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A magnetic resonance spectroscopy approach to quantitatively measure GABA and phosphorus level changes in the primary motor cortex elicited by transcranial direct current stimulation

Abstract: Several studies have presented molecular and neurophysiological evidence establishing a connection between synaptic plasticity, specific cognitive functions, energy metabolism, and metabolic syndrome in certain brain areas. As altered plasticity and energy regulation have been associated with neuropsychiatric disorders, studying the neurochemical mechanisms behind neuronal plasticity and energy metabolism simultaneously may support groundbreaking neuroscientific and therapeutic interventions. A favorable approach for investigating neuronal plasticity and energy metabolism is with the use of transcranial direct current stimulation (tDCS), a non-invasive brain stimulation technique that enables the modulation of neuronal excitability and energy in humans. The modulation in excitability and energy is likely mediated by the γ-aminobutyric acid (GABA), which is a potent inhibitor, and high-energy phosphates. Another well-established, non-invasive technique allowing the *in vivo* examination of the human brain and its functions is magnetic resonance spectroscopy (MRS). MRS is frequently used to quantify the concentration changes of various metabolites at the cellular level in the brain. Although proton-based measurements continue to be the standard, advancements in MRS methodologies and MR hardware have led to the ability to measure variations in neurotransmitters and high-energy phosphates using both proton and phosphorus MRS simultaneously. Owing to the complementary features of both tDCS and MRS, the simultaneous acquisition of data using both modalities offers a promising approach for gathering paired information concerning adaptive synthesis and energy consumption in both healthy and pathologically altered brains. This technique enables access to profound insights into the regulation of brain functions and to model the biochemical plasticity of the motor cortex.

Keywords: tDCS, MRI, MRS, brain, primary motor cortex, GABA, phosphorus

1 Introduction

The remarkable progress in brain stimulation and neuroimaging techniques made in recent decades has profoundly facilitated our understanding of the human brain. These developments enable both the exploration of neuroplasticity across various levels and the controlled replication of neuroplastic changes. Numerous neuroscientific studies have elucidated intricate connections between synaptic plasticity, cognition, and energy metabolism in specific brain regions [46]. Delving into this captivating aspect of the brain not only deepens our comprehension of its physiology and plasticity mechanisms but also holds substantial promise for innovative treatment strategies for neuropsychiatric disorders. An especially promising method for modulating neuronal excitability and energy in humans involves stimulating the brain using transcranial direct current stimulation (tDCS) and observing the subsequent effects on neurometabolites using magnetic resonance imaging (MRI) and/or spectroscopy (MRS).

Accurate modulation of brain energy and excitability can be attained through tDCS targeted at specific regions. *In vivo* studies with human subjects have demonstrated that anodal tDCS can elevate the spontaneous firing rates of cortical neurons, resulting in neuronal facilitation and increased energy consumption [28, 44]. The neuroplastic effects induced by tDCS in the brain are notably influenced by various parameters, such as current intensity, duration, and different montages. These formidable approaches have not merely shed light on various features characterizing brain functions, providing clinically pertinent insights, but have also shown potential as therapeutic modalities. As abnormalities in plasticity and energy regulation are associated with a range of neuropsychiatric diseases, examining the neurochemical mechanisms behind neuronal plasticity and energy metabolism simultaneously may yield breakthroughs for therapeutic interventions.

Studies have demonstrated alterations in γ-aminobutyric acid (GABA) and glutamate (Glu) using proton (^{1}H) MRS and changes in energy phosphates using phosphorus (^{31}P) MRS in the different areas of the brain following stimulation [11, 7]. The time courses of separately measured concentrations of GABA and Glu, adenosine triphosphate (ATP) and phosphocreatine (PCr) suggest that GABAergic activities within the selected region might be interconnected in modulating neuronal excitability and energy. The high-energy phosphates, such as ATP and PCr, serve as integral energy regulators in the human brain, playing a fundamental role in sustaining cerebral energy status by modulating the energy requirements for various cellular functions and physiological activities, including the sodium/potassium pump and the maintenance of brain energy homeostasis [12].

This chapter aims to introduce the brain stimulation technique, tDCS, which modulates levels of cortical activities. We will also outline methods for accessing and quantifying the effects of stimulation in the brain using MRS. Furthermore, this chapter provides examples of clinical and therapeutic applications of tDCS used simultaneously with MRS. Finally, we will conclude by discussing an outlook and future directions.

