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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 modulate 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, $M_{\text{age}} = 25$ 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.

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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 25years (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 cross-over 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 × 5 cm²) 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

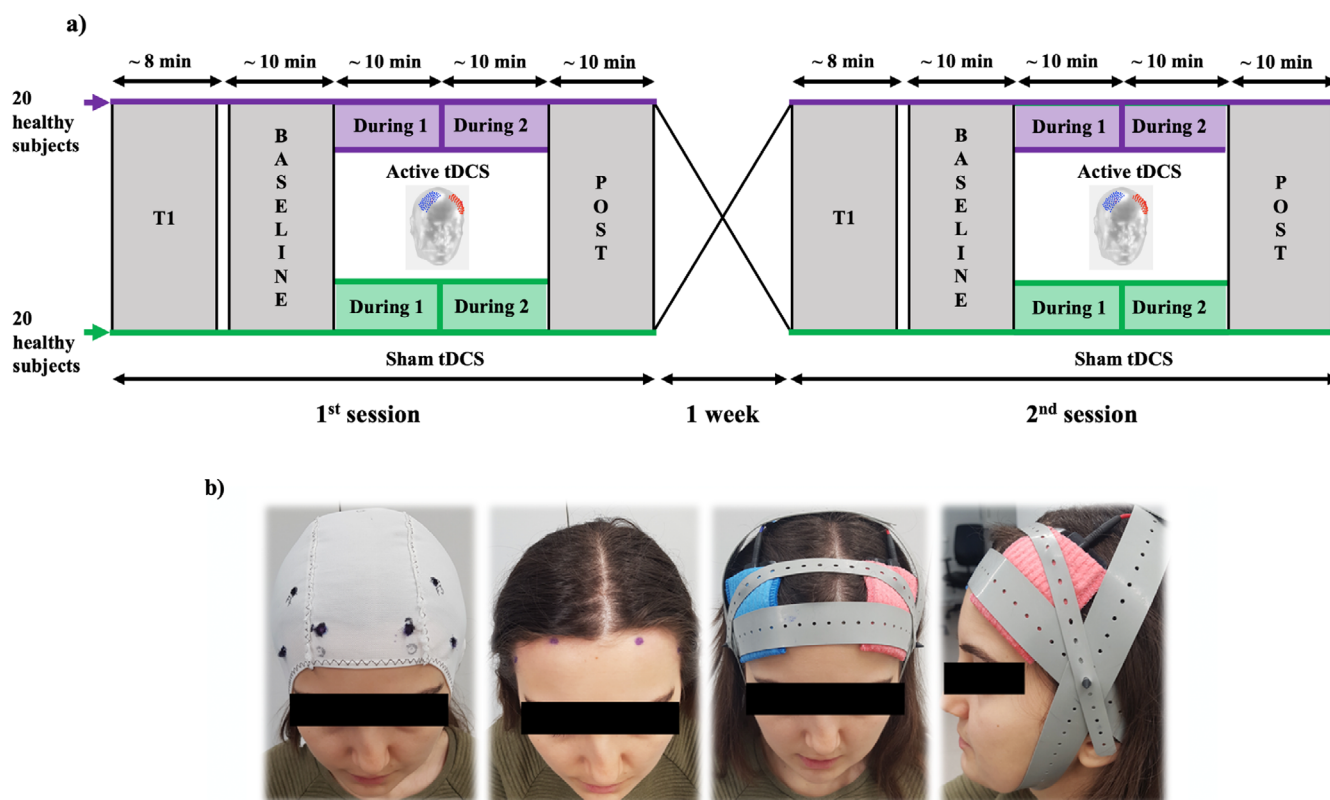


FIGURE 1 | Overview of the tDCS protocol. (a) Study Protocol: Assessment of 20-min, 2 mA active or sham prefrontal tDCS at four times, using an adapted MRS sequence. (b) tDCS Setup: Anodal electrode (red) positioned over F3 (left DLPFC) and cathodal electrode (blue) over F4 (right DLPFC), according to 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)=2300ms, echo time (TE)=2.98ms, and field of view (FoV)=256mm×240mm. The in-plane resolution for the T1-weighted imaging was 1mm×0.938mm, with a voxel size of 1mm×0.938mm×1mm. Additionally, the parameters for the T2-weighted sequence included TR=3200ms, TE=408ms, and FoV=230×230mm. The in-plane resolution for the T2-weighted imaging was 0.898mm×0.898mm, with a voxel size of 0.898mm×0.898mm×0.90mm.

