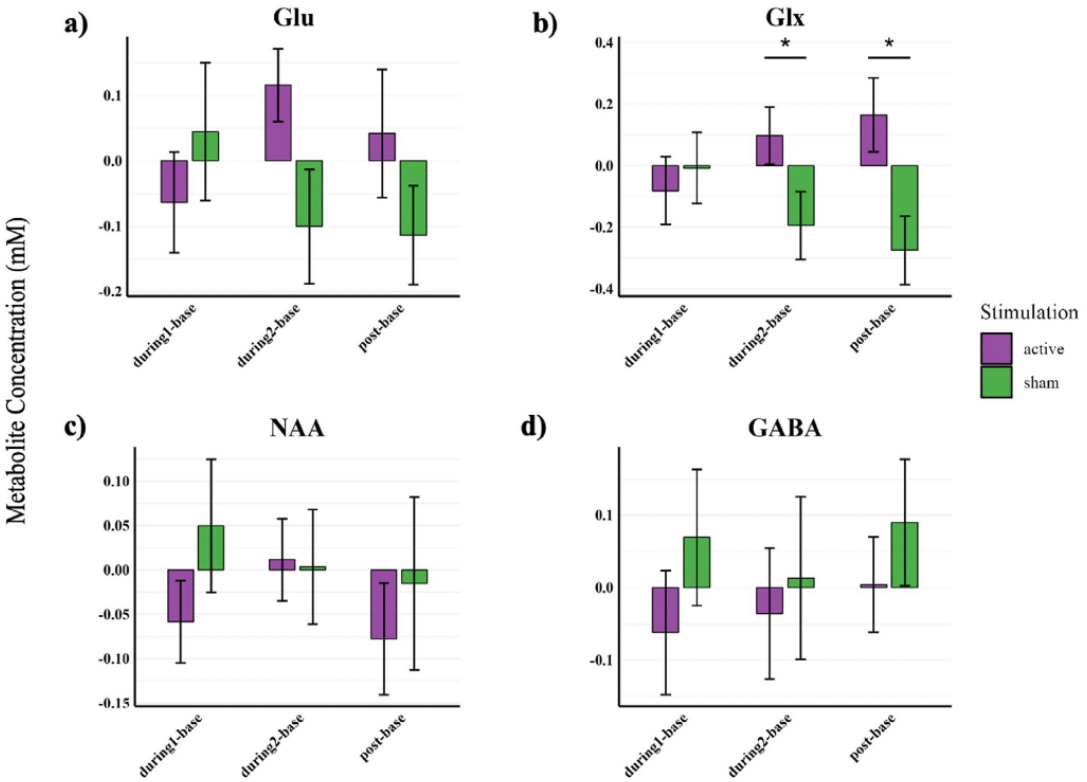

FIGURE 4 | Baseline-Corrected Concentrations of Glu, Glx, NAA, and GABA During and After Active and Sham tDCS Stimulation. This figure illustrates the relationship between baseline-corrected concentrations of metabolites (Glu, Glx, NAA, and GABA) and the administration of active versus sham tDCS. The observed effects on stimulation are derived from the left DLPFC as measured by MRS. †Error bars represent standard errors.

3.3 | Computational Modeling of Electrical Fields

We performed a correlation analysis between the 75th percentile magnitude of the e-fields and the difference between active and sham stimulation to examine whether field strength influences the strength of the stimulation effect. The reason we chose this intensity is because targeting the 75% magnitude of the electric field can help achieve more focused and localized stimulation (Saturnino et al. 2019).

Correlation analyses revealed a significant positive relationship between individual e-field strengths and the difference in Glx levels during the first interval, $r(35) = 0.38$, $p = 0.020$, 95% CI [0.06, 0.63]. However, this relationship was not significant for the second interval, $r(35) = 0.29$, $p = 0.081$, 95% CI [−0.04, 0.56], or post-stimulation, $r(35) = 0.05$, $p = 0.749$, 95% CI [−0.27, 0.37] (see Figure 5).

These findings suggest that individual differences in tDCS effects on metabolites cannot be explained by variations in the induced e-fields at least during the second or post-stimulation intervals.

3.4 | Additional Analysis of Sex Effects

In previous analyses, sex was incorporated as a covariate to consider its potential effects. Expanding on the sex differences identified by Mezger et al. (2021), sex was further included as an interaction term with "stimulation" and "time." Additionally, age and order were reintroduced as covariates in the LMMs separately for all dependent variables. As a result, there were no significant main effects or interactions, including the factor sex for Glx and Glu (all $ps > 0.05$). In contrast, significant stimulation × sex interactions were observed for NAA, $F_{(1, 173.66)} = 8.29$, $p = 0.004$, and GABA, $F_{(1, 176.79)} = 5.86$, $p = 0.016$. Bonferroni-corrected post hoc analysis revealed that in GABA levels, females demonstrated significant differences between the active and sham conditions, $t_{(172)} = -2.37$, $p = 0.019$, Cohen's $d = -0.2$, CI [−0.35, −0.05] (active $M = -0.18$, sham $M = 0.07$, mean difference = −0.24, SE = 0.1), indicating a decrease in GABA levels following active stimulation. For NAA levels, significant contrasts were found for females between conditions, $t_{(173)} = -2.21$, $p = 0.028$, Cohen's $d = -0.34$ (active = −0.04, sham = 0.11, mean difference = −0.15, SE = 0.07). Male participants on the other side, showed no significant

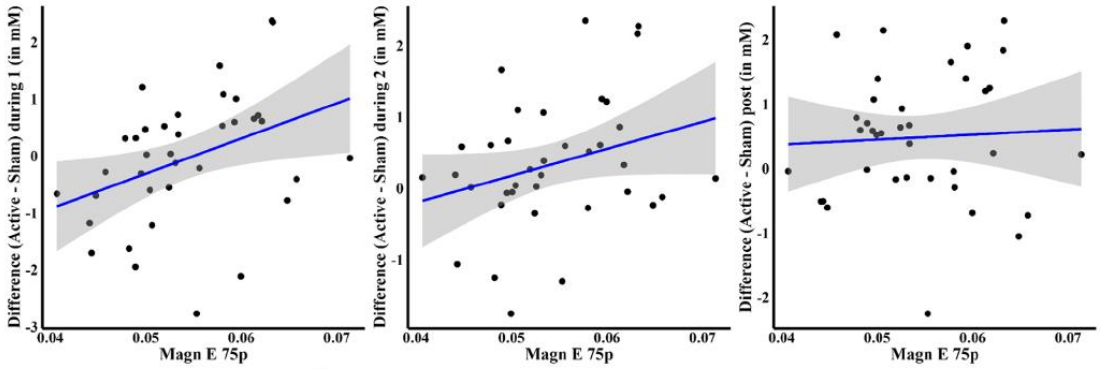

FIGURE 5 | Correlations between e-field magnitude (75th Percentile) and Glx Levels at tDCS time points. This figure illustrates the relationship between the 75th percentile of the e-field magnitude and the difference in Glx levels between active and sham conditions during the first and second intervals of tDCS and post-stimulation. The plots show the correlation for these differences, with each point representing a participant. The regression trend lines are displayed with shaded areas indicating the 95% confidence intervals.