2 Transcranial direct current stimulation

A variety of low-intensity electrical stimulation techniques are available, among which tDCS stands out as the most preferred method for brain stimulation [28, 34]. This preference is attributed to its non-invasive nature and the precision with which it can selectively manipulate neuronal excitability and inhibitory functions. Additionally, tDCS is adept at modulating brain energy activities in the target brain region and throughout various stages of progression. To stimulate the brain using tDCS, a weak direct current (typically, 1 or 2 mA) is consistently applied to the scalp between two electrodes. This application enables the modulation of levels of neuronal excitability and energy, thereby enabling the exploration of the neurological and neuroplastic networks of the brain.

Numerous studies have been conducted to examine the impact of tDCS on changes in brain metabolites, and the efficacy of tDCS appears to be intricately determined by various parameters [51, 10], including a) the dimension, location, and polarity of electrodes; b) the intensity, interval, and duration of applied current; and c) bespoke tDCS montages that can manipulate neurometabolites to a status of excitation or inhibition [20, 13].

Figure 18.1 shows three different montages, the most popular montage utilized in studies being anodal tDCS. In this context, anodal tDCS directs the current flow from the anodal electrode, positioned on the area of interest, such as the primary motor cortex (M1), toward the reference electrode (in this case, the cathodal electrode), which is usually situated on the contralateral supraorbital ridge or an extracranial location. Activation of the anodal electrode induces membrane depolarization, causing a shift in the resting membrane potential toward positive values [33]. Conversely, cathodal tDCS entails the opposite flow of current compared to anodal tDCS, with the current moving from the reference electrode to the cathodal electrode. Stimulation of the cathodal electrode results in membrane hyperpolarization [37]. Bihemispheric stimulation, on the other hand, refers to the current flowing from the anodal to the cathodal electrode when one electrode is placed on a specific region of interest in the brain and the other is positioned on the same region but on the contralateral side.

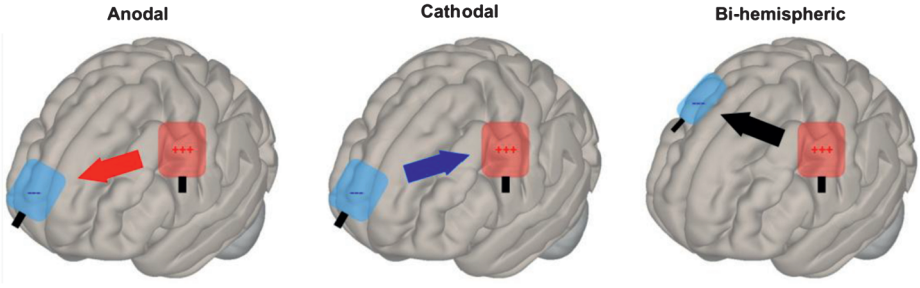

Figure 18.1: Various M1 targeting tDCS montages—anodal, cathodal, and bihemispheric tDCS montages showing the locations of electrodes and the directions of their current flow.

During the acute phase, the response of the brain to tDCS can be elucidated by considering the pre-excited synaptic activity levels and neuronal membrane properties [6]. It is important to emphasize that tDCS itself does not initiate an action potential; instead, it modulates a readiness for it [31]. In simpler terms, this modulation does not engage synaptic mechanisms but hinges on voltage-gated ion channels influenced by the electric field generated between the anodal and cathodal electrodes. According to computational simulations, this electrical field peaks somewhere between these electrodes. Regardless of the polarity of tDCS employed, specific portions of the neuronal membranes undergo depolarization, while others experience hyperpolarization. This is determined by cell morphology and their orientation toward the stimulating electrode [36].

Furthermore, tDCS not only induces acute or primary effects on the brain during the stimulation but also generates a secondary or after-effect that persists even after the stimulation terminates [5, 30]. These after effects on the synaptic efficacy can endure for up to an hour, and with carefully designed modulation time, intensity, repetition, and in combination with a specific task, in some cases, they may remain for several days [29]. This phenomenon resembles the exertion of long-term potentiation (LTP) and long-term depression (LTD)-like activities [45]. Similarly, to LTP and LTD, intracellular calcium levels, brain-derived neurotrophic factors and, most importantly, major excitatory and inhibitory neurotransmitters—Glu and GABA have been reported to be involved in the secondary effects of tDCS [20]. For instance, some studies have indicated the involvement of GABA in the after-effects of anodal tDCS, whereas both GABA and Glu concentrations have been shown to be modulated following cathodal stimulation [33].