2.5 | MRS Acquisition

The MEGA PRESS sequence was employed with the following parameters: TR=2000ms, TE=68ms, spectral bandwidth=2000Hz, 144 averages, and editing pulses applied to the GABA spins. These editing pulses were explicitly targeted at 1.9ppm to selectively refocus the GABA spins (ON-signal), while pulses at 7.5ppm 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 2.2cm³ 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 10s 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 0.2–4ppm. 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

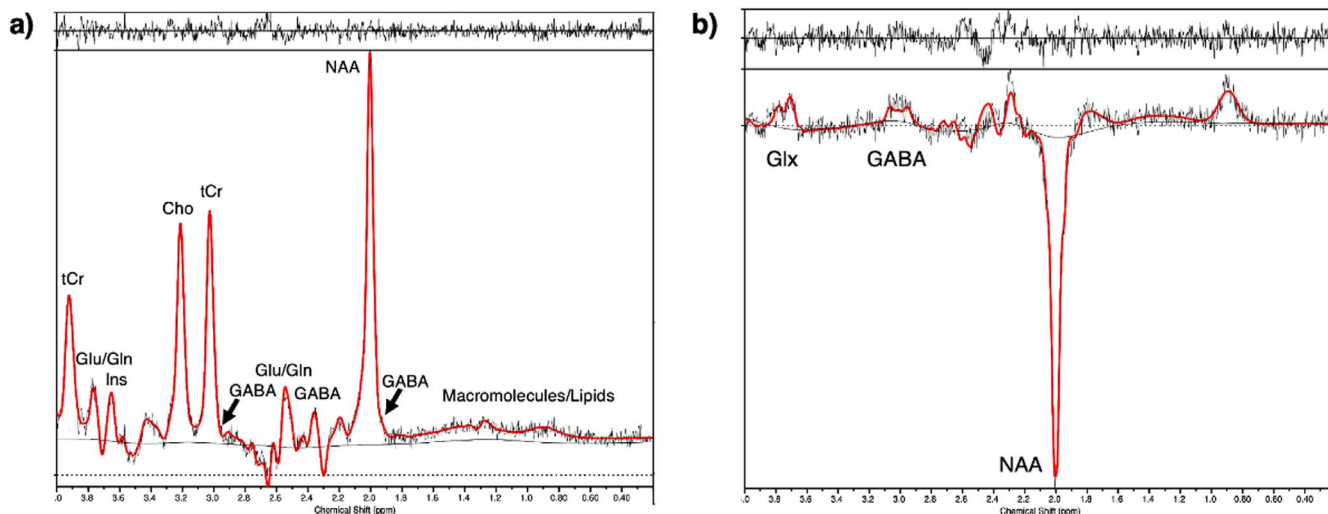


FIGURE 2 | Overview of spectroscopy analysis. (a) Example of an LCModel Output MR Spectrum: Display of metabolites, including Inositol (Ins), Total Creatine (tCr), Gamma-Aminobutyric Acid (GABA), N-acetylaspartic Acid (NAA), Choline (Cho), Glutamate/Glutamine (Glu/Gln). (b) Single Subject LCModel Output 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 $50\text{ mm} \times 70\text{ mm}$ 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 line-widths 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.

