Cortical excitation-inhibition homeostasis and energy metabolism following transcranial direct current stimulation using proton and phosphorus magnetic resonance spectroscopy

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Other Publications:

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Proton Magnetic Resonance Spectroscopy of the motor cortex reveals long term GABA change following anodal Transcranial Direct Current Stimulation

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Anodal transcranial direct current stimulation (tDCS) over the primary motor cortex (M1) has been reported to increase the firing rates of neurons and to modulate the gamma-aminobutyric acid (GABA) concentration. To date, knowledge about the nature and duration of these tDCS induced effects is incomplete. We aimed to investigate long-term effects of anodal tDCS over M1 on GABA dynamics in humans. Repeated magnetic resonance spectroscopy (MRS) was employed to measure relative GABA concentration in M1 for approximately 64 minutes after stimulation. The study was performed on 32 healthy subjects. Either anodal or sham tDCS were applied for 10 minutes with the active electrode over the left M1 and the reference electrode over the right supra-orbital region. Pre and post-tDCS MRS scans were performed to acquire GABA-edited spectra using 3T Prisma Siemens scanner. GABA signals showed no change over time in the sham tDCS group, whereas anodal tDCS resulted in a significant early decrease within 25 minutes after tDCS and then significant late decrease after 66 minutes which continued until the last test measurements. The late changes in GABA concentration might be related to long-term plasticity mechanism. These results contribute to a better understanding of the neurochemical mechanism underlying long-term cortical plasticity following anodal tDCS.

Transcranial direct current stimulation (tDCS) is a non-invasive stimulation technique that allows for the modulation of cortical excitability in humans. Animal studies[1], as well as studies on humans[2,3] have shown that anodal tDCS leads to cortical facilitation, that is increased overall excitability, and increases spontaneous firing rates of cortical neurons. The facilitation effect of anodal tDCS has also been associated with improved cognitive and behavioral skills as reported in healthy participants and patients[4–6].

So far, short- and long-term sustainability (i.e., after-effects) of increased excitability have been observed to depend on the duration of tDCS[2]. Previous neurophysiological studies have shown that tDCS application between 5 to 7 minutes over M1 led to short-term effects (increase of transcranial magnetic stimulation (TMS) elicited Motor Evoked Potential (MEP) amplitudes) for no longer than 5 minutes, whereas 9 to 13 minutes of stimulation have been found to induce a long lasting increase of excitability for up to 90 min[2,7]. Moreover, the increased duration of cortical excitability has been further demonstrated by applying two consecutive tDCS sessions on the M1 of healthy humans[8]. The second stimulation was applied without an interval, during the after-effects of the first stimulation, or after the after-effects of the first stimulation had vanished. The during after-effects condition resulted in an initially reduced, but then relevantly induced l-LTP like plasticity through prolonged excitability enhancement in M1. This increase of excitability is supposed to be associated in humans to the reduction

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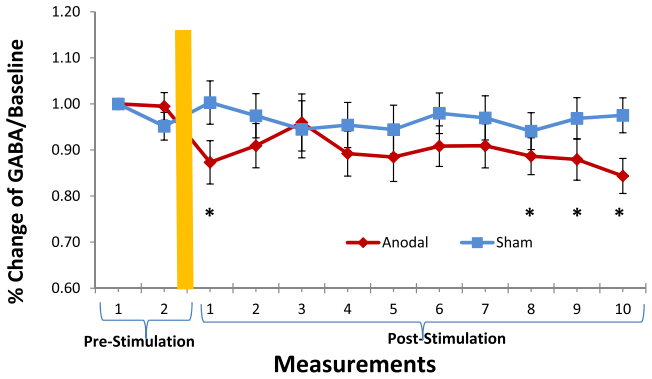

Figure 1. Mean GABA percentages of concentration for anodal and sham stimulation groups with 16 subjects per group. The graph shows a significant decrease of GABA concentration in the anodal stimulation group at first, eight, ninth and tenth post-stimulation measurements with respect to the first pre-stimulation measurement condition (*$p < 0.05$). Error bars reflect standard error of the mean.

of gamma-aminobutyric acid (GABA)-driven inhibition, as displayed in motor cortex studies[9,10]. GABA is an inhibitory neurotransmitter and has a prominent role in human motor learning[11,12]. However, knowledge about the long term effects of tDCS on GABA neurotransmission is incomplete. Until now, only few studies have investigated the role of GABA in cortical plasticity following anodal tDCS. Effects on cortical plasticity as a function of GABA modulation have been observed up to 20 minutes in healthy participants[13] and up to 25 minutes in chronic stroke recovery patients[14–16]. More recently, a study using a similar stimulation protocol displayed a depletion of ATP and Phosphocreatine over M1 for a longer time, that is approximately 90 minutes[17]. As ATP and Phosphocreatine are indexes of energy consumption in brain tissues, this localized decrease of their concentration has been associated to a long-term effect of increased energy consumption after anodal tDCS.

However, whether anodal tDCS can also produce long-term plasticity effects associated to reduced GABA concentration remains untested. In the present study we aimed at better understanding the mechanism of neural plasticity. Hence, we measure the effects of anodal tDCS on GABA concentration for one hour and more.

Results

Statistical analysis was conducted with SPSS (version 23.0 Armonk, NY, USA) with a $p = 0.05$ set as the threshold for significance. A mixed-design Analysis of Variance (ANOVA) was performed on GABA concentration with *Stimulation* (sham vs. anodal) as the between-participants factor and *Measurement* (12 measurements: 2 pre-stimulation and 10 post-stimulation measurements) as the within-participants factor, respectively. Paired-sample *t*-tests (confidence interval 95%) were conducted as post-hoc analyses of factors interaction. Partial eta-squared (η^2_p) was calculated within the ANOVA as measure of effect size. An open-source tool was used to compute Cohen's d_z effect size for the *t*-tests (https://webpower.psychstat.org/models/means01/effectsize.php). Figure 1 shows the time evolution of the normalized GABA concentration level induced by tDCS at different measurement times. The main effect of Stimulation was not significant [$F_{(1, 30)} = 1.157$, $p = 0.291$ $p_{rep} = 0.644$ $\eta^2_p = 0.037$]. The main effect of Measurement was significant [$F_{(11, 330)} = 2.378$, $p = 0.008$, $p_{rep} = 0.961$ $\eta^2_p = 0.073$]. Remarkably, the interaction Stimulation x Measurement was significant [$F_{(11, 330)} = 2.249$, $p = 0.012$ $p_{rep} = 0.950$ $\eta^2_p = 0.070$]. For the sham and anodal group, paired-sample *t*-test comparisons were performed between the first pre-stimulation measurement condition and each of the subsequent measurements (i.e., second pre-stimulation and first to tenth post-stimulation conditions). For the sham group, GABA concentration did not change significantly from the first pre-stimulation measurement to all the following measurements [$ts_{(15)} \leq 1.715$ $ps > 0.05$]. For the anodal tDCS group, relative to the first pre-stimulation measurement, GABA concentration did not change significantly at the second pre-stimulation [$t_{(15)} = 0.347$ $p = 0.733$ $d_z = 0.087$] but decreased significantly at the first post-stimulation measurement [$t_{(15)} = 2.226$ $p = 0.042$ $p_{rep} = 0.889$ $d_z = 0.556$]. Thereafter, GABA concentration returned close to baseline level from the second until the seventh post-stimulation measurements [$ts_{(15)} \leq 1.776$ $ps > 0.05$ $d_z s \leq 0.444$], and then further decreased significantly at the eighth to tenth post-stimulation measurements [$t(15) = 2.514$ $p = 0.024$ $p_{rep} = 0.922$ $d_z = 0.629$; $t_{(15)} = 2.410$ $p = 0.029$ $p_{rep} = 0.912$ $d_z = 0.602$; and $t_{(15)} = 3.483$ $p = 0.003$ $p_{rep} = 0.980$ $d_z = 0.871$] (Fig. 1). Thus, while in the sham group GABA concentration stayed at the pre-stimulation baseline level across all post-stimulation measurements, data from the anodal group suggested a decreasing pattern of GABA concentrations across different time points. GABA concentration first showed a decrease at the early first post-stimulation measurement, and then a more consistent decrease (approximately for 34 minutes) at the later eighth to the tenth post-stimulation measurements. To rule out the possibility that the observed GABA decrease depended on longitudinal changes in the fit of the MRS data specific to the stimulation group, we performed a mixed ANOVA on Cramér-Rao-Lower-Bounds (CRLB) values. This analysis revealed no significant effect of Stimulation [$F_{(1, 30)} = 0.387$, $p = 0.539$, $p_{rep} = 0.473$ $\eta^2_p = 0.013$], Measurement [$F_{(11, 330)} = 1.581$, $p = 0.103$, $p_{rep} = 0.808$ $\eta^2_p = 0.050$], and Stimulation x Measurement interaction [$F_{(11, 330)} = 1.566$, $p = 0.107$, $p_{rep} = 0.804$ $\eta^2_p = 0.050$], thus supported the hypothesis of decreased GABA concentrations produced by anodal stimulation.