Consequently, it is of great importance to comprehend the nature of the interference, the response mechanisms involved, and the controlled variables. Unraveling the temporal trajectory of modulation in the concentration of these neurotransmitters and understanding their dependence on the tDCS parameters are of utmost interest as this information can be harnessed for therapeutic purposes. The utilization of MRI and MRS (as discussed in Section 3) is highly suitable for the non-invasive assessment of the impact of tDCS on neurotransmitter levels, and as a result, the combined use of tDCS and MRS (in Section 4) has been steadily gaining prominence.

3 Magnetic resonance spectroscopy

MRI and MRS are well-established, non-invasive imaging and spectroscopic tools extensively employed in both clinical practice and neuroscientific research. MRI enables the *in vivo* examination of the human brain and its functions, delivering exceptional soft tissue contrast along with insights into metabolic and functional details. MRS is particularly valuable for assessing diverse metabolites in the brain *in vivo*, providing the quantification of metabolite concentrations with high levels of sensitivity. The metabolites in the ^{1}H spectrum include GABA, glutamine (Gln), Glu, N-acetyl aspartate (NAA),

choline-containing compounds (Cho), creatine (Cr), myo-inositol (mI), among others [16]. GABA and Glu function as the primary inhibitory and excitatory neurotransmitters in the brain, respectively, and play vital roles in the neurotransmitter cycle. They are also known to be closely involved with physiological processes and neurological disorders [27]. NAA serves as a useful indicator for the integrity of neurons and axons, reflecting membrane turnover and neuronal connections, and the analysis of NAA provides insights into functional neuronal loss in the brain [40]. Additionally, NAA plays a crucial osmoregulatory role by aiding in the removal of intracellular water from myelinated neurons against a water gradient [38]. Cho and Cr primarily contribute to cell membrane integrity and oxidative metabolism, respectively [40]. Cr is frequently utilized as a reference for normalizing the resonance intensities of other metabolites in ^{1}H MRS. Analyzing alterations in metabolite concentrations and their ratios offer complementary information to anatomical MR imaging, providing a comprehensive understanding of brain function and pathology.

The emergence of advanced MRI and MRS methods and improved MR systems is poised to enhance the comprehensive characterization of both known and undiscovered morphological structures and functions. Furthermore, it is anticipated that it will be possible to use metabolically and MR-sensitive nuclei, e. g., sodium-23, carbon-13, and phosphorus-31, to enhance our understanding of the human brain. As a counterpart to the proton, ^{31}P is an important nucleus in the brain and plays a crucial role in tissue energy metabolism and membrane synthesis [12, 4]. In a ^{31}P spectrum obtained using ^{31}P MRS, various spectral peaks of key metabolites are discernible, each exhibiting a relatively broad chemical shift range of approximately 30 ppm, facilitating clear differentiation between them. These peaks correspond to ATP, PCr, phosphodiester (PDE), inorganic phosphate (Pi), and phosphomonoester (PME). ATP, a vital high-energy phosphate substrate, is primarily synthesized by mitochondria and serves as the principal, readily available energy source for diverse cellular processes. It is distinguishable in the ^{31}P MR spectrum by three distinct, but multiple-let, α-, β-, γ-ATP peaks. PCr is a phosphorylated energy-rich molecule prevalent in the brain, functioning as a buffer to balance ATP levels in response to energy demand through the creatine kinase. Pi is also essential for the human brain, contributing to energy metabolism as a precursor for ATP synthesis. It plays an important role in cellular activities and provides information about intracellular and extracellular pH values, which are calculated based on its chemical shift relative to PCr [19, 47]. Moreover, ^{31}P MRS makes it possible to acquire information associated with free magnesium ions (Mg^{2+}) and nicotinamide adenine dinucleotide (NAD^{+}) [24]. Mg^{2+} is crucial for maintaining overall organismal health and function, and its concentration can be calculated using the peak separation between PCr and β-ATP. NAD^{+} appears in the α-ATP peak and is a crucial coenzyme, playing a pivotal role in energy production, particularly in cellular respiration, by participating in redox reactions. Thus, ^{31}P MRS offers unique insights into the dynamic aspects of energy metabolism.