condition differences in either NAA ($t_{(181)} = 1.86$, $p = 0.065$) or GABA metabolite ($t_{(183)} = 1.06$, $p = 0.292$). In other words, female participants showed a significant reduction when exposed to active stimulation compared to sham, suggesting a sex-dependent differential response to tDCS in NAA and GABA modulation. Importantly, this difference is not attributable to variations at specific time points (during, and after stimulation). See Figure S2 for baseline-corrected concentrations of NAA, Glu, Glx, and GABA by sex and time point. To further support these findings, we conducted additional one-sample t-tests to examine changes in active and sham stimulation conditions compared to zero when data were averaged across the time points for each sex group. Results indicated that female participants showed marginally significant reductions in mean NAA, $t(15) = -1.58$, $p = 0.07$, and GABA, $t(15) = -1.44$, $p = 0.08$, values in the active stimulation condition. No other significant effects were observed (all $ps > 0.05$). These additional analyses provide further support for the observed sex-specific effects in response to active stimulation. See Figure S3 for Baseline-Corrected Mean Concentrations of NAA and GABA by Sex and Type of tDCS.

The results from the e-field magnitudes to explore potential sex differences showed no significant differences in e-field magnitudes between males and females ($t(38) = 0.717$, $p = 0.477$, Cohen's $d = 0.23$), indicating that the observed sex differences in tDCS effects are not due to variations in e-field distribution.

4 | Discussion

In this study, we have investigated the impact of bifrontal tDCS on brain metabolites in the left DLPFC, a region crucial for cognitive processes and large-scale brain networks. Our results showed a significant increase in Glx levels during the second phase of prefrontal stimulation, persisting for at least 10 min post-stimulation, while no significant differences emerged in other neurotransmitter concentrations (GABA, NAA, and Glu). Moreover, our results showed sex-specific effects, with females displaying lower levels of NAA and GABA metabolites in response to tDCS consistent across the time points.

4.1 | Bifrontal tDCS Effect on Glu and Glx Concentrations

Compared to the sham condition, the increase in Glx observed following active stimulation suggests tDCS may modulate neuronal metabolism and neurotransmitter synthesis potentially by altering neuronal membrane potential, and increasing neuronal excitability (Ruffini et al. 2013; Modolo et al. 2018). However, the absence of a measurable change in Glu concentration indicates that other mechanisms may be involved. Some studies propose that tDCS could influence astrocytic function and, consequently, the Glu/Gln cycle (Ruohonen and Karhu 2012; Monai and Hirase 2018; Saidi and Firoozabadi 2021). Given that astrocytes rapidly uptake Glu and convert it into Gln (Norenberg and Martinez-Hernandez 1979; Pow and Robinson 1994), this may contribute to the increased Glx levels without significant changes in Glu. Further research is necessary to clarify the underlying mechanisms and the potential role of glial cells in tDCS-induced effects.

The delayed increase in Glx levels observed during and after tDCS without a significant change in the initial 10 min of stimulation aligns with the concept of time-dependent effects of tDCS. This is consistent with Mezger et al.'s (2021) findings, who reported a reduction in glutamate in female participants specifically during the second stimulation phase. This suggests that a temporal build-up is necessary for prefrontal tDCS to induce its effects on brain metabolites.

Few studies have measured Glx levels during stimulation without detecting any significant changes (Hone-Blanchet, Edden, and Fecteau 2016; Dickler et al. 2018). Prolonged tDCS effects, including increased Glx concentrations have been also reported in various regions, such as the frontal cortex (Mugnol-Ugarte et al. 2022) and the right parietal lobe

(Clark et al. 2011). However, differences in study protocols, and methodologies, complicate direct comparisons with our results.

The observed changes in Glx levels in the present study may offer therapeutic potential for conditions with glutamatergic dysfunction, such as SCZ and MDD. The sustained effects on Glx suggest ongoing neurometabolic alterations, highlighting tDCS's potential in conditions requiring continuous neurometabolic modulation. This warrants further investigation through longitudinal and clinical studies using MRS imaging.

4.2 | Bifrontal tDCS Effect on NAA Concentrations

There was no effect of active tDCS on NAA levels. This finding is consistent with previous studies focusing on other brain areas, including the motor cortex (Nwaroh et al. 2020; Rango et al. 2008; Ryan et al. 2018; Stagg et al. 2009; Tremblay et al. 2014), as well as research examining the parietal (Clark et al. 2011) and temporal cortices (Koolschijn et al. 2019). Similar observations were made in a study by Dickler et al. (2018), which employed bifrontal tDCS with F4 as the anodal site in patients with gambling disorder. They used a current intensity of 1 mA for 30 min and reported no significant changes in NAA levels in active tDCS compared to sham, further supporting our findings.

To our knowledge, only one study conducted by (Hone-Blanchet, Edden, and Fecteau 2016) investigated the effects of bifrontal tDCS on NAA levels at the F3 site in the DLPFC. This study reported an increase in NAA levels during active tDCS over the left DLPFC, a change that did not persist post-stimulation. The reason of this difference may be due to variations in stimulation intensity and duration. While Hone-Blanchet et al. used a current intensity of 1 mA for 30 min, our study applied 2 mA over the left DLPFC for 20 min. We based our choice of intensity and duration on what is recognized as standard practice in prior tDCS studies targeting major depression and schizophrenia (Boggio et al. 2008; Dondé et al. 2017; Loo et al. 2012). Additionally, the 20-min duration aligns with our previous research and common practices in the field (Loo et al. 2012; Mezger et al. 2021; Palm et al. 2012, 2016; Wörsching et al. 2018). In summary, the impact of tDCS on NAA metabolites appears to be modulated by several factors, including the stimulation site, the targeted brain region, and specific stimulation parameters.