Discussion

The purpose of this study was to explore the neurochemical mechanism underlying after effects of anodal tDCS and their long-term duration by means of repetitive MRS measurements of GABA levels. Our results suggest a drop in GABA levels within 25 minutes following anodal stimulation (at first post-stimulation measurement) which is consistent with previous reports[13]. Furthermore, our results also suggest late after effects of anodal tDCS, which emerged about 66 minutes (i.e. at the eighth post-stimulation measurement) and remained stable until about 90 minutes (at the last post-stimulation measurement). Our finding that anodal, but not sham, tDCS delivered to the motor cortex leads to a significant decrease in MRS-GABA within the stimulated region, shows that the excitatory effects of anodal tDCS would be associated with a modulation of GABAergic interneurons[12,18,19], which might be due to downregulation of the enzyme GAD-67 activity[20,21]. Excitatory effects of anodal tDCS seem to trigger compensatory changes in neuronal excitation. One example of such synaptic homeostasis is the down regulation of the enzyme GAD-67, which critically controls GABA synthesis while likely gating glutamatergic plasticity of the M1. Hence, enhanced glutamatergic activity following anodal tDCS might be one of the mechanism gated by GABA down regulation. The significant reduction of cortical GABA seems not to be involved only in early phase but also late phase effects after the end of tDCS over a period of approximately 90 minutes. It would occur (i) in the form of short-time plasticity after the end of tDCS within 25 minutes after the end of tDCS and (ii) a consolidated long term effect after 66 minutes following tDCS which continues until the end (90th minutes) of the last measurement. Noteworthy, the time-line of GABA depletion is similar to the reduction pattern of high-energy phosphates as demonstrated by phosphorus spectroscopy with a similar anodal tDCS protocol[17]. Such a similarity between the time course of reduced GABA concentration and high energy phosphates concentration suggests that anodal tDCS would be able to induce an active neural process, most probably gated by GABA-ergic plasticity as one source as seen in our results.

A study from dyke and colleagues[22] reported that concentrations of GABA measured by MRS, and GABA-mediated physiological inhibition indexed by 3 ms SICI (short-interval intracortical inhibition) are unrelated; which replicated previous results for 3 ms SICI[23] and 2.5 ms SICI[24]. The physiological mechanism underlying 2.5 ms and 3 ms involves post-synaptic inhibition mediated through GABA-A receptors[25]. Lack of correlation between GABA measured by MRS and GABA indexed by SICI suggests that one of the possible source of MRS-GABA modulation following anodal stimulation in our study might be due to extracellular GABA levels and may not be associated with synaptic transmission[26,27]. However, a study from Stagg and colleagues[24] reported a significant correlation between MRS-GABA and 1 ms SICI effects, which may be related to refractory period of inter-neurons[28] or synaptic processes[29]. However, the exact mechanism underlying correlation of MRS-GABA and 1 ms SICI remained unclear.

The current study suggests first evidence for a long term after effects on GABA modulation following anodal tDCS. Results deliver a first move towards the understanding of long term effects of anodal tDCS and raise the question on how long the GABA concentration takes to return to baseline values. Our data do not provide a clear explanation on the return of GABA to baseline following depletion. Longer duration investigations including several measurements (e.g across more than three hours) might shed some light on the return of GABA concentration at baseline levels following significant decrease after anodal tDCS. The GABA and glutamate are closely linked in the human brain and this relationship has been confirmed by MRS studies[13]. However, due to technical reasons the GABA edited MEGA PRESS sequence does not allow us to separate glutamate and glutamine and reliably quantify the composite measure of glutamate and glutamine (Glx). A study, for example at 7 T MRI scanner, directly investigating long term glutamate modulation has yet to be performed to explore more about long term plasticity-like mechanism.

Although the implementation of a cross-over design (i.e., one group having both anodal and sham stimulations) would have provided some increased statistical power, it could have generated cumulative excitability effects[30,31] and would have exposed the participants to experience different skin reactions or sensations[32] between the two sessions, thus introducing the risk of significant biases on the final data. For this reason, we chose a parallel design to avoid any carry over effect from active anodal to sham stimulations and vice versa.

Chew and colleagues[33] investigated cortical excitability after M1 anodal tDCS (10 min duration, 16 cm^2 target reference electrodes) and did not observe a main effect of intensity; however no sham condition was tested. Intra-individual reliability of 0.5 over 30 minutes following stimulation was reported (poor ICC (2,1) = −0.5), and participants responded strongly to 0.2 and 2 mA, only. Moreover, reliability of prefrontal tDCS-induced resting state function MRI modulation (20 min 35 cm^2 target/reference 2 mA, bipolar stimulation) was investigated: low reliability was observed for active tDCS compared to baseline and sham groups[34]. A study investigating 1.0 mA anodal tDCS (13 min duration, 35 cm^2 target/reference electrodes) reported good intra-individual reliability over the first 30 min (ICC(2,1) = 0.565), although measurements obtained during the 30 min afterwards showed poorer reliability (ICC(2,1) = − 0.028)[35]. One further study (15 min duration, 35 cm^2/100 cm^2 target/reference) investigated intra-individual reliability in 1.0 mA anodal tDCS and showed stronger reliability, both over early and late measurement periods (ICC(2,1) = 0.74 and 0.64, between 0–30 and 60–120 min, respectively)[36]. Methodological differences are present between these studies, such as sample sizes and stimulation parameters, which may promote to different findings. Indeed, it is known that MEP amplitude change is affected by the size of the electrode[37–39], as well as by the duration of the tDCS[40,41]. Moreover, we have also investigated the fit quality of the MRS data specific to the stimulation group because the decrease in GABA levels following anodal stimulation might results from intra-individual changes in the fit quality of GABA levels. The statistical analysis, as mentioned in the result section, revealed neither significant main effects of Stimulation and Measurement, nor their significant interaction. Another possible reason for low reliability may be due to elevated drowsiness associated with participants during the MRS measurements which may affect cortical excitability and corresponding GABA modulation. In an effort to regulate these factors, all participants in the study were informed about the measurement duration before the start of the MRS measurement and their level of vigilance was monitored during the

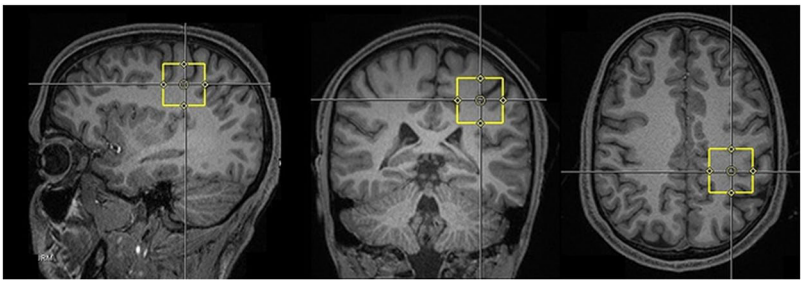

Figure 2. Representative sagittal, coronal and axial T1-weighted MRI brain images of a subject depicting the $3 \times 3 \times 3$ cm voxel (in yellow) within the primary motor cortex.

whole experiment. Furthermore, as also different time lags between sessions may affect vigilance and attention of the participants and bias the results, we kept the day time of measurements rather constant between sessions.

Regarding application-relevant aspects of the results of this study in stroke rehabilitation, it is of much interest to foster motor learning to aid the development and implementation of adjunctive therapies. Thus, reduction in GABA concentrations via tDCS might gate glutamatergic plasticity, which could be used to investigate motor learning effects. Our experimental approach might furthermore provide a methodological approach to non-invasively investigate if patients with neurodegenerative diseases express abnormal forms of long-lasting plasticity in M1.

It is also important to understand the interaction of tDCS and GABA with pharmacological treatment in clinical practice to a larger extent. Given that GABAergic cortical inhibitory interneurons play a role in the early stage of Alzheimer's disease[42], modulation of these GABA interneurons by tDCS could be a potential disease-modifying mechanism for restoring working memory and cognition. Furthermore, the transferability of results from motor cortex experiments to other critical targets, such as the dorsolateral prefrontal cortex, is unclear at present. Hence, further research is needed to determine if mechanisms found in studies investigating M1 are also relevant to other brain target regions. So far, GABA has not been widely explored with regard to tDCS induced plasticity, but it might be an important contributor to cognitive neuroscience research which deserves higher consideration.

We have demonstrated that anodal tDCS induces long-lasting effects of anodal tDCS on GABA concentrations, and to measure the corresponding early and delayed GABA changes at the molecular level for nearly one and half hour. Here we propose long term GABA reduction as one potential elements contributing to local cortical changes in M1 following anodal tDCS, which might gate glutamatergic plasticity.

Materials and Methods

Participants. Thirty-two healthy volunteers (mean age 26 ± 4, range 22–30 years) with no history of neurological or psychiatric diseases participated in this single blind randomized control study. Participants were assigned to two groups by counterbalancing the sex, and matching their age as much as possible between the groups. Sixteen participants were assigned to the *Anodal stimulation group* (8 females, mean age = 27.25, SD = 6.48; 8 males, mean age = 25.63, SD = 3.58), and sixteen to the *Sham stimulation group* (8 females, mean age = 26.75, SD = 3.92; 8 males, mean age = 25.25, SD = 3.28), that is, the control group for possible changes of GABA concentrations unrelated to the anodal stimulation. All participants were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). All assessments were conducted at the Division of Clinical Cognitive Sciences of the RWTH Aachen University, Germany, and after participants gave their written informed consent for participation in this study, which was approved by the ethics committee of the Medical Faculty-RWTH Aachen University. All methods were performed in accordance with the relevant guidelines and regulations of the institution.

Transcranial direct current stimulation (tDCS) and sham stimulation. A DC-Stimulator (neuroConn GmbH, Ilmenau, Germany) delivered 1 mA of electric current to the brain *via* two rubber electrodes (5×7 cm) covered by saline-soaked (0.9% NaCl) sponges. One electrode was positioned 5 cm lateral from Cz and 2 cm anterior to the mid-pre-central position of the left hemisphere, to stimulate left primary motor cortex (M1), and the other over the contralateral supraorbital ridge using convention of EEG 10/20 system. To ensure accuracy, during the positioning of the electrodes, we adhered to EEG 10–20 system; a method that provides reproducible and consistent placement of electrodes for different head sizes[43,44], and kept a distance of not less than 6 cm between the sponges. Indeed, tDCS with electrode covering area of 35 cm^2 is not focal, and variation of displacement of the electrode by 1 cm should not have significant impact on current flow[45].