Similar to tDCS localization, MRS necessitates precise voxel selection because metabolite concentrations vary across different brain regions. The use of 3-dimensional

(3D) pulse-acquire or chemical shift imaging (CSI) sequences is particularly common for collecting ^{31}P signals within the selected volume, incorporating the phase encoding gradient in each dimension [39]. However, obtaining high-quality spectra poses challenges due to the substantially low signal intensity of certain metabolites and due to interference by J-coupling. This makes it difficult to distinguish some metabolite peaks, such as Gln and Glu, in a ^{1}H spectrum at 3 tesla (3 T), as their chemical shifts are in a similar frequency range. In order to address these challenges, specially customized sequences, such as MEGA-PRESS and Semi-LASER [26, 41], modified from the conventional single-voxel spectroscopy sequences, have been developed to focus on specific metabolites. The choice of the MR acquisition method depends on the metabolites under investigation. Utilizing highly sensitive multichannel radiofrequency (RF) coils and multituned coils [9] can also improve access to X-nuclei, i. e., ^{31}P, by leveraging the nuclear Overhauser effect for ^{31}P, enhancing signal-to-noise ratio (SNR) via irradiation of ^{1}H, and significantly improving the spectra quality [32]. Furthermore, the application of well-established metabolite fitting algorithms and software packages, such as LCModel [35] and jMRUI [49], is crucial for accurate data analysis and the precise quantification of metabolite concentrations.

4 Transcranial direct current stimulation magnetic resonance spectroscopy

Simultaneous MR spectroscopic data acquisition during the application of tDCS could facilitate profound insights into the regulation of brain functions and provide the benefit of paired information. Typically, tDCS MRS experiments are designed with both an active condition, e. g., anodal or cathodal, and a control condition, i. e., sham, where tDCS is programmed to deliver a controlled, weak direct current to the brain via two electrodes (e. g., red and blue electrodes in Figure 18.2). The stimulation using the MR-compatible tDCS sets is often conducted inside the MRI scanner, during which a series of MR data is acquired, depending on the predetermined study protocol. The MR acquisition begins with a calibration and anatomical scan. Subsequently, a user selects a specific voxel (e. g., the yellow boxes in Figure 18.2) or a group of multiple voxels which be positioned onto the pre-acquired, 3D structural MR image (i. e., background head images in Figure 18.2). Careful adjustment of the volume of the voxel-of-interest (VOI) is necessary and should be sufficiently small so as to avoid any signal interference from neighboring voxels, yet large enough to increase its detectable signal level for reliable quantification. This selected MRS voxel is usually aligned with the region where the anodal tDCS electrode is located. After an advanced static magnetic field shimming process to improve the MR spectrum quality [14], a set of MRS data is recorded before and after the stimulation (i. e., pre- and post-stimulation). This data set is then processed by the means of the above-mentioned fitting software. Examples of spectra fitting are shown in Figure 18.3.

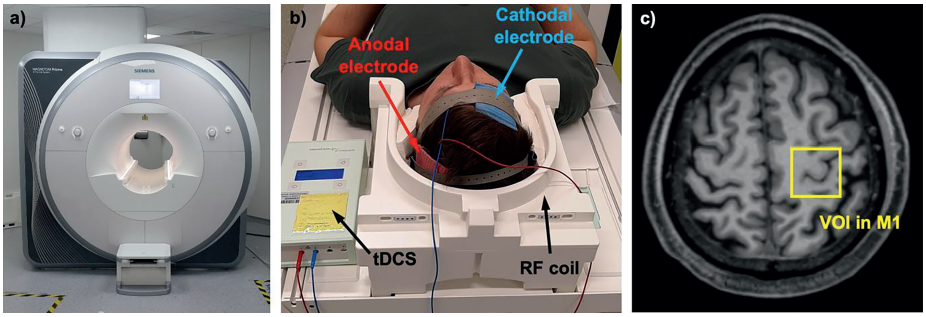

Figure 18.2: Display a) A photograph of 3 T clinical MRI scanner without the patient bed. Display b) An image of the overall tDCS MR experiment setup, displaying the commercially available MR-compatible tDCS device, two electrodes placed on the volunteer's head (with the anodal electrode in red centered over the left M1 and the cathodal electrode in blue centered over the contralateral supraorbital ridge) and the top part removed MRI coil. Display c) A representative T_1-weighted, axial slice, brain MR image of a healthy subject, depicting the MRS VOI (i. e., the yellow box) in the M1 region.