4.3 | Bifrontal tDCS Effect on GABA Concentrations

Previous research has primarily focused on the effects of motor cortex stimulation, demonstrating a decrease in GABA levels following tDCS (Antonenko et al. 2017, 2019; Bachtiar et al. 2015; Kim et al. 2014; O'Shea et al. 2017; Stagg et al. 2009). In contrast, a significant body of research has reported no change in GABA levels from stimulation of the motor cortex (Nwaroh et al. 2020; Tremblay, Lee, and Rudy 2016), occipital lobe (O'Shea et al. 2017), right cerebellum (Jalali et al. 2018), and left posterior superior temporal gyrus (Dwyer et al. 2019). The heterogeneity in these findings could be attributed to several factors, including variations in stimulation intensity (1–3 mA), duration (10–30 min), GABA analysis methods (LCModel, JMRUI, TARQUIN, and Gannet), editing techniques (MEGA-PRESS, MEGA-SLASER, and MEGA-SPECIAL) and sample sizes (ranging from 8 to 69). Accurately measuring GABA metabolites is challenging because of their uneven distribution and low concentrations in the brain (Chang, Cloak, and Ernst 2003; Rothman et al. 1993). Furthermore, the overlap of GABA signals with macromolecules like creatine necessitates various editing techniques to ensure accurate detection (Andreychenko et al. 2012). These methodological variabilities underscore the need for cautious interpretation of results across different studies.

While numerous studies have quantified GABA concentration following motor cortex stimulation, research on DLPFC positioning remains limited. In our study, no significant changes in GABA concentration were observed during or after stimulation compared to the sham condition, a finding consistent with Hone-Blanchet, Edden, and Fecteau (2016), who also reported no significant changes in the left DLPFC. This may suggest that the DLPFC exhibits a different response to stimulation than the motor cortex. Its extensive connectivity and association with cognitive functions could make detecting neurochemical changes difficult. However, the inability to detect neurotransmitter changes does not necessarily imply that tDCS is ineffective on these metabolites. It highlights the necessity to explore optimal stimulation parameters that could induce detectable changes.

4.4 | Sex Differences in NAA and GABA

The investigation of sex-specific variations in brain morphology is of significant scientific interest, primarily due to the potential influence of hormones on GABA-ergic and glutamatergic neurotransmission (Zheng 2009; O'Gorman et al. 2011; Spurny-Dworak et al. 2022). Our study found significant sex-by-stimulation interactions for NAA and GABA levels, indicating different tDCS effects in males and females. Females showed significant differences between active and sham conditions for both metabolites, while males did not. These results suggest that active tDCS significantly reduces GABA and NAA levels in females. Importantly, no three-way interaction was found between sex, stimulation, and time. This indicates that these effects do not significantly vary across the specific time points (pre, during, and post-stimulation) and are attributable to tDCS itself.

Previous research by Mezger et al. (2021) also reported stronger Glu reductions in females from the cathodal side, emphasizing the need to consider sex as a critical factor in neurostimulation research. The lack of significant Glu and Glx changes in our study could be attributed to the specific MRS voxel location and the variability in individual responses to tDCS, as noted by Mezger et al. (2021). These findings highlight the importance of conducting sex-matched studies in MRS research (Endres et al. 2016; Spurny-Dworak et al. 2022).

4.5 | Variability in tDCS Response

Interpreting the effects of DC stimulation on neural activity can be challenging due to inter-individual variability influenced by factors such as anatomical differences, tissue properties, hormonal levels, age, sex, and brain state (Tremblay, Lee, and Rudy 2016; Krause, Márquez-Ruiz, and Kadosh 2013; Bhattacharjee et al. 2022). As clarified in the previous section, the reported sex differences were not attributable to specific time points in the study. In addition, observed sex differences in tDCS effects are unlikely to be explained by differences in e-field magnitudes, as no significant differences were found between males and females in this study. Regarding the hormonal effects, we ensured that female participants were not menstruating during data acquisition. However, the study did not systematically control for all phases of the menstrual cycle (e.g., follicular, ovulation, and luteal). This could have introduced variability in the neurometabolic data (Chrzan, Tomaszuk, and Urbanik 2013; De Bondt et al. 2015; Epperson et al. 2005).

Finally, all participants were advised to abstain from alcohol and caffeine before MRI sessions to avoid confounding effects on brain metabolism. While one study (Oeltzschner et al. 2018) found that acute caffeine intake does not significantly impact certain metabolite levels (GABA, Glu, Glx, and NAA), habitual caffeine consumption may influence baseline levels. Future studies should investigate the interaction between caffeine intake and tDCS effects with MRS to determine its impact on the metabolites measured and to better understand its role in brain chemistry.

4.6 | Limitations and Future Research

The study focused exclusively on the acute effects during and immediately after tDCS administration and disregarded possible long-term changes in brain metabolites. Future research should address these limitations through longitudinal studies and explore the lasting effects of tDCS on brain metabolism.

Non-smoking was specified as an exclusion criterion in our recruitment materials; however, six regular smokers were unintentionally included in the study (see Table S3). This inclusion could affect brain metabolite profiles, emphasizing the complexities of participant selection and its impact on research outcomes as noted by O'Neill et al. (2023).

Higher magnetic fields like 7T offer better signal-to-noise ratio and spectral resolution, allowing clearer separation of overlapping metabolites. Our study successfully used 3T MRI with the MEGA-PRESS technique to achieve our research goals, effectively editing, and enhancing specific metabolite signals. However, future studies might benefit from using 7T MRI. This could improve the separation of glutamate and glutamine for more precise Glx quantification, enable more accurate measurement of low-concentration GABA, and potentially detect subtle NAA level changes. These advantages could further clarify the time-dependent effects of tDCS on brain metabolites, including the delayed Glx increase we observed.

Finally, this study primarily focused on metabolite concentrations at the stimulation site, with limited consideration for the surrounding areas. However, tDCS effects can extend beyond the targeted region (Hone-Blanchet, Edden, and Fecteau 2016). Multivoxel MRS methods could offer a deeper understanding of the wider impacts of tDCS on brain metabolites.

4.7 | Strengths

This study is noteworthy for several reasons, including its implementation of placebo control, a double-blind cross-over design, and large sample size. This study includes one of the largest sample sizes to date for investigating *prefrontal* tDCS with simultaneous MRS in healthy individuals. With the help of our sample size, we created comparably sized male and female subgroups, enabling the observation of stimulation effects across both sexes.

Importantly, the study integrates a real-time MRS approach as it allows for assessing the acute effects of stimulation. Moreover, including baseline measurements and two separate time windows during the tDCS session allowed us to correct metabolite levels for pre-existing differences and observe the gradual accumulation of stimulation effects on metabolites, offering a statistical insight into the temporal dynamics of the response.