For real (anodal) stimulation, the current was ramped-up for 10 s, kept constant at 1 mA for 10 min, and finally ramped down over a period of 10 s. For sham stimulation, the current was ramped up for 10 s and then immediately ramped-down and kept off for the next 10 minutes.

MRS acquisition. All measurements were performed with a 3 T Siemens PRISMA MR scanner (Siemens, Erlangen, Germany). The system was equipped with a dockable patient bed and participants were asked to lay at rest on it throughout the experimental session. Each experimental session started with the acquisition of sagittal T1-weighted images, which were used to carefully place a $3 \times 3 \times 3$ cm voxel of interest within the hand area of the left M1 as shown in Fig. 2. Voxel size of $3 \times 3 \times 3$ cm is frequently used to acquire MRS data reliably

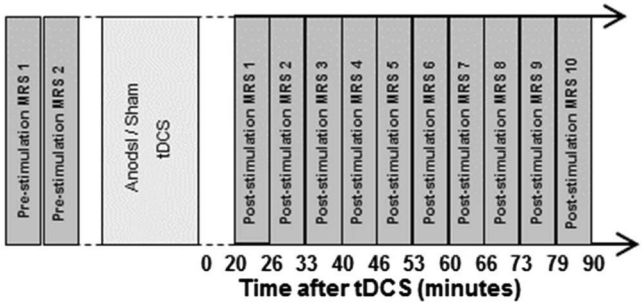

Figure 3. Layout of the experimental procedure depicting the GABA spectroscopy measurements performed before (pre-stimulation) and after (post-stimulation) the application of transcranial direct current stimulation.

and offer trade-off between localization and data quality[46]. Then, a careful shimming procedure was performed using a FASTEST map sequences. In order to achieve good field homogeneity, linewidths of water at full width at half maximum (11 ± 1 Hz in M1) was obtained by shimming maximum three times before first pre- and post-stimulation measurement. To assess the creatine and N-acetylaspartate acid (NAA) line widths, a standard PRESS sequence was used to acquire an unedited spectrum with 32 averages. A MEGA-PRESS editing sequence[47] (with parameters repetition time = 2000 ms, echo time = 68 ms, averages = 96 and editing pulses centered at 1.9 and 7.5 ppm on each alternate scan) was used to acquire GABA spectra. The acquisition and analysis protocol used in this study followed recently published guidelines for GABA MRS at 3 T using MEGA-PRESS[46]. Each acquisition took approximately 6 minutes and 25 seconds.

It has been observed that within-participants stimulations over consecutive days can cause cumulative and larger excitability effects[30,31]. No standard guidelines have been established yet on the amount of time that should be left between tDCS sessions to ensure that any stimulatory effects have washed out[48]. For these reasons, stimulations were administered between participants with separate stimulation groups. Furthermore, a cross-over design can bring the participants to notice different skin reactions or sensations[32] between the two sessions, thus potentially introducing significant biases on the final data.

Two baseline GABA measurements were performed before tDCS (*pre-stimulation* measurements). Successively, subjects were asked to hold their position, while the patient table was undocked from the scanner and moved outside of the magnet room. This allowed to minimize the movements of the participant's head between the pre- and post-stimulation MRS measurements. The participants were asked not to move while the stimulation electrodes were placed on pre-marked areas of the head. The stimulation was then started, and after 10 minutes, electrodes were removed carefully from the head of the participants, and the table docked again to the MR scanner. Electrode removal from participant head, participant table docking in the scanner, running localizer, anatomical acquisition, voxel replacement and shimming were performed between the end of tDCS and the start of the first post-stimulation measurement. Thus the first post-stimulation measurement started 20 min after the end of tDCS. A second, faster 3D T1-weighted acquisition was performed in order to place the spectroscopy voxel in the position identical with that of pre-stimulation. After shimming, ten consecutive post-stimulation GABA measurements were sampled at every 6 minutes interval for approximately sixty-four minutes (*post-stimulation* measurements). Correct placement of voxels after tDCS was confirmed using a screenshot of the voxel location taken before stimulation measurements. A graphical description of the experimental layout is depicted in Fig. 3. The participants were asked to keep their eyes open during the whole experimental procedure and were informed about the start and duration of each measurement before acquisition.

All GABA concentration levels as shown in Table 1 were normalized to the first baseline pre-stimulation measurement before running statistical analyses. The second pre-stimulation measure served as control for spontaneous fluctuations of GABA concentration irrespective of experimental manipulations. To verify that the voxels of interest (VOI) were equivalent pre- and post-tDCS, a set of mixed ANOVAs was conducted to examine any changes in grey and white matter fractions (as shown in Table 2) within each VOI. The ANOVA included the within-subject factor time (pre- vs. post-tDCS) and the between-subject factor tDCS polarity (anodal vs. sham). The analyses confirmed that in all cases there were no significant main or interaction effects (all $p > 0.05$). These analyses confirm that tissue fractions pre- and post-tDCS did not differ.

MRS data analysis. The freely available software package TARQUIN (Totally Automatic Robust Quantitation in NMR, version 4.3.5) was used to quantify RDA files containing MRS data. This tool utilizes a linear combination of basis functions to perform a fully automatic analysis of spectra[49] and its reliability is comparable with other spectral quantification methods (O'Gorman *et al*., 2011). The free induction decay signal (FID) was zero filled to twice its original length to obtain reference at a higher precision. The residual water signal was then filtered out by fitting and removing Gaussian peaks around the water frequency using hankel singular value decomposition techniques. The spectrum was then frequency and phased corrected with respect to both the zero- and first-order phase. To analyze MEGA-PRESS data, TARQUIN models the GABA peak as composed by two single Gaussians[46]. The unsuppressed water scan was used as internal reference to find the absolute value of GABA concentration. The 3-ppm GABA peak in the difference spectrum was fitted using a two single Gaussian and quantified relative to water. The final spectral quality was assessed using Cramér-Rao-Lower-Bounds[50] minimum possible variance on a fit parameter. Only data (as shown in Fig. 4) that had Cramér-Rao-Lower-Bounds

	Measurements	Mean_GABA Conc [mM(±SD)] (Anodal group)	Mean_GABA Conc [mM(±SD)] (Sham group)
Before stimulation	1	1.68(±0.34)	1.76(±0.19)
	2	1.67(±0.29)	1.67(±0.18)
After Stimulation	1	1.47(±0.45)	1.76(±0.23)
	2	1.53(±0.42)	1.71(±0.25)
	3	1.61(±0.34)	1.66(±0.20)
	4	1.50(±0.48)	1.68(±0.25)
	5	1.49(±0.44)	1.66(±0.24)
	6	1.53(±0.46)	1.72(±0.24)
	7	1.53(±0.52)	1.70(±0.22)
	8	1.49(±0.45)	1.65(±0.22)
	9	1.48(±0.48)	1.70(±0.31)
	10	1.42(±0.45)	1.71(±0.27)

Table 1. Mean GABA concentrations related to anodal and sham tDCS.

	Subjects	Grey Matter	White Matter	CSF
Mean(±SD)	32	0.45(±0.08)	0.42(±0.05)	0.13(±0.08)

Table 2. Mean Grey Matter, White Matter and CSF values.

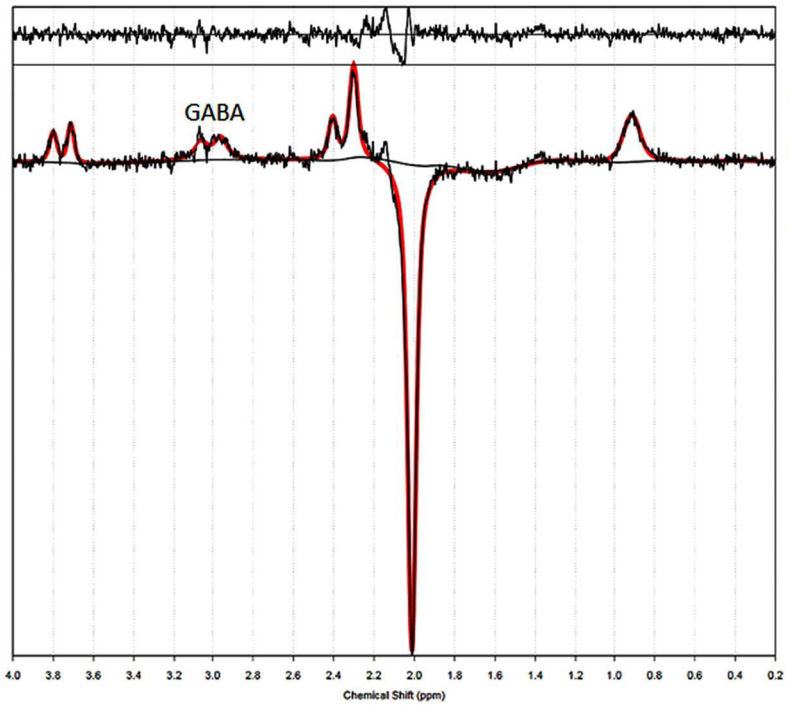

Figure 4. An edited spectrum from a single subject shows characteristic peaks for GABA after application of a linear combination model using TARQUIN to perform a fully automatic fit of spectra. The acquired spectrum is plotted in black and the fit in red. Below and above the acquired spectrum are the baseline and residual shown respectively.

values of less than ≤20% were included in the analysis. The concentration of GABA was corrected for the proportion of grey matter within the voxel using the MPRAGE anatomical image[13]. The voxel fraction of CSF, gray matter, and white matter were calculated after generation of a binary mask of the MRS voxel created with the same imaging matrix as the T1-weighted anatomical image, using Gannet's[51] integrated voxel-to-image coregistration and segmentation of the anatomical image using Segment in SPM12[52].