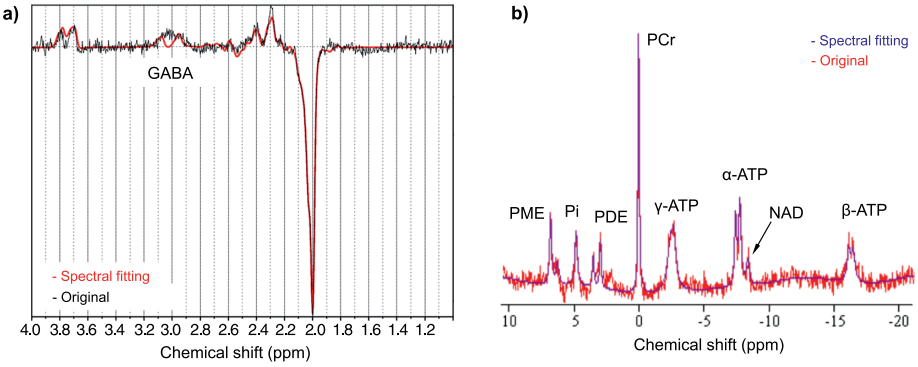

Figure 18.3: Display a) A GABA peak obtained using a GABA-edited ^{1}H MRS sequence (MEGA-PRESS). Display b) ^{31}P metabolite peaks acquired via a ^{31}P MRS sequence (3D CSI). The original ^{1}H and ^{31}P spectra were spectrally fitted by means of LCModel and jMRUI software, respectively.

In the realm of tDCS effects, the predominant research focus centers on anodal stimulation within M1, with a primary emphasis on investigating alterations in GABA and Glu levels accessible using ^{1}H MRS, as well as parameters related to energy metabolism, such as ATP, PCr, and Pi, available through ^{31}P MRS. Findings from these studies consistently indicate a tendency toward reduced GABA concentration and a bipolar response involving both a decrease and an increase in the phosphorate metabolites following the stimulation.

The prevailing assumption is that the diminished GABA concentration aligns with an augmented firing rate of neurons and an increased predisposition for changes in plasticity. There is strong evidence suggesting that the LTP-like effects induced by stimulation are associated with a decrease in inhibitory activity [44, 30, 29, 45, 1, 2, 43, 18].

Similarly, the LTD-like effect linked with cathodal modulation may arise from reduced excitatory neurotransmitter levels. Instances of decreased Glu following cathodal stimulation coincide with a simultaneous reduction in GABA [45]. The impact of stimulation on GABA levels is evident both at the immediate time point and also extends to a later time point, approximately 65 minutes afterwards, as shown in Figure 18.4a. This observation suggests a potential intricate connection to long-term cortical plasticity mechanisms [30].

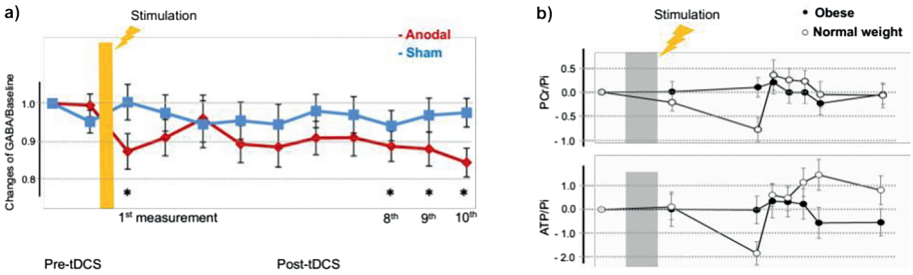

Figure 18.4: Display a) A trend of biphasic decrease in GABA concentration in healthy volunteers following anodal tDCS relative to a sham condition in the left M1, which was shown at the time points of the first, eighth, ninth, and tenth post-stimulation measurements. Display b) An overall reduction in cerebral energy content, marked by a subdued response in ATP and PCr levels in right hemisphere of the brain following stimulation with ^{31}P MRS in obese individuals compared to normal-weight counterparts. [Figure 18.4a) is reused based on [30] and b) is redrawn from [17] with the copyright permission granted through RightsLink.]

While studies utilizing ^{31}P MRS are less abundant than those employing ^{1}H MRS, efforts have been made to explore the impact of anodal tDCS on high-energy phosphates in M1. Analogous to the observed biphasic pattern in GABA response, the levels of ATP and PCr have been shown to exhibit a similar trend, i. e., an immediate decrease post-tDCS, followed by an increase after a certain duration [7, 50]. In investigations focused on obesity, anodal tDCS demonstrated a muted effect on high-energy phosphate concentration. As shown in Figure 18.4b, there was only a delayed drop in PCr/Pi and ATP/Pi values at around 85 minutes after anodal tDCS, compared to the biphasic behavior observed in normal-weight individuals, highlighting potential variations in the effects based on obesity conditions [17].