5 | Conclusion

Our investigation suggests that prefrontal tDCS may modulate Glx levels within the left DLPFC, highlighting its potential for neuromodulatory interventions. The modulation observed beyond the stimulation period indicates that tDCS could be further explored as a possible approach for targeted treatment of psychiatric disorders and personalized medicine. Future research should focus on understanding the factors underlying sex differences, optimizing stimulation protocols, and evaluating the broader clinical implications of these findings.

Author Contributions

Gizem Vural: investigation, data curation, formal analysis, writing original draft. **Aldo Soldini:** investigation, data curation, review and editing. **Frank Padberg:** supervision, conceptualization, funding acquisition. **Berkhan Karslı:** review and editing. **Artyom Zinchenko:** formal analysis, review and editing. **Stephan Goerigk:** formal analysis, review and editing. **Alexander Soutschek:** formal analysis, review and editing. **Eva Mezger:** review and editing. **Sophia Stoecklein:** review and editing. **Lucia Bulubas:** review and editing. **Antonia Šušnjar:** data curation, writing-review and editing, supervision. **Daniel Keeser:** supervision, conceptualization, review and editing.

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Conflicts of Interest

This work is a part of G.V.'s PhD program at Munich Medical Research School. F.P. is a member of the European Scientific Advisory Board of Brainsway Inc., Jerusalem, Israel, and the International Scientific Advisory Board of Sooma, Helsinki, Finland. He has received speaker's honoraria from Mag&More GmbH, the neuroCare Group, Munich, Germany, and Brainsway Inc. His lab has received support with equipment from neuroConn GmbH, Ilmenau, Germany, Mag&More GmbH, and Brainsway Inc. A.S., B.K., A.Z., S.G., A.S., E.M., S.S., L.B., A.S., and D.K. reported no potential conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in MRSDC2 at https://osf.io/3axvf/?view_only=40cd66fa864e4f959b81e80a7f8ef196.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

7. Paper II

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ORIGINAL PAPER

Neurocognitive function as outcome and predictor for prefrontal transcranial direct current stimulation in major depressive disorder: an analysis from the DepressionDC trial

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Abstract
Transcranial direct current stimulation (tDCS) of the prefrontal cortex might beneficially influence neurocognitive dysfunctions associated with major depressive disorder (MDD). However, previous studies of neurocognitive effects of tDCS have been inconclusive. In the current study, we analyzed longitudinal, neurocognitive data from 101 participants of a randomized controlled multicenter trial (DepressionDC), investigating the efficacy of bifrontal tDCS (2 mA, 30 min/d, for 6 weeks) in patients with MDD and insufficient response to selective serotonin reuptake inhibitors (SSRI). We assessed whether active tDCS compared to sham tDCS elicited beneficial effects across the domains of memory span, working memory, selective attention, sustained attention, executive process, and processing speed, assessed with a validated, digital test battery. Additionally, we explored whether baseline cognitive performance, as a proxy of fronto-parietal-network functioning, predicts the antidepressant effects of active tDCS versus sham tDCS. We found no statistically significant group differences in the change of neurocognitive performance between active and sham tDCS. Furthermore, baseline cognitive performance did not predict the clinical response to tDCS. Our findings indicate no advantage in neurocognition due to active tDCS in MDD. Additional research is required to systematically investigate the effects of tDCS protocols on neurocognitive performance in patients with MDD.

Keywords Transcranial direct current stimulation · Non-invasive brain stimulation · Major depressive disorder · Depression · Cognition · Neurocognitive tests

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Introduction

Transcranial Direct Current Stimulation (tDCS) is a form of non-invasive brain stimulation (NIBS) that utilizes electrodes on the scalp to create a weak electrical current in order to modulate cortical excitability [1]. In the treatment of major depressive disorder (MDD), anodal tDCS is usually applied over the left dorsolateral prefrontal cortex (DLPFC) [2], a brain area which contributes to frontoparietal network (FPN) function [3]. The FPN plays a central role for several cognitive domains, like attention[4], working memory [5], memory span [6] executive function [7], processing speed[8], and cognitive control [9]. Poor performance in these cognitive domains has also been associated with depressive disorders [10–14]. Therefore, it seems plausible that stimulation of the FPN could influence performance in these domains and that baseline cognitive performance, as a proxy of FPN functioning, could predict the clinical effects of stimulation.

Previous studies have investigated the neurocognitive effects of tDCS when applied to the DLPFC in patients with MDD reporting significant time-dependent improvements in attention/vigilance, working memory, executive functioning, processing speed, and social cognition when compared to placebo [15–18]. On the other hand, multiple studies report no statistically significant group-by-time interaction effects [19–27]. A recent meta-analysis of the cognitive effects of tDCS across multiple disorders revealed that active tDCS elicited improvements in attention/vigilance, and working memory when compared to sham tDCS [28]. This meta-analysis was based on studies that were very heterogeneous in designs, sample sizes, outcomes, and main findings. Thus, a study with a large sample size would be warranted to further test the effects of tDCS on cognition in patients with MDD. To the best of our knowledge, no studies have investigated baseline cognitive testing as a predictor of affective response to tDCS.

In this ancillary analysis of a triple-blind, randomized, sham-controlled multicenter trial, we investigated whether a standard bifrontal tDCS protocol compared to sham tDCS alters cognitive performance across the domains of memory span, working memory, selective attention, sustained attention, executive functioning, and processing speed. Additionally, we explored whether baseline cognitive performance as a proxy of FPN functioning predicts the antidepressant effects of tDCS versus sham tDCS.

Methods and materials

Study population

We analyzed data from the DepressionDC trial (trial registration number: NCT02530164); a triple-blind, randomized, sham-controlled clinical trial carried out across eight psychiatric centers in Germany [29]. The study investigated the efficacy and safety of tDCS as a treatment for MDD in patients that did not respond to conventional pharmacological treatment with selective serotonin reuptake inhibitors (SSRIs). Patients were originally randomized to receive 24 sessions within 6 weeks of either active or sham tDCS. The montage employed in tDCS involves placing the anode over F3 and the cathode over F4. Active stimulation consisted of a constant 2 mA direct current that lasted for 30 min. The sham paradigm consisted of a ramp-up and ramp-down sequence to induce similar skin sensations as active tDCS. tDCS was applied using a DC-stimulator ('Mobile', neuroConn GmbH, Ilmenau, Germany). Inclusion and exclusion criteria are reported in the supplement. Local ethics committees approved the study at each study site. All participants gave their written informed consent before inclusion in the study. From an initial sample total of 150 patients (intention-to-treat sample), we analyzed the data from 101 patients that had available neuropsychological assessments. Data from 49 patients were missing due to technical errors, organizational difficulties at the local treatment sites, and refusal to participate.