Data Availability

The datasets generated during and/or analyzed during the current study are freely available from the open access *OSF* repository (https://osf.io/). https://osf.io/bkzey/?view_only=320b254a563b4b5d8cd9c6891a6d110a.

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Author Contributions

H.J.P.: Design, acquisition, analysis and interpretation of data; F.B.: Design and interpretation of data; M.N.: Interpretation of data; A.P.: Interpretation of data: S.R.: Acquisition of data; K.R.: Acquisition of data.

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A study of long-term GABA and high-energy phosphate alterations in the primary motor cortex using anodal tDCS and $^{1}H/^{31}P$ MR spectroscopy

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Introduction: Anodal transcranial direct current stimulation (tDCS) has been reported to modulate gamma-aminobutyric acid levels and cerebral energy consumption in the brain. This study aims to investigate long-term GABA and cerebral energy modulation following anodal tDCS over the primary motor cortex.

Method: To assess GABA and energy level changes, proton and phosphorus magnetic resonance spectroscopy data were acquired before and after anodal or sham tDCS. In anodal stimulation, a 1 mA current was applied for 20 min, and the duration of ramping the current up/down at the start and end of the intervention was 10 s. In the sham-stimulation condition, the current was first ramped up over a period of 10 s, then immediately ramped down, and the condition was maintained for the next 20 min.

Results: The GABA concentration increased significantly following anodal stimulation in the first and second post-stimulation measurements. Likewise, both ATP/Pi and PCr/Pi ratios increased after anodal stimulation in the first and second post-stimulation measurements.

Conclusion: The approach employed in this study shows the feasibility of measuring long-term modulation of GABA and high-energy phosphates following anodal tDCS targeting the left M1, offering valuable insights into the mechanisms of neuroplasticity and energy metabolism, which may have implications for applications of this intervention in clinical populations.

KEYWORDS

tDCS, GABA, ^{31}PMRS , ^{1}HMRS, primary motor cortex, neuroplasticity, energy metabolism

Introduction

Several studies have demonstrated molecular and neurophysiological evidence linking altered neuronal plasticity to neurological disorders (Di Lazzaro et al., 2010) and altered energy metabolism to metabolic disorders (Soares et al., 2011) in brain areas. It has been suggested that metabolic disorders are associated with neurological disorders (Crabtree and Gogos, 2014; Penninx and Lange, 2018). For instance, abnormal neuroplasticity mediated by altered neurotransmission (Harrison, 1999) has been observed in the prefrontal cortex in schizophrenia patients (Hulshoff Pol and Kahn, 2008). One suggested explanation is that hypofunction of the NMDA-type glutamate receptor cause a decrease in the excitation of GABAergic interneurons, resulting in glutamatergic neurons disinhibition (Kondziella et al., 2007; Moghaddam et al., 1997; Olney et al., 1999; Stone et al., 2009). This disinhibition of glutamatergic neurons may result in excessive glutamate stimulation, which may cause neuronal damage or death via excitotoxicity leading to a hyperdopaminergic state and psychosis. In an EEG study comparing healthy subjects with schizophrenia, variation in the P3a distribution was found which shows differences in the attention system activity (Mugruza-Vassallo and Potter, 2019). Functional imaging studies that assess regional cerebral blood flow (rCBF) can help identify cerebral activity associated with schizophrenia (Tamminga, 1999). For example, Liddle et al. (1992a) and Liddle et al. (1992b) found a negative correlation between the psychomotor poverty symptoms of schizophrenia and rCBF in the lateral prefrontal cortex and overactivity in the striatum.

Pharmacological studies have discovered genes involved in the pathophysiology of schizophrenia (De Jong et al., 2016; Gaspar and Breen, 2017; Rodriguez-Lopez et al., 2020; Ruby et al., 2014), such as GRM3 expression in astrocytic cells, which is required to protect neurons against NMDA induced neurotoxicity (Hikmah et al., 2023). This suggests that diminished GRM3 functionality, which may result in hyper-glutamatergic signalling, may cause more damage to the neurons as a result of the abnormal signaling exhibited in schizophrenia (Saini et al., 2017). In schizoaffective disorder, both schizophrenic and affective occur at the same time (Werner and Covenas, 2016). The mesolimbic system (the hippocampus and prefrontal cortex) plays an important role in the symptoms, which involves alteration in a multi-neurotransmitter system such as dopamine, serotonin, GABA and glutamate (Kulkarni et al., 2022) due to susceptibility genes such as neuregulin-1 dysbindin-1, GAD67 catechol-O-methyl transferase and monoamine oxidase (Haller et al., 2014; Ruby et al., 2014; Werner and Coveñas, 2013).

Several studies using magnetic resonance spectroscopy (MRS) have indicated that major depressive disorder (MDD) individuals have lower GABA concentrations in occipital cortex than healthy controls (Sanacora et al., 2004; Sanacora et al., 1999; Sanacora et al., 2003). MDD, along with GABA, is also related to altered serotonin activity. In many research studies, the binding potential of serotonin receptor, 5-hydroxytryptamine or (5-HT) was found to be lower in patients with MDD than in control subjects (Bhagwagar et al., 2004; Drevets et al., 1999; Hirvonen et al., 2008; Sargent et al., 2000). Selective serotonin reuptake inhibitors (SSRI) are most widely used to treat MDD and have shown significant change in the dorsolateral prefrontal cortex (DLPFC) volume and resting-state functionality (Lee et al., 2023).

Altered neurotransmission as seen in MDD is multifaceted and has been associated with the risk of developing severe medical disorders. i.e. it increases the risk of cardiovascular disorders by 1.5–2 fold (Van der Kooy et al., 2007) for stroke 1.8 fold (Ramasubbu and Patten, 2003) for Alzheimer's disease by 2.1 fold (Green et al., 2003) and diabetes by 60% (Mezuk et al., 2008). The relationship between metabolic disorder and other psychiatric disorders is evident in the above-mentioned studies. Moreover psychiatric disorders are often accompanied by cognitive impairment in various domains, such as attention, executive functions, memory, and processing speed, as highlighted in studies (Grundman et al., 2004; McIntyre et al., 2015; Millan et al., 2012; Reichenberg, 2010). GABA neurotransmitter plays an important role in cognition and several studies have shown a link between dorsolateral prefrontal cortex GABA to working memory (Duncan et al., 2014; Yoon et al., 2016) and supplementary motor area GABA levels to motor distraction (Duncan et al., 2014; Mullins et al., 2014) and to alterations in cognitive processing with age (Harris et al., 2017; Porges et al., 2017). The interaction between altered neuroplasticity and metabolism has implications on cognitive functions or impairment. Therefore, simultaneously studying the neurochemical mechanisms behind neuronal plasticity and energy metabolism may offer significant breakthroughs for therapeutic interventions. With the advanced assessment of high-energy phosphate and neurotransmitters like gamma amino butyric acid (GABA) we can get a deeper insight into the mechanism of such changes in the brain of patients suffering from psychiatric and metabolic disorders.

A well-established approach for modulating neuronal plasticity and energy in humans is a non-invasive brain stimulation technique known as transcranial direct current stimulation (tDCS). By targeting specific brain regions, tDCS can be used to modulate brain energy metabolism and neuronal plasticity. Human *in vivo* studies have demonstrated that the application of anodal tDCS in the primary motor cortex can lead to spontaneous firing rates of cortical neurons, inducing neuronal excitation and altering energy consumption (Binkofski et al., 2011; Nitsche and Paulus, 2000). This modulation in excitability and energy is likely mediated by an altered concentration of the inhibitory neurotransmitter GABA (Stagg et al., 2009) and high-energy phosphates, e.g., adenosine triphosphate (ATP) and phosphocreatine (PCr).

Owing to ongoing advancements in magnetic resonance (MR) techniques (Choi et al., 2021; Henning, 2018; Wilson et al., 2019), changes in GABA and high-energy phosphates in M1 following tDCS can be monitored using MR spectroscopy (MRS). Specifically, it has been shown that proton (^{1}H)-MRS (Patel et al., 2019) and phosphorus (^{31}P)-MRS can be used to measure changes in GABA and energy phosphates in the M1, for example, following anodal tDCS as compared to sham tDCS. The time courses of separately measured concentrations of GABA and ATP/PCr in the aforementioned studies indicated that GABAergic activity and bioenergetics inside the M1 might work together to modulate neuronal excitability and energy. Consecutive measurement of both ^{1}H- and ^{31}P-MRS, before and after tDCS, could offer a promising approach to yield information related to plastic adaptation and energy consumption in both healthy and diseased brains.

In this study, we hypothesize that the application of anodal tDCS to the M1 region modulates GABA concentration and brain energy consumption. Notably, this work presents a measurement approach

that integrates one pre-tDCS, ^{1}H- and ^{31}P-MRS measurement and two consecutive post-anodal tDCS, ^{1}H- and ^{31}P-MRS measurements to demonstrate the viability of using MRI and MRS to measure the long-term modulation of GABA and high-energy phosphates following anodal tDCS targeting the left M1 for the first time.

Materials and methods

Forty-four healthy subjects (22 anodal and 22 sham) with no history of neurological or psychiatric diseases were recruited for this single-blind, randomized control pilot study. The subjects (Mean age: 28 ± 7; Gender: 26 Female and 18 Male) were all right-handed, as evaluated by the Edinburgh Handedness Inventory (Oldfield, 1971). All examinations were conducted at the Division of Clinical Cognitive Sciences of the RWTH Aachen University, Germany, and subjects provided their written informed consent for participation in this pilot study, which was agreed upon by the local ethics committee.