The applications of tDCS MRS encompass the investigation of the biochemical and physiological brain activity in humans. Its non-invasive nature offers tremendous potential, as it carries almost no side effects, unlike the conventional pharmacological interventions. Additionally, the use of the technique for therapeutic purposes serves as an alternative treatment strategy for Parkinson's disease or psychiatric conditions, such as depression or schizophrenia [3, 8]. tDCS also finds application in the ethically challenging domain of human biological enhancement, where it has been reported to modulate motor learning, memory, and even creativity [18, 25].

5 Outlook and future directions

The convergence of advanced methodologies and cutting-edge technologies holds the promise of unlocking new horizons, fostering a deeper understanding of the physiology and metabolic mechanisms of the complex brain.

Ultra-high field (UHF) strengths, such as 7 T or higher, bring about several advantages [22] for MR measurements, including enhanced accuracy, efficiency, and specificity, notwithstanding the necessity of a UHF MR-compatible tDCS device for simultaneous examinations. The increased SNR afforded by measurements at UHF is particularly beneficial for ^{31}P MRS due to its inherently low MR sensitivity, facilitating more detailed investigations by enabling the selection of a smaller voxel size. In turn, this allows for a more precise focus on the volume of interest in the brain with reduced contamination from unwanted areas. The heightened SNR and improved spectra quality not only enhance the accuracy of MRS data fitting but also reduce overall acquisition time. This helps multiple baseline and post-stimulation data acquisitions, leading to enhanced temporal resolution as the temporal dynamics of GABA and phosphorus concentration following tDCS exhibit a coupled relationship. The decrease in GABA concentration induces heightened neuronal activity and plasticity, leading to an escalation in energy consumption and a concurrent decline in high-energy phosphates. Furthermore, the increased chemical shift at UHF enables the isolation of metabolites from one another. As previously stated with the example of Glu and Gln, this separation benefits specificity in understanding synaptic plasticity by allowing the distinct examination of the major excitatory neurotransmitter. Ultimately, the increased field strength, coupled with an advanced, low-loss double-tuned RF coil [9], will enable the execution of, e. g., simultaneous tDCS ^{1}H and ^{31}P MRS experiments with improved data quality.

High-definition (HD) tDCS represents a relatively recent approach characterized by a central electrode surrounded by several oppositely polarized electrodes in a ring configuration. In comparison to traditional simple montages, this arrangement has proven to be more effective [21]. Recent studies utilizing HD-tDCS have highlighted its improved specificity and precision in targeting modulation within the designated region, leading to improved responses [23, 15]. While the conventional single-probe tDCS method effectively targets specific brain areas, it may still lack the capacity to provide a comprehensive understanding of the intricate and interconnected functions of the human brain. The promising development in simultaneous multivoxel stimulation and MR signal detection encourages further exploration and research into the intricate dynamics of brain function and the implications of stimulation.

The widespread adoption of artificial intelligence (AI) is infiltrating numerous domains, and its integration into brain research has immense potential. The transformative synergy between AI and MRS provides both efficiency gains and enables the comprehensive exploration of intricate relationships [48, 42]. This approach affects entire workflows, enhancing the accuracy and precision of metabolite concentration estimates and providing a more personalized MR and tDCS parameter optimization to support the

diagnosis and treatment of diseases. Incorporating AI into the quantification process enables the exploration of complex relationships within spectral data that may be challenging to discern through traditional methods. This addresses the inherent variability in metabolite profiles, and the automated analysis pipeline further streamlines the complex task of interpreting MRS results, making the process more efficient and allowing researchers to focus on the nuanced aspects of brain metabolism and function. This multidimensional integration of AI and MRS not only advances methodological precision but also holds the promise of unlocking new dimensions of knowledge relating to the intricacies of brain function and metabolism.

6 Conclusions

The integration of tDCS and MRS offers a robust, noninvasive methodology for investigating the intricate relationship between neuronal plasticity and energy metabolism. This combined approach holds significant promise for advancing our understanding of brain function and for the development of novel therapeutic interventions for neuropsychiatric disorders. By concurrently modulating and measuring neuronal excitability, energy consumption, and metabolite concentrations, this technique enables us to gain profound insights into the biochemical and physiological processes underlying both healthy and pathological brain states. Consequently, this could lead to the development of more effective treatments and better mental health outcomes.

Bibliography

[1] D. Antonenko, F. Schubert, F. Bohm, B. Ittermann, S. Aydin, D. Hayek, U. Grittner, and A. Flöel. tDCS-induced modulation of GABA levels and resting-state functional connectivity in older adults. *The Journal of Neuroscience*, 4065–4073, 2017.