Neurocognitive test battery

Neurocognitive function was assessed longitudinally during the study at baseline, post-treatment (week 6), and at the 6-month follow-up using the EmoCogMeter, a digitalized, validated cognitive test battery developed at the Charite Berlin [30–32]. The EmoCogMeter examines the domains of memory span, working memory, selective attention, sustained attention, executive function, and processing speed. Memory span is tested by a digit-span assessment [33]. Working memory was assessed by an n-back task [13]. A variant of the Stroop test and a working memory component were used to assess selective attention and sustained attention, respectively [34]. executive function was measured by both the Trail Making B [35] and Tower of Hanoi tests [36]. Finally, processing speed was measured using a symbol letter modalities test, a variation of the symbol digit modality test. For additional technical information about the tests, please refer to the supplement.

Further outcome measures

The severity of the depressive episode was assessed by trained clinical staff utilizing the Montgomery-Åsberg Depression Rating Scale (MADRS), which was also chosen for the primary outcome of the study [37]. Severity is classified as an absence of symptoms (0–6 points), mild depressive episode (7–19 points), moderate depressive episode (20–34 points), or severe depressive episode (35–60 points). State and trait anxiety were measured utilizing The State-Trait Anxiety Inventory (STAI) [38], with a threshold of 39–40 for identifying clinically significant anxiety symptoms [39].

Statistical analysis

Statistical analyses were conducted in R, version 4.2.1. results [40]. Results were considered significant at $\alpha = 0.05$. We compared baseline characteristics between treatment groups using Pearson's χ^2 tests and Wilcoxon-rank-sum tests as appropriate. To reduce the effect of extreme test performances, we identified values below the 1% and above the 99% percentile on each task and set them to the respective percentile values (winsorization).

To assess potential treatment effects of active tDCS on cognitive performance, we fitted linear mixed models using the lme4 package [41] to predict change from baseline to week 6 on each cognitive test. Treatment group (active tDCS versus sham tDCS) was included as a fixed effect while controlling for the respective baseline cognitive test score (formula: change in cognitive performance ~ treatment group + baseline cognitive performance). Sensitivity analyses included additional models with sex, age, and baseline MADRS as covariates.

To assess potential predictive influences of baseline cognitive performance on antidepressant treatment effects of active tDCS, we again fitted linear mixed models to predict change from baseline to week 6 on the MADRS. Treatment group, performance on the respective cognitive domain, and their interaction were included as fixed effects while controlling for baseline MADRS scores (formula: MADRS change ~ treatment group x cognitive performance at baseline + baseline MADRS score).

All models included the treatment site as a random effect (formula: ~ 1| site). Significance of the model factors was determined using omnibus tests (Type III ANOVA) with Satterthwaite approximation to degrees of freedom. We did not use imputation since linear mixed models are able to handle missing data. Standardized effect sizes for regression coefficients were computed using the emmeans::eff_size() approach, with the sigma parameter being directly extracted from the regression model [42]. We corrected for multiple testing across predictors using the false-discovery-rate (FDR) method [43].

Results

Sample characteristics

We analyzed data from 101 patients (active tDCS, n = 50; sham tDCS, n = 51). Mean age (active tDCS 39 [SD 14]; sham tDCS 39 [SD 14]; p = 0.76). Sex: active tDCS 40% male; sham tDCS 40% male. Primary baseline and clinical features across the active and sham-tDCS groups were similar (Table 1 and Supplementary Table 1). Winsorized mean test performances and the number of winsorized measurements per cognitive test are reported in supplementary Table 5 and 6.

Treatment effects on neurocognitive test scores

We observed no significant group-by-time interactions between treatment group and memory span, working memory, selective attention, sustained attention, executive function, or processing speed. Pre- and post-treatment performance across neurocognitive tests for active tDCS and sham tDCS is shown in Fig. 1, and Table 2 provides further statistical information. Results for additional models including sex, age and baseline MADRS yielded similar results (supplementary Table 2–4).

Prediction of clinician-rated depression (MADRS)

We did not detect significant interactions, when predicting MADRS change, between treatment group and memory span, working memory, selective attention, sustained attention, executive function, or processing speed. Table 3 provides the effect size of each neurocognitive test at baseline and Fig. 2 depicts the association between baseline cognitive performance and changes in MADRS scores.

Discussion

In this ancillary analysis of the DepressionDC trial, a randomized, sham-controlled multicenter study assessing the antidepressant efficacy of a prefrontal tDCS as acute treatment in patients with MDD and SSRI treatment, we found no statistically significant group differences between active tDCS and sham tDCS for the change of performance in FPN-associated cognitive domains (i.e. memory span, working memory, selective attention, sustained attention, executive function and processing speed) from baseline to week 6. Furthermore, baseline performance in these domains was not

Table 1 Baseline patient characteristics

Characteristic	tDCS, $n=50$[1]	Sham, $n=51$[1]	p value[2]
Sex			0.76
Female	30 (60%)	29 (57%)	
Male	20 (40%)	22 (43%)	
Age (years)	39 (14)	39 (14)	0.98
Age of onset of depression (years)	32 (12)	34 (15)	0.85
Duration of current episode (weeks)	62 (69)	58 (69)	0.66
Schooling (years)	11.84 (1.93)	11.66 (1.72)	0.56
MADRS score	22.8 (6.1)	23.2 (5.3)	0.60
BDI score	27 (12)	28 (11)	0.52
WHO/DAS score	22 (9)	24 (11)	0.32
GAF score	55 (10)	56 (9)	0.98
SHAPS-D score	4.6 (3.0)	5.7 (3.5)	0.14
State-trait anxiety inventory state score	53 (11)	55 (9)	0.53
State-trait anxiety inventory trait score	57 (10)	55 (10)	0.73
CD-RISC score	16 (7)	17 (7)	0.68