The non-invasive DC-Stimulator (neuroConn GmbH, Germany) used to stimulate the M1 region was programmed to deliver constant, direct current to the brain via two rubber electrodes (5 × 7 cm) covered by saline-soaked (0.9% NaCl) sponges, which modulate brain activity. One electrode was centered over the left M1 (5 cm lateral to Cz, C3) and the other over the contralateral supraorbital ridge using the conventional EEG 10/20 system. To accomplish the electrical contact between the electrodes and the scalp, 0.9% NaCl solution was used as a conducting medium. In anodal stimulation, a 1 mA current was applied for 20 min, and the duration of ramping the current up/down at the start and end of the intervention was 10 s. In the sham-stimulation condition, the current was first ramped up over a period of 10 s, then immediately ramped down, and the condition was maintained for the next 20 min.

All MR measurements were carried out on a 3 T PRISMA MRI scanner (Siemens Healthineers, Erlangen, Germany) with a dockable patient bed. A quadrature double-tuned head coil (RAPID Biomedical, Wuerzburg, Germany) was used to achieve both ^{1}H and ^{31}P acquisitions. Figure 1 shows a tabular representation of the overall experimental design. Each experimental session started with a localizer followed by the acquisition of whole-brain anatomical images using an MP-RAGE MRI sequence [parameters: voxel size = 1.5 × 1.5 × 1.5 mm^3, repetition time (TR) = 2,000 ms, echo time (TE) = 2.05 ms, inversion time (TI) = 900 ms, acquisition time (TA) = 4:32 min], with RF power and global static magnetic field (B_0) shimming calibrations. Figure 2 shows the 3D anatomical image used to locate a 30 × 30 × 30 mm^3 voxel-of-interest (VOI) within the hand-knob area of the left M1. Prior to the MRS measurements, an additional advanced shimming procedure was employed using a FASTEST map MRS sequence (Gruetter and Tkáč, 2000) to improve the B_0 homogeneity in the selected VOI. A full-width at half-maximum (FWHM) of the ^{1}H resonance peak in the VOI was achieved at approximately 15 Hz. A MEGA-PRESS MRS sequence (Mescher et al., 1998; parameters: voxel size = 30 × 30 × 30 mm^3, TR = 5,350 ms, TE = 68 ms, TA =17:53 min, averages = 96, vector size = 1,024, flip angle = 90°) and a 3D chemical shift imaging MRS sequence (Wenger et al., 2020; parameters: voxel size = 30 × 30 × 30 mm^3, TR = 3,730 ms, TE = 2.3 ms, TA = 14:33 min, averages = 6, vector size = 1,024, flip angle = 90°) were applied to acquire ^{1}H-GABA and ^{31}P spectra, respectively. A standard PRESS MRI sequence with eight averages was also included to record an unedited spectrum for the assessment of the creatine and N-acetylaspartate acid (NAA) linewidths. The nuclear Overhauser effect enhancement technique was employed to improve the quality of the spectra in the ^{31}P acquisition. Reference ^{1}H and ^{31}P spectra as shown in Figure 3 were attained before the application of tDCS (pre-stimulation measurements), which took approximately 35 min. After obtaining the baseline MRS scans, the whole patient table, including the subject and the coil, was undocked from the scanner and moved outside the magnet room for stimulation. The stimulation was delivered for 20 min, during which participants were asked to remain still. Following the stimulation, the patient table was docked back to the MR scanner for the subsequent measurements. Undocking and docking of the table procedure helped to mitigate subject movement between the pre- and post-stimulation MRS measurements. The same MP-RAGE MRI sequence was used post-stimulation to ensure that the location of the VOI was identical to the pre-stimulation position. Two consecutive post-stimulation ^{1}H and ^{31}P MRS measurements were conducted. The ^{1}H MRS measurement was conducted for 17:53 min, and then ^{31}P MRS was conducted for 14:33 min twice, in a constant order post-stimulation. All subjects were informed about the duration of the measurement and were asked to remain awake and not to move during the whole experimental procedure.

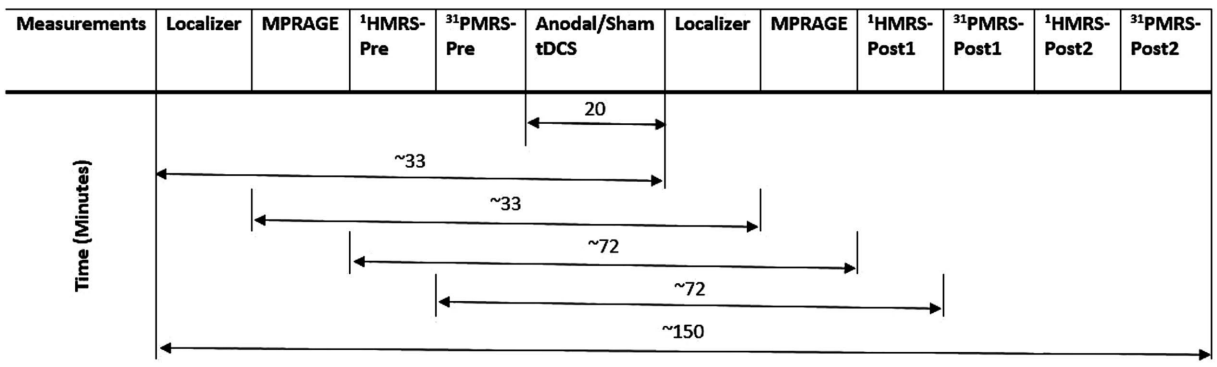

FIGURE 1
The table shows the ^{1}H GABA, ^{31}P MRS and other MR measurements performed before and after tDCS.

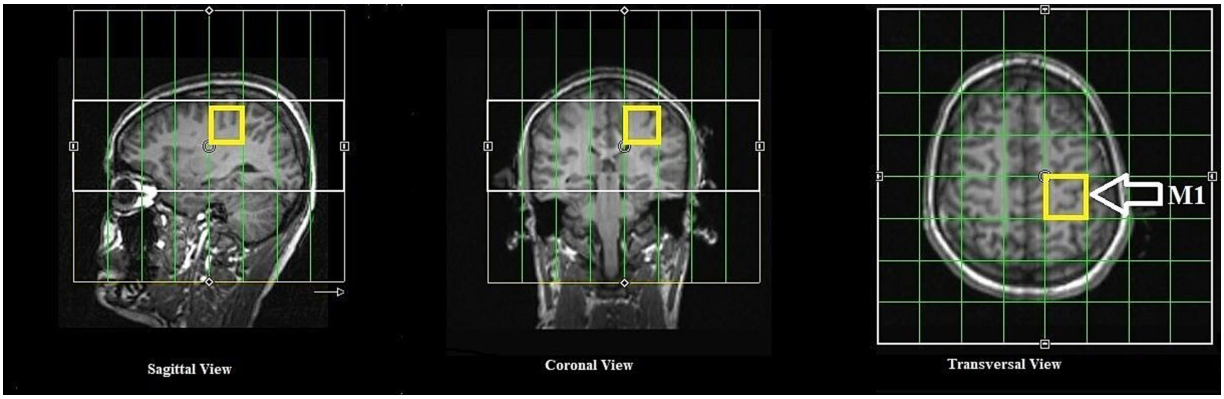

FIGURE 2
Anatomical MR images in axial, coronal and sagittal slices and the overlaid voxel of interest (in yellow) for ^{1}H and ^{31}P MRS.

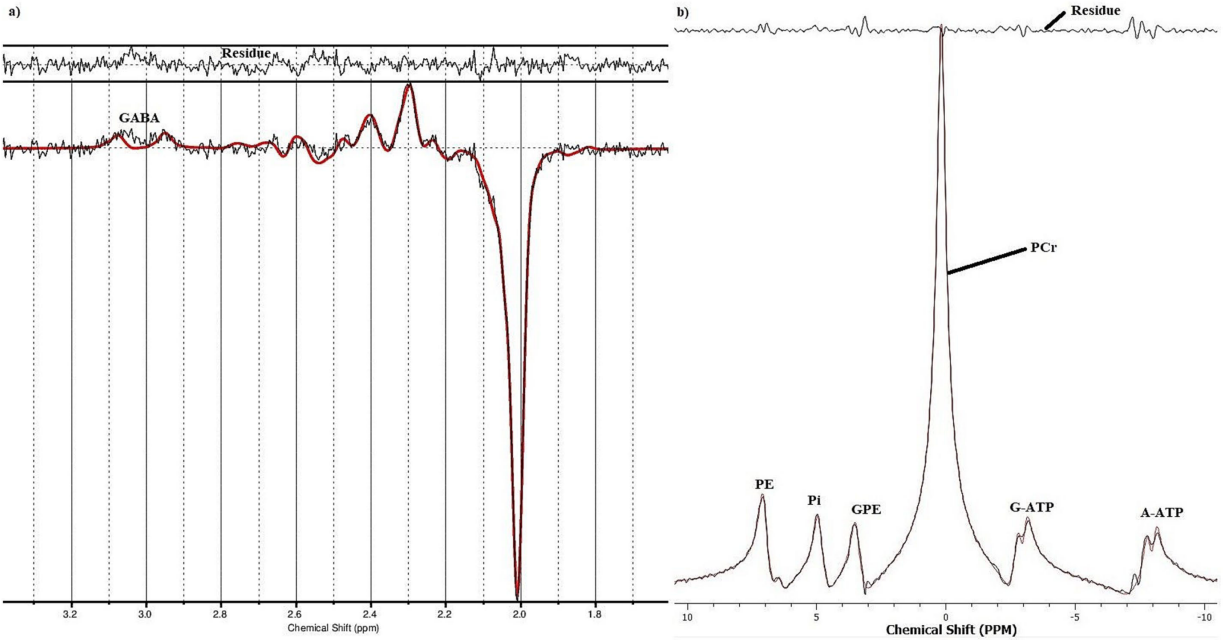

FIGURE 3
An example of a fitted **(A)** 1H GABA and **(B)** 31P spectrum from a healthy subject.