[2] V. Bachtiar, A. Johnstone, A. Berrington, C. Lemke, H. Johansen-Berg, U. Emir, and C. J. Stagg. Modulating regional motor cortical excitability with noninvasive brain stimulation results in neurochemical changes in bilateral motor cortices. *The Journal of Neuroscience*, 38(33):7327–7336, 2018.

[3] D. H. Benninger and M. Hallett. Non-invasive brain stimulation for Parkinson's disease: Current concepts and outlook 2015. *NeuroRehabilitation*, 37(1):11–24, 2015.

[4] Z. M. Bhujwalla, C. L. McCoy, J. D. Glickson, R. J. Gillies, and M. Stubbs. Estimations of intra- and extracellular volume and pH by 31P magnetic resonance spectroscopy: effect of therapy on RIF-1 tumours. *British Journal of Cancer*, 78(5):606–611, 1998.

[5] M. Bikson, Z. Esmaeilpour, D. Adair, G. Kronberg, W. J. Tyler, A. Antal, A. Datta, B. A. Sabel, M. A. Nitsche, C. Loo, D. Edwards, H. Ekhtiari, H. Knotkova, A. J. Woods, B. M. Hampstead, B. W. Badran, and A. V. Peterchev. Transcranial electrical stimulation nomenclature. *Brain Stimulation*, 12(6):1349–1366, 2019.

[6] M. Bikson, M. Inoue, H. Akiyama, J. K. Deans, J. E. Fox, H. Miyakawa, and J. G. R. Jefferys. Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *The Journal of Physiology*, 557(1):175–190, 2004.

[7] F. Binkofski, M. Loebig, K. Jauch-Chara, S. Bergmann, U. H. Melchert, H. G. Scholand-Engler, U. Schweiger, L. Pellerin, and K. M. Oltmanns. Brain energy consumption induced by electrical stimulation promotes systemic glucose uptake. *Biological Psychiatry*, 70(7):690–695, 2011.

[8] J. Brunelin, M. Mondino, L. Gassab, F. Haesebaert, L. Gaha, M.-F. Suaud-Chagny, M. Saoud, A. Mechri, and E. Poulet. Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. *The American Journal of Psychiatry*, 169(7):719–724, 2012.

[9] C.-H. Choi, S.-M. Hong, J. Felder, and N. J. Shah. The state-of-the-art and emerging design approaches of double-tuned RF coils for X-nuclei, brain MR imaging and spectroscopy: A review. *Magnetic Resonance Imaging*, 72:103–116, 2020.

[10] C.-H. Choi, E. Iordanishvili, N. J. Shah, and F. Binkofski. Magnetic resonance spectroscopy with transcranial direct current stimulation to explore the underlying biochemical and physiological mechanism of the human brain: A systematic review. *Human Brain Mapping*, 42(8):2642–2671, 2021.

[11] V. P. Clark, B. A. Coffman, M. C. Trumbo, and C. Gasparovic. Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: A 1H magnetic resonance spectroscopy study. *Neuroscience Letters*, 500(1):67–71, 2011.

[12] F. Du, X.-H. Zhu, Y. Zhang, M. Friedman, N. Zhang, K. Uğurbil, and W. Chen. Tightly coupled brain activity and cerebral ATP metabolic rate. *Proceedings of the National Academy of Sciences*, 105(17):6409–6414, 2008.

[13] B. R. Foerster, T. D. Nascimento, M. De Boer, M. A. Bender, I. C. Rice, D. Q. Truong, M. Bikson, D. J. Clauw, J.-K. Zubieta, R. E. Harris, and A. F. Da Silva. Brief report: excitatory and inhibitory brain metabolites as targets of motor cortex transcranial direct current stimulation therapy and predictors of its efficacy in fibromyalgia. *Arthritis and Rheumatology*, 67(2):576–581, 2015.

[14] R. Gruetter and I. Tkáč. Field mapping without reference scan using asymmetric echo-planar techniques. *Magnetic Resonance in Medicine*, 43(2):319–323, 2000.

[15] Z. Guo, Y. Gong, H. Lu, R. Qiu, X. Wang, X. Zhu, and X. You. Multitarget high-definition transcranial direct current stimulation improves response inhibition more than single-target high-definition transcranial direct current stimulation in healthy participants. *Frontiers in Neuroscience*, 16:905247, 2022.