[1] *n* (%); mean (SD). [2] Pearson's Chi-squared test; Wilcoxon rank sum test

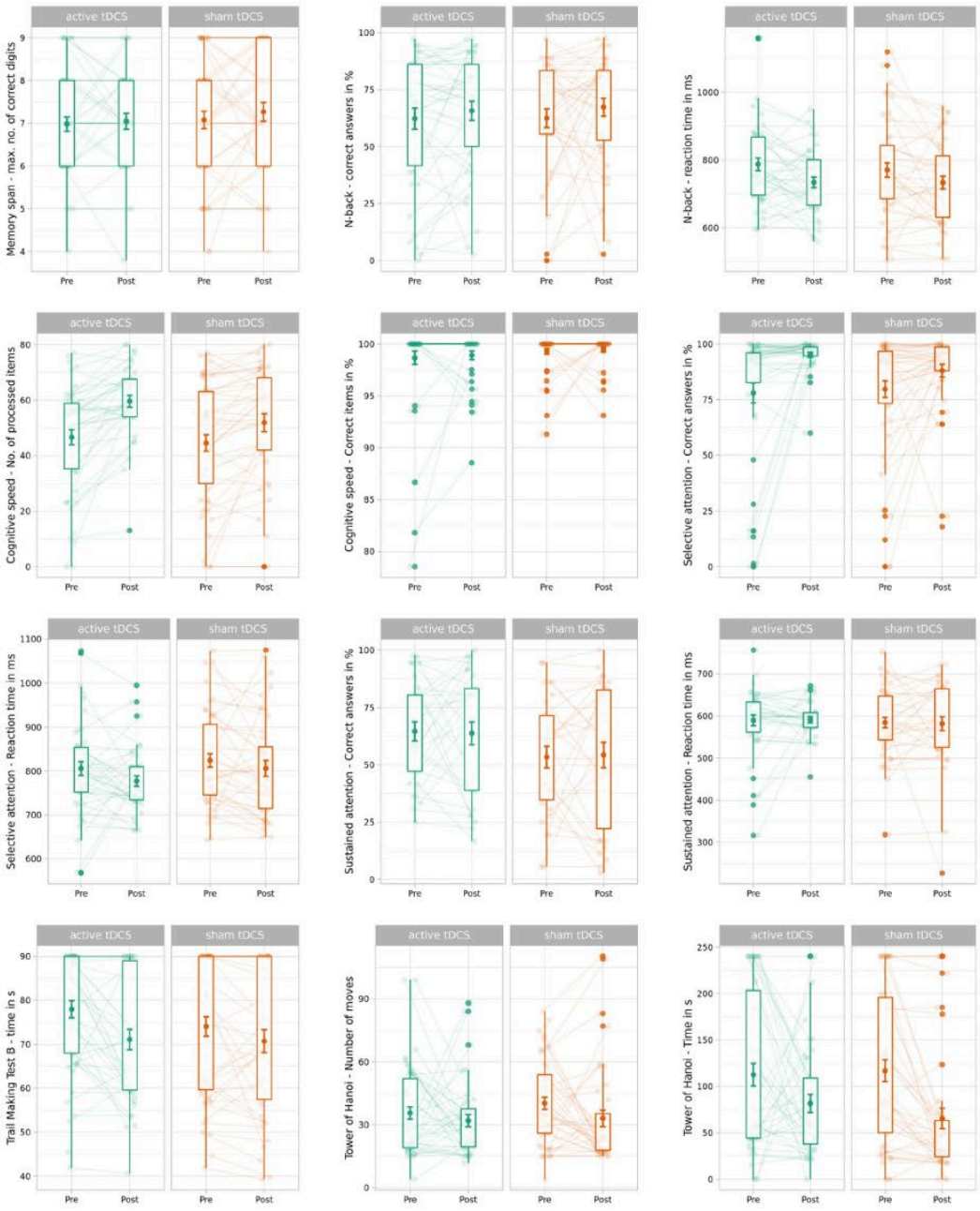
MADRS Montgomery–Åsberg Depression Rating Scale, *BID* Beck Depression Inventory, *WHO/DAS* The World Health Organization Disability Assessment Schedule, *GAF* Global Assessment of Functioning, *SHAPS-D* self- reported anhedonia assessed with the Snaith Hamilton Anhedonia Pleasure Scale, *CD-RISC* Connor-Davidson Resilience Scale

differentially associated with a change in depression severity for active tDCS compared to sham tDCS.

Our results are in contrast to a recent meta-analysis that found significant effects of tDCS on working memory and attention [28]. This meta-analysis was based on studies with sample sizes between n = 18 [15] and n = 127 [26] the number of treatment sessions (one [24] up to 22 [26] and tDCS dosages(0.5 mA [21, 27], 1 mA [15, 20] and 2 mA [16–18, 22–26]) was highly heterogeneous. Among single studies included in this meta-analysis, several authors reported an improvement of attention/vigilance, working memory, executive functioning, processing speed, and social cognition [15, 17], spatial working memory [18] or processing speed [16]. However, other studies in this meta-analysis are rather in line with our findings and did not show significant effects of tDCS on performance in neurocognitive domains [20–27]. The ELECT-TDCS trial, a clinical study with identical stimulation parameters and a larger sample size, did not find significant effects on cognition either [26].

There are several potential reasons for these negative findings. First, our multicenter trial tested only one set of tDCS parameters with the aim of reducing depressive symptoms. However, dose–response curves for single domains of neurocognitive performance have not been established. They may be non-linear and could theoretically vary from one domain to another [44, 45] as well as from dose–response curves of antidepressant effects. While being in line with previous studies on antidepressant tDCS, the administered dosage in our trial might have been insufficient to optimally modulate specific prefrontal cognitive functions. Second, the main trial did not show beneficial antidepressant effects of active tDCS over sham tDCS. Thus, the applied tDCS protocol might have also been not potent enough to modulate neuroplasticity changes in general. Third, high levels of arousal, estimated by using the State-Trait Anxiety Inventory (STAI), have been reported to diminish cognitive practice effects elicited by tDCS, [46] underlining the potential role of arousal in shaping responses to neuromodulation. In our study, both groups had high baseline STAI scores, and such high baseline anxiety could have reduced the effects of tDCS on neurocognitive performance. Lastly, several studies have reported that tDCS might only elicit procognitive effects when simultaneously combined with specific cognitive tasks [47–52]. Thus, passive stimulation, as administered in our trial, might not be sufficient to enhance cognition in patients with MDD.

To the best of our knowledge, this is the first study that investigates whether cognition at baseline may be used to predict improvement of depression during a course of tDCS. Our study has multiple strengths. The study followed the highest possible trial design standards by being triple-blinded, placebo-controlled, and multicenter. We applied a tDCS protocol (2 mA, 30 min) established in previous studies which showed a superior antidepressant efficacy of active over sham tDCS, i.e. the SELECT-TDCS [53] and ELECT-TDCS [26] trials, and our data-set is one of the biggest samples in the field to date (n = 101). Furthermore, we used a validated digital assessment battery that has successfully been used in other previous studies [31, 32, 54]. While efforts are being made to digitize previously validated



Note: Error bars indicate mean (SE). Boxplots include the IQR with whiskers indicating 1.5 times IQR. Thin lines represent patient-individual changes.