All ^{1}H-GABA-MRS data processing was performed using the LCModel (Provencher, 1993), a spectral quantification tool that fits each spectrum as a weighted linear combination of basis spectra from individual brain metabolites. The difference basis set included GABA, Glu, glutamine, glutathione, and NAA. Based on an 8 × 8 CSI grid, the ^{31}P MR spectra from one voxel were analyzed with TARQUIN 4.3.10(Wilson et al., 2011). All data underwent a Fourier transformation, as well as zero and first-order phase correction, and were fitted as linear combinations of the simulated metabolic basis set, including PCr, ATP, Pi, PE, GPC and GPE for ^{31}P-MRS. While PCr, Pi, PE, GPC and GPE are observed as singlet Lorentzian peaks, the signals from the α- and γ-ATP were modelled as doublets. However, the signal from ATP-ß was excluded from the analysis due to phasing instabilities (Novak et al., 2014). The quality of the final spectra was assessed using the Cramér-Rao-Lower-Bounds (Rao, 1947) minimum possible variance on a fit parameter. Only data that had Cramér-Rao-Lower-Bounds values of less than ≤20% were included in the analysis (Kreis, 2016; Öz et al., 2014; Peek et al., 2023; Wilson et al., 2019).

Statistical analysis

In order to investigate the effects of tDCS on the inhibitory neurotransmitter and energy metabolites in the left M1, changes in proton and phosphorus metabolites in pre- and post-stimulation scans were calculated separately to obtain the information about GABA, ATP/Pi and PCr/Pi. Each ratio was normalized (post1-tDCS/pre-tDCS & post2-tDCS/pre-tDCS) with its reference measurement (pre-tDCS). Statistical analysis was conducted with SPSS (version 29.0 Armonk, NY, USA), with $p = 0.05$ set as the significance threshold. A

mixed-design Analysis of variance was performed for GABA concentration, ATP/Pi and PCr/Pi metabolites. Stimulation (sham vs. anodal) was considered as the between-subjects factor, and measurement (three measurements: one pre-stimulation and two post-stimulation measurements [post1 and post2]) was considered as the within-subject factor. Paired sample t-test comparisons ($p > 0.05$) were performed in the cases of significant ANOVA results.

Results

Effect of tDCS on GABA

For GABA, the main effect of the stimulation was found to be significant [$F_{(1, 42)} = 4.742$, $p = 0.035$]; however, the main effect of the measurement was not significant [$F_{(2, 84)} = 2.855$, $p = 0.063$]. That being said, the stimulation × measurement interaction was significant [$F_{(2,84)} = 3.387$, $p = 0.038$]. Paired sample *t*-test comparisons show that concentration did not change significantly from pre-tDCS measurement to post1- and post2-tDCS measurements [$ts_{(21)} \leq 0.277$ $ps > 0.05$] for the sham group. Conversely, relative to the pre-tDCS, GABA concentration in the anodal tDCS group significantly increased after 14 min at the post1-tDCS measurement [$ts_{(21)} = 3.380$ $p = 0.001$], and also after 48 min at the post2-tDCS measurement [$ts_{(21)} = 1.893$ $p = 0.036$]. Thus, in the sham group, GABA concentration stayed at the pre-tDCS baseline level across all post-tDCS measurements. In contrast, data from the anodal group suggest an increase in GABA concentration across the two time points in the post-tDCS phase. Figure 4A shows the changes in normalized GABA concentration levels induced by tDCS at pre-tDCS, post1-tDCS and post2-tDCS spectroscopy measurements.

Effect of tDCS on energy phosphates (ATP/pi & PCr/pi)

For ATP/Pi ratios, neither the main effect of stimulation [$F_{(1,42)} = 1.573$, $p = 0.217$] nor the main effect of the measurement [$F_{(2,84)} = 0.259$, $p = 0.772$] was significant. Furthermore, the interaction stimulation × measurement was also not significant [$F_{(2,84)} = 0.887$, $p = 0.416$]. Figure 4B shows changes in normalized ATP/Pi ratios for pre-tDCS, post1-tDCS and post2-tDCS spectroscopy measurements. For PCr/Pi ratios, neither the main effect of stimulation [$F_{(1,42)} = 0.218$, $p = 0.643$] nor the main effect of the measurement [$F_{(2,84)} = 0.045$, $p = 0.956$] was significant. Additionally, the interaction stimulation × measurement was also not shown to be significant [$F_{(2,84)} = 852$, $p = 0.430$]. Figure 4C shows tDCS-induced effects on normalized PCr/Pi ratios for pre-tDCS, post1-tDCS and post2-tDCS spectroscopy measurements.

Discussion

The human brain functions as a dynamic system, continually adjusting its metabolic interactions to accommodate the activation status and energy requirements of the entire organism. Therefore, static MRS measurements obtained in single sessions necessarily deliver an incomplete picture of the state of the brain. To address this limitation, our feasibility study demonstrates the acquisition of combined measurements using ^{1}H-GABA-MRS, ^{31}P-MRS and anodal stimulation to provide unique information relating to adaptive plasticity and energy consumption in the human brain during extended time periods after plasticity-inducing brain stimulation.

Our results indicate a notable increase in GABA levels measured by ^{1}H-MRS following 20 min of anodal stimulation over the M1 at both the initial post-tDCS measurement and the second post-tDCS measurement, which contradicts previous reports showing a reduction in GABA levels at these time points (Agboada et al., 2019; Patel et al., 2019). Studies have shown that changes in cortical excitability due to tDCS are calcium-dependent (Grundey et al., 2018; Nitsche et al., 2003) and that calcium concentration within a specific range is required for LTP induction (Lisman, 2001). Therefore, it could be argued that previous studies (Patel et al., 2019; Stagg et al., 2009) utilizing relatively low tDCS intensities and/or short durations might have been operating at the lower limit of the calcium concentrations required for inducing the aforementioned neuroplastic changes. Within the range given in Lisman (2001), increasing calcium concentration should, theoretically, increase the efficacy of LTP induction. Hence, prolonging the duration of stimulation beyond a critical time point may lead to a saturation of the after-effects, possibly caused by calcium overflow (Monte-Silva et al., 2013). Consequently, higher calcium concentration might activate counteracting homeostatic mechanisms, such as the activation of potassium channels or the saturation of NMDA receptors, thereby limiting the amount of plasticity (An et al., 2000; Lau and Zukin, 2007; Misonou et al., 2004). These potential mechanisms require further exploration. To the best of our knowledge, there are very few studies investigating the mechanism underlying these non-linear effects (Batsikadze et al., 2013; Jamil et al., 2017; Monte-Silva et al., 2013). In line with this notion, studies using different non-invasive brain stimulation techniques have also reported non-linear cortical excitability effects post-tDCS (Batsikadze et al., 2013; Kuo et al., 2013) with respect to varying intensity (Moliadze et al., 2012) and duration (Choi et al., 2021; Heimrath et al., 2020; Monte-Silva et al., 2013).

In an apparent contradiction to the results obtained in our study, a study from Agboada et al. (2019) reported that, having applied anodal tDCS with 1 mA current for 20 min, cortical excitation could be subsequently monitored for two hours by measuring motor evoked potentials (MEPs) using TMS. However, the reason for these opposing results might be due to a difference in the study population. Furthermore, the amount of extracellular GABA measured by MRS might not correlate with TMS-induced activity in the GABAergic synapses. As neurotransmitters are active in intracellular and extracellular space (Belelli et al., 2009; Martin and Rimvall, 1993), and MRS is not sensitive enough to differentiate between these sources, it is only possible to speculate about mechanisms that are known to change GABA concentration. Moreover, animal studies conducted by Purpura and McMurtry (1965) have shown that direct stimulation in the deep cortical layers leads to the activation of neurons through cathodal stimulation and deactivation through anodal stimulation. Hence, the longer tDCS protocol used in the present study might have more significant effects on GABAergic neurons, which are less affected under the weaker electric field induced by a shorter stimulation duration. It is also possible that longer tDCS protocols may affect the target neurons as well as neighbouring non-target neurons, which might change the direction of plasticity in the target regions.