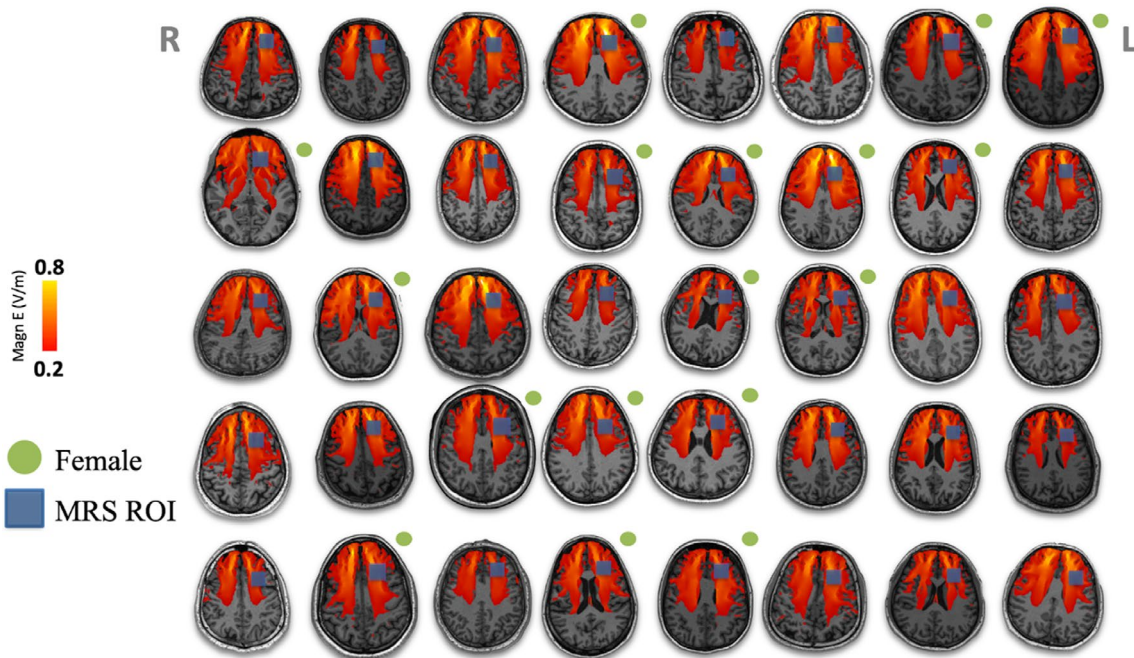


FIGURE 3 | Electric field distribution variability and MRS ROI alignment. Axial MRI slices from a cohort of healthy subjects, each of whom underwent computational modeling of tDCS using SimNIBS 4.0. The simulations were performed with a 2 mA current applied via electrodes positioned at the F3 and F4 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 mm² 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 p s > 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 p s > 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” $t(190) = 1.973$, $p_{\text{Bonferroni}} = 0.049$ and “post” $t(190) = 2.930$, $p_{\text{Bonferroni}} = 0.01$ 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).