Fig. 1 Pre- and post-treatment performance across neurocognitive tests for active tDCS and sham tDCS. Note: Error bars indicate mean (SE). Boxplots include the IQR with whiskers indicating 1.5 times IQR. Thin lines represent patient-individual changes

Table 2 Treatment effects on neurocognitive test scores

Cognitive measure	Slope active tDCS (95% CI)	Slope sham tDCS (95% CI)	F (df)	*p*	p_{fdr}	Standardized effect size (95% CI)
Memory span (maximum number of correct digits)	0.02 (– 0.63 0.66)	0.20 (– 0.44 0.84)	0.72 (1, 73)	0.40	0.80	– 0.19 (– 0.75, 0.37)
Working memory (correct answers in %)	4.86 (– 9.41, 19.1)	6.68 (– 7.47, 20.8)	0.13 (1, 71)	0.72	0.81	– 0.08 (– 0.66, 0.50)
Working memory (reaction time in ms)	-32.8 (– 109, 43.6)	– 38.4 (– 117 40.2)	0.07 (1, 69)	0.79	0.81	0.06 (– 0.71, 0.83)
Cognitive speed (number of processed items)	10.91 (4.12, 17.7)	5.71 (– 1.19, 12.6)	4.69 (1, 73)	**0.03**	0.18	0.49 (– 0.12, 1.09)
Cognitive speed (correct items in %)	0.22 (– 2.90, 3.34)	0.68 (– 3.31, 4.66)	1 (1, 73)	0.32	0.77	– 0.23 (– 1.86, 1.4)
Selective attention (correct items in %)	14.58 (– 3.33, 32.5)	7.83 (– 14.52, 30.2)	4.88 (1, 77)	**0.03**	0.18	0.50 (– 0.92, 1.91)
Selective attention (reaction time in ms)	– 16.9 (– 126, 92.7)	– 11.5 (– 194, 170.9)	0.08 (1, 73)	0.77	0.81	– 0.07 (– 1.96, 1.83)
Sustained attention (correct items in %)	1.52 (– 19.2, 22.2)	– 1.34 (– 27.5, 24.8)	0.18 (1, 56)	0.67	0.81	0.11 (– 0.87, 1.09)
Sustained attention (reaction time in ms)	16.01 (– 75.4, 107)	2.85 (– 155.2, 161)	0.47 (1, 61)	0.50	0.81	0.172 (– 1.61, 1.95)
Trail making B (time in s)	– 5.69 (– 24.6 13.2	– 2.54 (– 25.1, 20.0)	1.78 (1, 75)	0.19	0.76	– 0.31 (– 2.12, 1.51)
Tower of Hanoi (number of moves)	– 4.2 (– 19.7, 11.3)	– 3.05 (– 19.6, 13.5)	0.06 (1, 74)	0.81	0.81	– 0.05 (– 0.73, 0.63)
Tower of Hanoi (time in s)	– 29.8 (– 112, 52.9)	– 44.0 (– 158, 70.4)	1.02 (1, 74)	0.32	0.77	0.23 (– 1.31, 1.77)

p values computed using Type III analyses of variance with Satterthwaite's method. Slope active tDCS = standardized slope parameter for active tDCS. Slope sham tDCS = standardized slope parameter for sham tDCS

Table 3 Prediction of changes MADRS

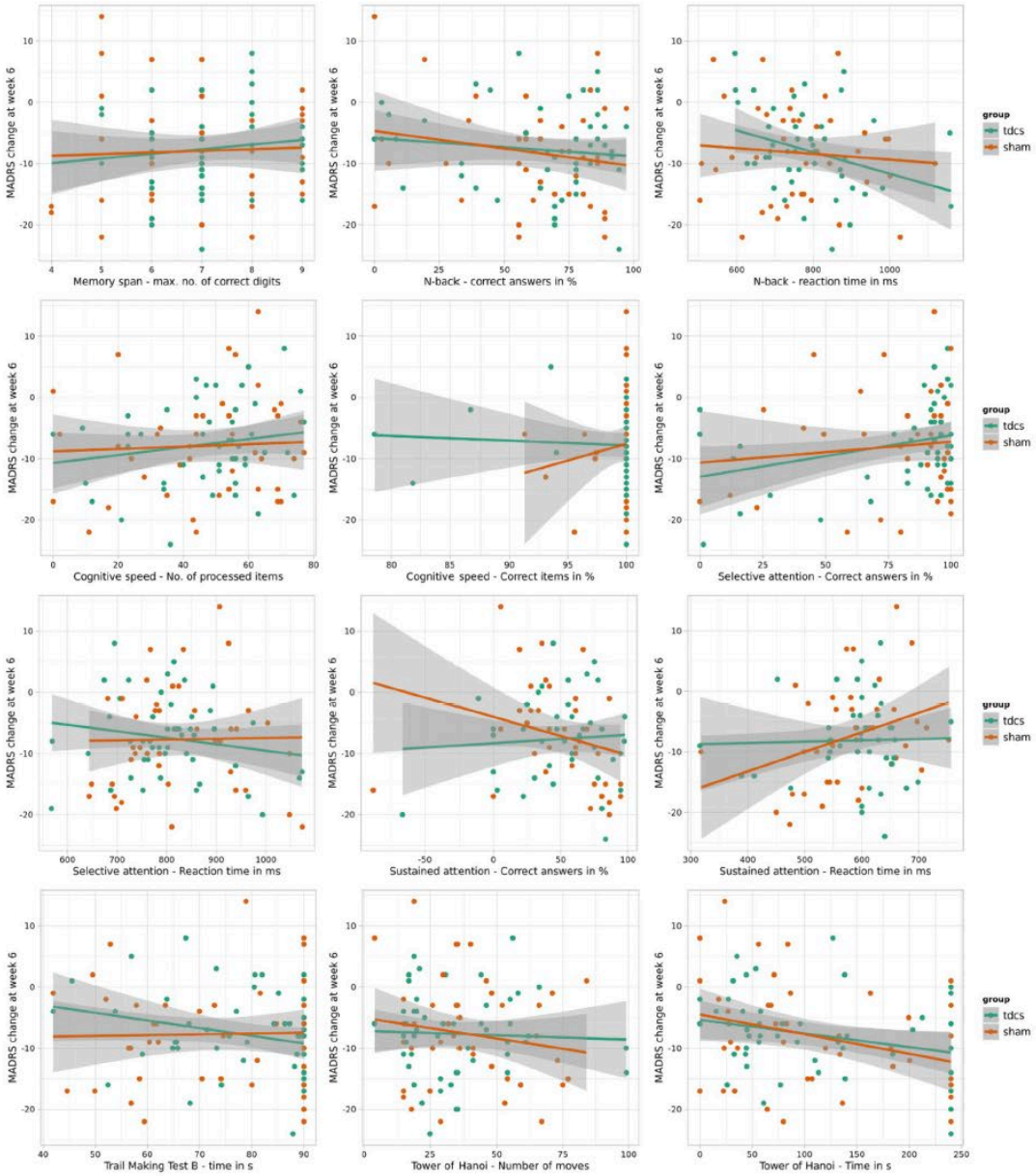
Measure	Cognitive tests							
	Group		Cognitive test score		Group × cognitive test score			
	F (df)	*p*	F (df)	*p*	F (df)	*p*	p_{FDR}	η^2
Memory span (maximum number of correct digits)	0.12 (1, 89)	0.74	0.66 (1, 89)	0.42	0.14 (1, 89)	0.71	0.71	0.001
Working memory (correct answers in %)	0.20 (1, 88)	0.65	2.66 (1, 88)	0.11	0.42 (1, 88)	0.52	0.63	0.005
Working memory (reaction time in ms)	1.54 (1, 81)	0.22	3.10 (1, 64)	0.08	1.43 (1, 81)	0.24	0.63	0.02
Cognitive speed (number of processed items)	0.29 (1, 89)	0.59	0.87 (1, 88)	0.35	0.43 (1, 89)	0.51	0.63	0.005
Cognitive speed (correct items in %)	0.93 (1, 85)	0.34	0,87 (1, 85)	0.35	0.92 (1, 85)	0.34	0.63	0.01
Selective attention (correct items in %)	0.32 (1, 89)	0.57	3.77 (1, 88)	0.06	0.47 (1, 89)	0.49	0.63	0.005
Selective attention (reaction time in ms)	0.40 (1, 85)	0.53	0.42 (1, 85)	0.52	0.40 (1, 84)	0.53	063	0.005
Sustained attention (correct items in %)	0.66 (1,73)	0.42	0.03 (1, 75)	0.86	0.62 (1, 74)	0.43	0.63	0.008
Sustained attention (reaction time in ms)	1.17 (1, 73)	0.28	2.24 (1, 74)	0.14	1.36 (1, 73)	0.25	0.63	0.02
Trail Making B (time in s)	1.59 (1, 85)	0.21	0.80 (1, 87)	0.37	1.59 (1, 85)	0.21	0.63	0.02
Tower of Hanoi (number of moves)	0.47 (1, 88)	0.49	1.37 (1, 88)	0.25	0.55 (1, 88)	0.46	0.63	0.006
Tower of Hanoi (time in s)	0.12 (1, 87)	0.73	8.32 (1, 87)	0.005	0.031 (1, 87)	0.58	0.63	0.004