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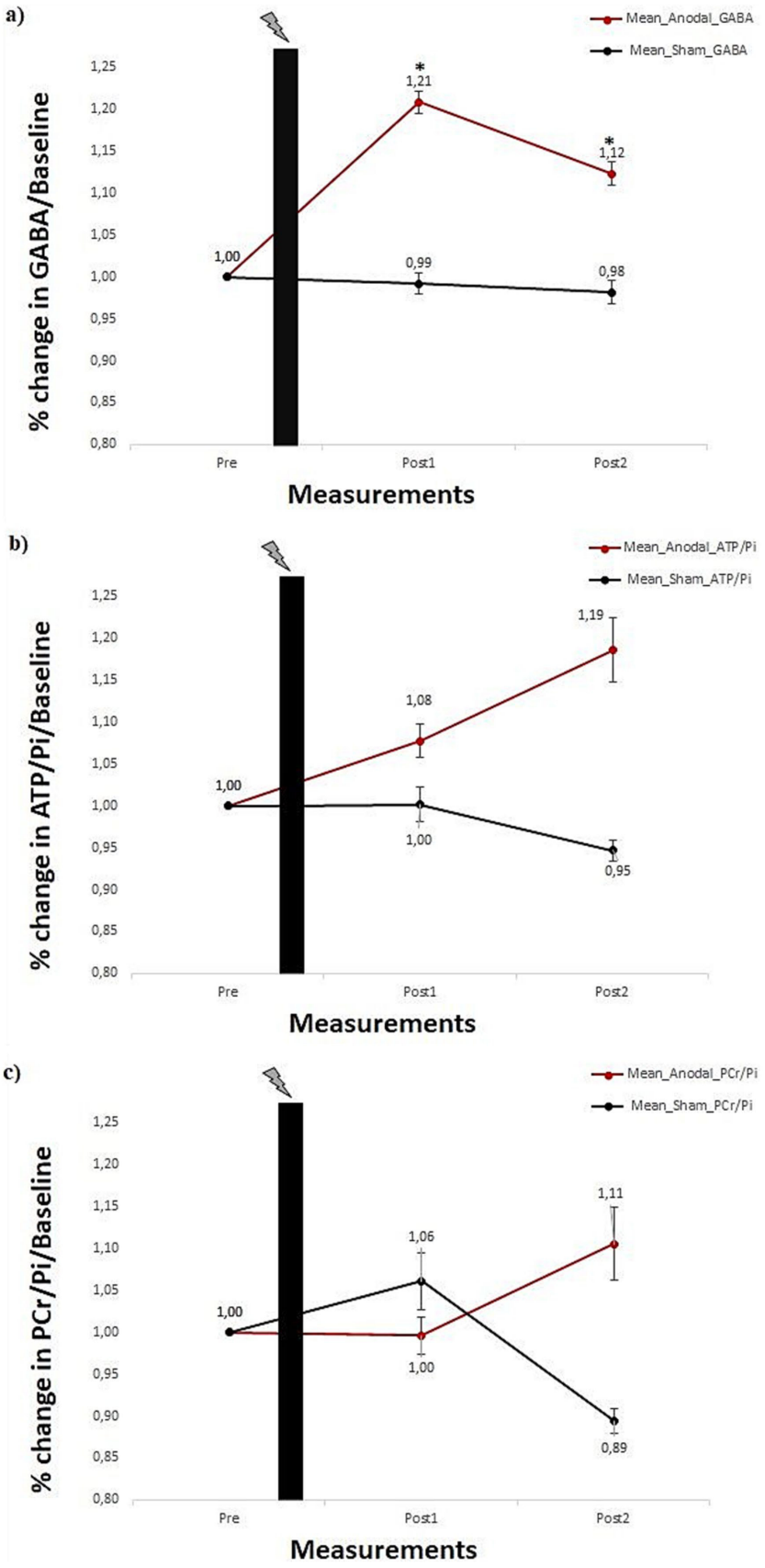

FIGURE 4
(A) Percentage change in mean GABA concentration. **(B)** ATP/Pi ratio, and **(C)** PCr/Pi ratio for anodal and sham stimulation groups, each comprising 22 subjects. Error bars reflect the standard error of the mean. Black plot: anodal/sham stimulation. *Show significances ($p < 0.05$) for a paired sample *t*-test.

The levels of MRS GABA have been linked to behavioural measures in M1, where higher GABA levels were associated with greater inhibition and slower reaction times (Stagg et al., 2011). Changes in GABA levels in the dorsolateral prefrontal cortex (DLPFC) have also been observed in studies on impulsivity, a personality trait associated with various psychiatric disorders in the DSM. Studies have indicated that higher GABA levels in the DLPFC are linked to lower urgency scores, suggesting a role in self-control for the GABA-mediated inhibitory mechanism (Boy et al., 2011). Additionally, research by Kühn et al. (2016) and Yoon et al. (2016) demonstrated that higher GABA levels in the DLPFC and anterior cingulate cortex (Rossini et al., 1994) are associated with less performance degradation under higher working memory load compared to subjects with lower GABA levels. Furthermore, elevated GABA levels in the visual cortex have been associated with improved cognitive and perceptual performance in visual discrimination tasks (Cook et al., 2016; Edden et al., 2009; Porges et al., 2017; Sumner et al., 2010). van Vugt et al. (2020) investigated the influence of GABA modulation on audio perception discrimination and mapping motor responses with perceived sounds. Elevated GABA levels in the left sensorimotor cortex (SM1) during training were associated with enhanced behavioural learning, highlighting the role of GABA modulation in forming unique audio-motor learning tasks. The elevated GABA levels in the ACC during situations involving conflict or uncertainty (Bezalel et al., 2019) contribute to decision-making by maintaining inhibition and preventing excessive excitement. A further study investigated the relationship between GABA levels within the sensorimotor cortex and measures of sensory discrimination and demonstrated that healthy subjects who had higher GABA levels showed greater frequency discrimination (Puts et al., 2011). Higher GABA levels were associated with improved performance in orientation discrimination task as seen in studies of the primary visual cortex (Edden et al., 2009). Similar relationships between a variety of task-specific behaviours and levels of GABAergic inhibition have also been demonstrated in a number of brain regions outside the sensorimotor cortex (Boy et al., 2010; Boy et al., 2011; Jocham et al., 2012; Sumner et al., 2010). This implies that MRS-derived measurements of GABA are behaviorally relevant in a variety of cortical areas, and not limited to M1.GABA modulation is not exclusive to learning and has been observed in memory consolidation as well. For an increase in GABA levels in the occipital lobes following a visual learning task helps in fast memory consolidation and protects against interference (Shibata et al., 2017). The use of Zolpidem, a GABAA agonist, during sleep has been shown to enhance episodic memories, indicating the role of GABA in memory consolidation. The Increased GABA activity during sleep supports memory consolidation (Mednick et al., 2013; Zhang et al., 2020).

Coxon and colleagues discovered that engaging in high-intensity interval exercise resulted in elevated GABA levels in SM1 (Coxon et al., 2018). In a study by Maddock, it was demonstrated that vigorous cycling increased GABA/Cr levels in the primary visual cortex post-exercise (Maddock et al., 2016). This suggests that physical exercise influences regional GABA levels beyond SM1, but the regional specificity of GABA changes due to exercise require further investigation.

It has been well reported that GABA and Glu interact along the same biochemical pathway, as Glu is the primary precursor for GABA synthesis (Petroff, 2002; Rae et al., 2003). Given that GABA is synthesized from the alpha decarboxylation of Glu by glutamic acid decarboxylase (GAD-67), the activity-driven expression of GAD-67 controls GABA synthesis and determines the concentration of GABA in interneurons (Lau and Murthy, 2012). It is possible that anodal tDCS may subtly interfere with GAD-67 activity and consequently affect this pathway, resulting in a change in the GABA concentration. However, we were only able to access the Glx information at 3 T, which combined glutamate and glutamine signals due to their close chemical shift range. A study, for example, using 7 T MRI, might help to investigate long-term glutamate modulation and allow further exploration of the mechanism of excitatory glutamatergic neurons in long-term plasticity.

The observed increase in ATP/Pi and PCr/Pi ratios shown in our study can be partially compared to findings from Binkofski et al. (2011), who reported their first non-significant post-tDCS measurement between the end of tDCS and before around 65th minute of MRS measurement, thus also showing an initial rise in energy followed by a subsequent decline after the 65th minute. The increase in ATP/Pi ratio and PCr/Pi ratio in our study could be attributed to a higher rate of energy phosphate synthesis compared to its consumption as a consequence of the longer tDCS duration. ATP concentration has been observed as a potential modulator of neurotransmission (Miller et al., 1980; Miller et al., 1977), and the neurotransmitter glutamate is a known precursor of GABA synthesis and uses glutamic acid decarboxylase (GAD) to synthesize GABA. At least 50% of GAD is present in the brain as apoenzyme (apoGAD), thereby providing a reservoir of inactive GAD that can be drawn on when additional GABA synthesis is required (Itoh and Uchimura, 1981; Miller et al., 1980; Miller et al., 1977). It seems that GAD plays an important role in regulating the transmitter pool of GABA. Studies indicate that the increase in the ATP/Pi ratio accelerates the formation of apoGAD (Meeley and Martin, 1983; Wu and Martin, 1984), inhibits its activation (Porter and Martin, 1988; Sze et al., 1983) and stabilizes apoGAD against thermal denaturation (Meeley and Martin, 1983; Porter and Martin, 1988). It appears that the activation of GAD is regulated by energy metabolism, and increased neural activity leads to a higher turnover of energy metabolites. Moreover, it is plausible that these energy metabolites play a vital role in linking GAD activity with neuronal activity. Thus, it is plausible that elevated levels of ATP might stimulate the activation of apoGAD, thereby promoting GABA synthesis.