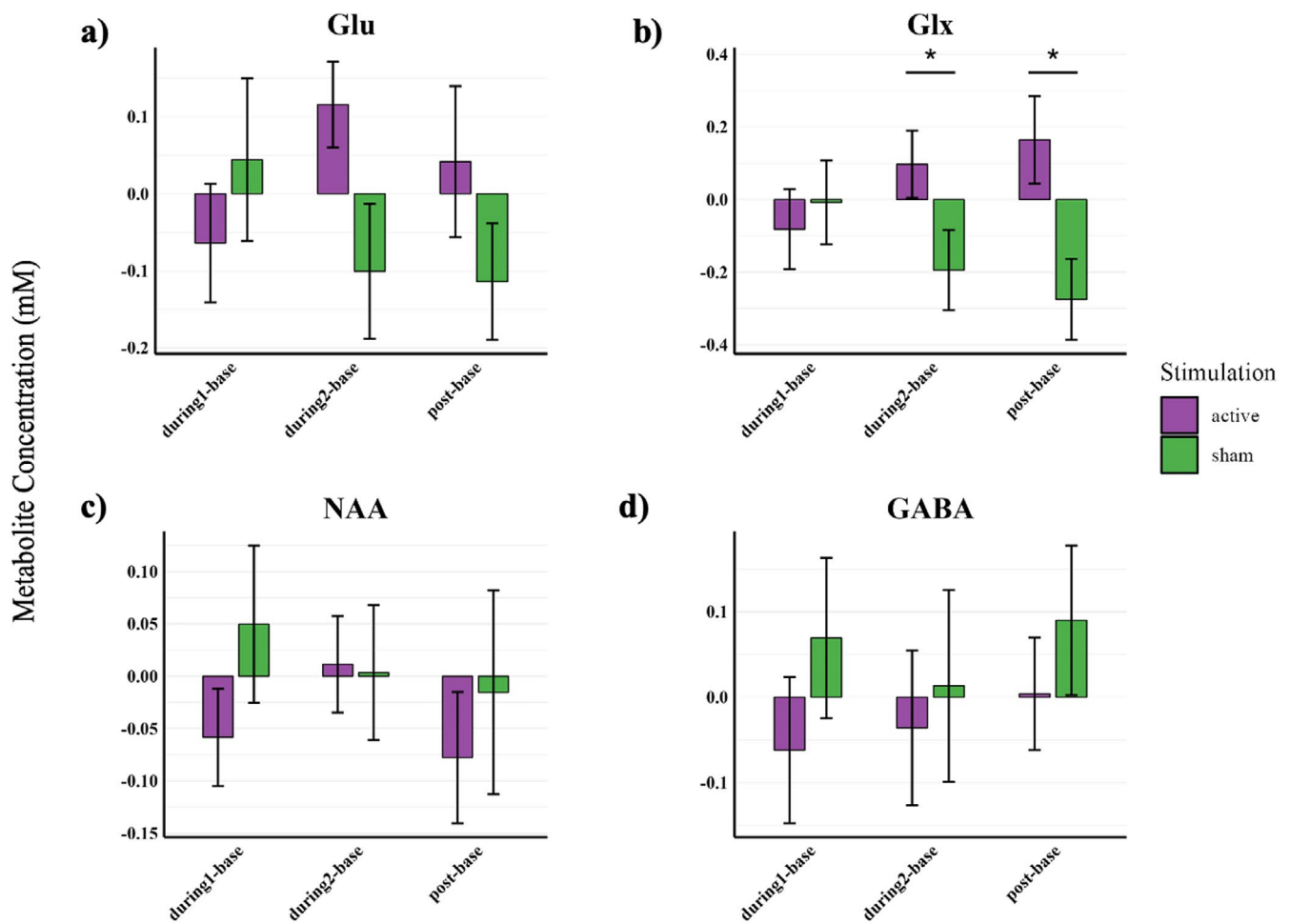


FIGURE 4 | Baseline-Corrected Concentrations of Glu, Glx, NAA, and GABA During and After Active and Sham tDCS Stimulation. This figure illustrates the relationship between baseline-corrected concentrations of metabolites (Glu, Glx, NAA, and GABA) and the administration of active versus sham tDCS. The 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 \times 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

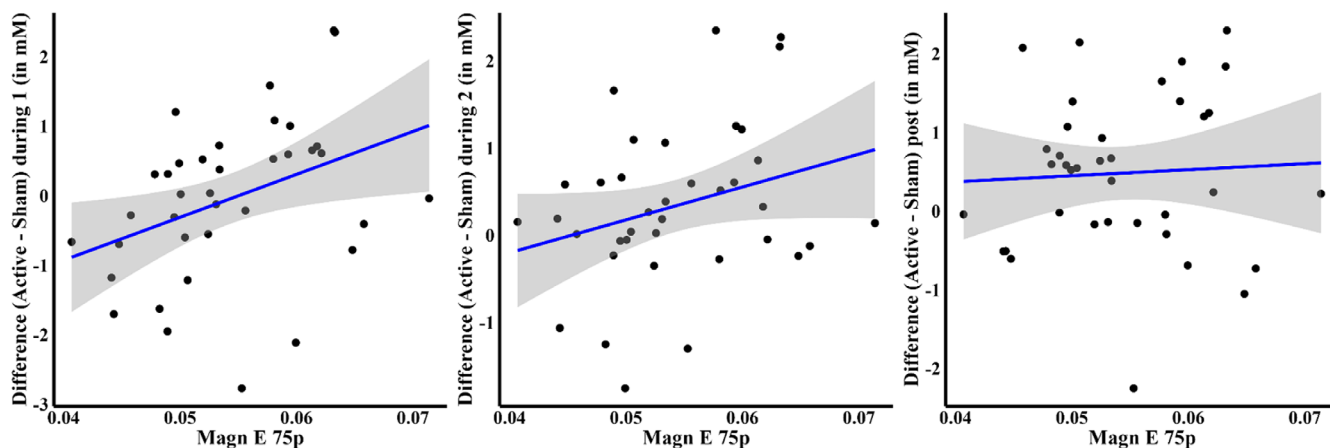


FIGURE 5 | Correlations between e-field magnitude (75th Percentile) and Glx Levels at tDCS time points. This figure illustrates the relationship between the 75th percentile of the e-field magnitude and the difference in Glx levels between active and sham conditions during the first and second intervals 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 Karşlı:** 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.