p values computed using Type III analyses of variance with Satterthwaite's method. MADRS = Montgomery-Åsberg Depression Rating Scale. $\eta^2 = 0.01 \leq 0.06$ (small effect), $0.06 \leq 0.14$ (moderate effect) and ≥ 0.14 (large effect)

cognitive tests [55, 56], such tools which also reduce documentation errors [57, 58], are still underused.

Limitations

First, there is no uniform consensus on what neurocognitive tests are better used to evaluate the performance in domains



Note: MADRS=Montgomery-Åsberg Depression Rating Scale.

Fig. 2 Association between baseline cognitive performance and MADRS change across the trial. *MADRS* Montgomery-Åsberg Depression Rating Scale

associated with FPN function. Our battery included some of the most common tests and slight variations of them. However, other standardized tests could have a higher sensitivity and specificity for detecting neuromodulation effects on cognitive performance [59]. Second, digital tools present a few caveats such as failure of the equipment, corruption of data, and loss of information when retrieving the data. This limited the availability of data in our study. Third, the evaluation of procognitive effects of tDCS and the potential predictive effects of baseline cognition on treatment response were ancillary investigations. Though this data was well balanced across both conditions, there may be latent selection biases making the sample not representative for the whole study population. In addition, the current analysis was likely underpowered to detect small treatment and prediction effects. Lastly, all patients were on a stable SSRI medication for at least 4 weeks prior to inclusion, but not antidepressant-free. Thus, our conclusions regarding the differential effects of SSRI medication and tDCS on performance in distinct neurocognitive domains are limited.

Conclusion

In conclusion, our analysis does not support the notion that acute treatment with active tDCS compared to sham tDCS leads to an improvement in FPN-related neurocognitive functions. In addition, neurocognitive functioning at baseline did not predict the change of MADRS scores over the course of tDCS. Future research should aim at identifying tDCS protocols with optimal dose–response curves for effects on specific neurocognitive domains. Most promising candidates could then be further optimized by adjusting parameters at an individual patient's level.

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Data availability The de-identified individual patient data in this paper will be made accessible after its publication for non-commercial academic projects that have a legitimate research topic and a clearly stated hypothesis. If the application is accepted, researchers will be asked to get the study approved by their institution's ethics board. The authors will subsequently provide the de-identified data sets via a safe data transfer system. You may find the DepressionDC research protocol as well as further extra information at https://osf.io/cpw6f/.

Declarations

Conflict of interest FP has received consulting fees from Brainsway Inc. (Jerusalem, Israel) as a member of the European Scientific. Advisory Board and from Sooma (Helsinki, Finland) as a member of the International Scientific. Advisory Board; honoraria for workshops from Mag&More GmbH (Munich, Germany); and honoraria for lectures from neuroCare Group (Munich, Germany) and Brainsway Inc. (Jerusalem, Israel); and has received equipment from Mag&More GmbH (Munich, Germany), neuroCare Group (Munich, Germany), and Brainsway Inc. (Jerusalem, Israel). BL received honoraria for consultancy and speakers' fees from ANM, AstraZeneca, Autifony Therapeutics, Decibel Therapeutics, Desyncra, Gerson Lehmanns Group, Lundbeck, Merz, MagVenture, Medical Tribune, Neurolite, Neuromod, Novartis, Pfizer, Rovi, Schwabe, Sea Pharma, Servier, Sonova and Sound Therapeutics; research funding from the Tinnitus Research Initiative, Bayhost, the German Research Foundation, the German Federal Ministry of Education and Research, the American Tinnitus Association, AstraZeneca, cerbomed, Neuromod and the European Union; and has received equipment from MagVenture and Deymed Diagnostic. CP is managing partner of PsyKit GmbH, Tübingen, Germany. AS, UV, GV, LF, AJF, GB, CSL, SG, SA, CP, CN, LF, PZ, TK, and MB declare no competing interests.

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