GABA appears to have two major functions in nervous tissue, acting both as a neurotransmitter and as an intermediate in the energy metabolism of GABAergic neurons (Shelp et al., 2012). While much attention is given to the role of GABA as a neurotransmitter, its role as a metabolic intermediate is also important and may help to explain the link between neuronal plasticity and energy metabolism. This metabolic function of GABA arises from its relationship with the tricarboxylic acid (TCA) cycle (Baxter, 1970). The TCA cycle is a series of processes that produce high-energy phosphate, such as ATP, following glycolysis. Three enzymes of GABA pathways, GAD, GABA-a-oxoglutarate transaminase and succinic semialdehyde dehydrogenase, facilitate a bypass known as the GABA shunt. This bypass allows for the circumnavigation of two steps of the TCA cycle, leading to the production of glutamate for GABA synthesis. In this way, GABA is metabolized via the GABA shunt within the TCA cycle, thus emphasizing the role of GABA synthesis in maintaining adequate GABA levels. Based on this neurochemical mechanism, it

might be possible that the longer stimulation protocol demands higher energy phosphate synthesis and also higher GABA synthesis, which may work together to regulate the higher demand for energy phosphate and the corresponding GABA synthesis in the brain. Our study demonstrates that anodal tDCS leads to an increase in ATP resynthesis to meet the energy demand, which is similar to the energy regulation observed after physical exercise (Hargreaves and Spriet, 2020). Modulation of energy following physical activity has shown to improve memory, reasoning, planning, motor skill (Nanda et al., 2013; Statton et al., 2015) and promote cognitive functioning in both healthy as well as clinical populations such as stroke, multiple sclerosis and depression (Moriarty et al., 2019; Moriya et al., 2016; Sandroff et al., 2015; Sibley et al., 2006; Vasques et al., 2011; Wang et al., 2016). Physical activity boosts brain-derived neurotrophic factor (BDNF), promoting neuroplasticity (Loprinzi and Frith, 2019), and consequently enhancing memory and learning (Piepmeier and Etnier, 2015), while also offering protection against Alzheimer's and depression (Bjornebekk et al., 2005; Erickson et al., 2011; Liu and Nusslock, 2018). Elevated BDNF levels are associated with the survival and growth of neurons (McAllister et al., 1999). Lower BDNF levels are linked to ageing and neurodegenerative diseases (Holsinger et al., 2000). These findings reveal the usefulness of physical exercise or alteration of energy regulation as shown in our study can be employed as an auxiliary tool for rehabilitation because it appears to affect the neurological system for learning and skill acquisition.

The results of this study suggest that the new combined approach introduced here has strong implications for the measurement of energy and neural plasticity in both healthy subjects and neuropsychiatric patients (Gobel et al., 2013; Jauch-Chara et al., 2015; Stagg et al., 2011; Stagg and Johansen-Berg, 2013). For instance, psychiatric and metabolic diseases have been found to be interconnected, often acting as a precursor to each other (Estrov et al., 2000; Zuccoli et al., 2017). In this context, combined ^{1}H- and ^{31}P-MRS together with anodal tDCS may represent a two-pronged approach to better understand impaired glucose metabolism and psychiatric disorders without the need for pharmacological intervention. Furthermore, this approach can be employed in numerous additional experiments aiming to optimize the described experiment from a clinical perspective. For instance, improvements in terms of shortening acquisition time and increasing the signal-to-noise ratio can be achieved at ultra-high field strengths (Pradhan et al., 2015), particularly with the use of double-tuned, multi-channel array coils (Rowland et al., 2020). Additionally, further investigation of the intensity and duration of the stimulation in order to understand the neurochemical mechanisms involved could also extend the impact of the tDCS. Apart from tDCS, future research may explore other transcranial electrical stimulation options, including transcranial alternating current stimulation (tACS), transcranial pulsed current stimulation (tPCS) or transcranial random noise stimulation (tRNS), to investigate neurochemical pathways in the brain. This feasibility study serves as a potential tool for expanding our understanding of the neurochemistry underlying energy metabolism and neuronal plasticity. Moreover, our experimental approach represents an encouraging nonpharmacological alternative to investigate altered neuronal plasticity and energy metabolism in neurological and metabolic disorders.

Limitations of the study

The current study is limited by the relatively short duration of the post-tDCS measurements, i.e., 67 min following tDCS. This narrow timeframe could be one possible reason for the absence of late significant recovery of energy phosphates. To address this shortcoming and to capture the late energy effects following tDCS, as shown in the study by Binkofski et al. (2011), future studies should aim to measure energy metabolites over a longer time. The follow-up period may not be sufficient to assess the long-term effects of tDCS. Longer-term studies are necessary to determine the durability of the observed changes and their potential implications for clinical applications. Moreover, as this feasibility study is only based on the motor cortex, it cannot be generalized to other brain regions. More studies are needed to explore other brain areas with similar quantitative and statistical investigations.

As studies using identical tDCS protocols have demonstrated different effects in healthy young adults (Alexandersen et al., 2022; Boayue et al., 2020; Willmot et al., 2024), the results obtained from this study are not one-to-one transferable to different age populations or patient groups. However, investigating inter-individual variability was not the aim of the study, and hence, subtle differences between individuals may have been concealed due to the small sample size. For future studies that aim to study inter-individual variability, a larger sample size would be useful for detecting subtle differences. One-to-one comparison with other studies is not possible due to differences in the method, material, acquisition protocols, etc. Further research is needed to investigate the relevance of calcium dynamics at deeper cortical layers and neighbouring brain regions to evaluate the effects of prolonged stimulation protocols. While we have proposed some reasonable explanations and possible molecular mechanisms underlying the aftereffects of prolonged tDCS duration, the precise neurobiological framework regarding neuroplasticity and energy metabolism remains unclear and requires further study.

Conclusion

We have demonstrated that it is possible to induce long lasting effects by anodal tDCS and measure the corresponding GABA and energy phosphate change in left primary motor cortex using proton and phosphorus magnetic resonance spectroscopy. Hence, this new approach may help to better understand the neurochemical mechanism underlying neurological and metabolic disorders.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethical committee of the Faculty of medicine, RWTH Aachen University. The studies were conducted in accordance with the local legislation and

institutional requirements. The participants provided written informed consent to participate in this study.

Author contributions

HP: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software. L-SS: Data curation, Writing – review & editing. C-HC: Data curation, Resources, Writing – review & editing. MN: Writing – review & editing. NS: Resources, Supervision, Writing – review & editing. FB: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

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Conflict of interest

Michael A. Nitsche is Scientific Advisory Boards of Neuroelectrics and Precisis.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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Glossary

ATP	Adenosine triphosphate
B_0	Static magnetic field
CSI	Chemical shift imaging
FWHM	Fullwidth half maximum
GABA	Gamma amino butyric acid
Glu	Glutamate
Glx	Glutamate-glutamine
GAD	Glutamic acid decarboxylase
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAA	N-acetylaspartate acid
PCr	Phosphocreatine
Pi	Inorganic phosphate
GPC	Glycerophosphocholine
GPE	Glycerophosphoethanolamine
TCA	Tricarboxylic acid
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
MEP	Motor evoked potential
VOI	Volume of interest
TR	Repetition time
TE	Echo time
TA	Acquisition time
PRESS	Point resolved spectroscopy,
MEGAPRESS	Meshcher-garwood point resolved spectroscopy
MDD	Major depressive disorder
DLPFC	Dorsolateral prefrontal cortex
SM1	Sensorimotor cortex
ACC	Anterior cingulate cortex
tACS	Transcranial alternating current stimulation
tPCS	Transcranial pulsed current stimulation
tRNS	Transcranial random noise stimulation

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Affidavit according to § 5 (1) for Data Retention

I hereby declare that the original data forming the basis of this doctoral thesis are stored in the **Division of Clinical Cognitive Science, Department of Neurology, RWTH Aachen University Hospital.**

Aachen, Date: **10.02.2025** **Harshal Jayeshkumar Patel**

Affidavit according to § 5 (1) and (2), and § 11 (3) 12 of the doctoral studies regulations[1]

I, **Harshal Jayeshkumar Patel,** hereby declare on oath, that I have contributed a significant part, and thus majority, of the publication:

PATEL HJ, Romanzetti S, Pellicano A, Nitsche MA, Reetz K, Binkofski F. Proton Magnetic Resonance Spectroscopy of the motor cortex reveals long term GABA change following anodal Transcranial Direct Current Stimulation. Scientific Reports. 2019 Feb 26;9(1):2807.

The contributions to the publication were as follows:

Names -→	Patel	Romanzetti	Pellicano	Nitsche	Reetz	Binkofski	Sum (%)
Study supervision	50					50	100
Study design/conception	60					40	100
Examination of the study participants	60	10	30				100
Collection of data	70	10	10		10		100
Data analysis	100						100
Performing experiments tDCS MR Spectroscopy	100						100
Statistical evaluation	60		30	10			100
Delivery of materials	60	10	10		10	10	100
Interpretation of data evaluation	60	5	10	10	5	10	100
Writing/draft of the manuscript	**100**						100
Correction of the manuscript		10	20	20		50	100

The position as the first author obviously arises from this significant contribution.

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Signature of the doctoral candidate

As supervisor and / or corresponding author I confirm the statements of Harshal Jayeshkumar Patel

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Signature of the doctoral supervisor

As co-author I endorse the statement of Prof. Dr. F. C. Binkofski

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Names and signatures of all German speaking co-authors (allowed on separate pages, each with table)

Affidavit according to § 5 (1) and (2), and § 11 (3) 12 of the doctoral studies regulations[1]

I, **Harshal Jayeshkumar Patel,** hereby declare on oath, that I have contributed a significant part, and thus majority, of the publication:

PATEL HJ, Stollberg LS, Choi CH, Nitsche MA, Shah NJ, Binkofski F. A study of long-term GABA and high-energy phosphate alterations in the primary motor cortex using anodal tDCS and $^{1}H/^{31}P$ MR spectroscopy. Frontiers in Human Neuroscience. 2024 Dec 13;18:1461417.

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Names -→	Patel	Stollberg	Choi	Nitsche	Shah	Binkofski	Sum (%)
Study supervision	50					50	100
Study design/conception	60		20			20	100
Examination of the study participants	70	15	15				100
Collection of data	70	10	10		10		100
Data analysis	100						100
Performing experiments tDCS MR Spectroscopy	100						100
Statistical evaluation	70		20	10			100
Delivery of materials	60		20	10	10		100
Interpretation of data evaluation	60		15	15		10	100
Writing/draft of the manuscript	**100**						100
Correction of the manuscript			30	20		50	100

The position as the first author obviously arises from this significant contribution.

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As supervisor and / or corresponding author I confirm the statements of Harshal Jayeshkumar Patel

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