[16] A. Henning. Proton and multinuclear magnetic resonance spectroscopy in the human brain at ultra-high field strength: A review. *NeuroImage*, 168:181–198, 2018.

[17] K. Jauch-Chara, F. Binkofski, M. Loebig, K. Reetz, G. Jahn, U. H. Melchert, U. Schweiger, and K. M. Oltmanns. Blunted brain energy consumption relates to insula atrophy and impaired glucose tolerance in obesity. *Diabetes*, 64(6):2082–2091, 2015.

[18] S. Kim, M. C. Stephenson, P. G. Morris, and S. R. Jackson. tDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: A 7T magnetic resonance spectroscopy study. *NeuroImage*, 99:237–243, 2014.

[19] A. Korzowski, N. Weinfurtner, S. Mueller, J. Breitling, S. Goerke, H.-P. Schlemmer, M. E. Ladd, D. Paech, and P. Bachert. Volumetric mapping of intra- and extracellular pH in the human brain using 31P MRSI at 7T. *Magnetic Resonance in Medicine*, 84(4):1707–1723, 2020.

[20] B. Krause, J. Márquez-Ruiz, and R. Cohen Kadosh. The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance? *Frontiers in Human Neuroscience*, 7(24):602, 2013.

[21] H.-I. Kuo, M. Bikson, A. Datta, P. Minhas, W. Paulus, M.-F. Kuo, and M. A. Nitsche. Comparing cortical plasticity induced by conventional and high-definition 4×1 ring tDCS: A neurophysiological study. *Brain Stimulation*, 6(4):644–648, 2013.

[22] M. E. Ladd, P. Bachert, M. Meyerspeer, E. Moser, A. M. Nagel, D. G. Norris, S. Schmitter, O. Speck, S. Straub, and M. Zaiss. Pros and cons of ultra-high-field MRI/MRS for human application. *Progress in Nuclear Magnetic Resonance Spectroscopy*, 109:1–50, 2018.

[23] K. Lengu, S. Ryan, S. J. Peltier, T. Tyszkowski, A. Kairys, B. Giordani, and B. M. Hampstead. Effects of high definition-transcranial direct current stimulation on local GABA and glutamate levels among older adults with and without mild cognitive impairment: an exploratory study. *Journal of Alzheimer's Disease*, 84(3):1091–1102, 2021.

[24] M. Lu, X.-H. Zhu, and W. Chen. In vivo 31P MRS assessment of intracellular NAD metabolites and NAD+/NADH redox state in human brain at 4 T. *NMR in Biomedicine*, 29(7):1010–1017, 2016.

[25] L. E. Mancuso, I. P. Ilieva, R. H. Hamilton, and M. J. Farah. Does transcranial direct current stimulation improve healthy working memory?: A meta-analytic review. *Journal of Cognitive Neuroscience*, 28(8):1063–1089, 2016.

[26] M. Mescher, H. Merkle, J. Kirsch, M. Garwood, and R. Gruetter. Simultaneous in vivo spectral editing and water suppression. *NMR in Biomedicine*, 11(6):266–272, 1998.

[27] M. O. Nava-Mesa, L. Jiménez-Díaz, J. Yajeya, and J. D. Navarro-Lopez. GABAergic neurotransmission and new strategies of neuromodulation to compensate synaptic dysfunction in early stages of Alzheimer's disease. *Frontiers in Cellular Neuroscience*, 8:167, 2014.

[28] M. A. Nitsche and W. Paulus. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology*, 527(3):633–639, 2000.

[29] J. O'Shea, P. Revol, H. Cousijn, J. Near, P. Petitet, S. Jacquin-Courtois, H. Johansen-Berg, G. Rode, and Y. Rossetti. Induced sensorimotor cortex plasticity remediates chronic treatment-resistant visual neglect. *eLife*, 6:e26602, 2017.

[30] H. J. Patel, S. Romanzetti, A. Pellicano, M. A. Nitsche, K. Reetz, and F. Binkofski. Proton magnetic resonance spectroscopy of the motor cortex reveals long term GABA change following anodal transcranial direct current stimulation. *Scientific Reports*, 9(1):2807, 2019.

[31] W. Paulus. Transcranial electrical stimulation (tES – tDCS; tRNS, tACS) methods. *Neuropsychological Rehabilitation*, 21(5):602–617, 2011.

[32] T. H. Peeters, M. J. van Uden, A. Rijpma, T. W. J. Scheenen, and A. Heerschap. 3D 31P MR spectroscopic imaging of the human brain at 3 T with a 31P receive array: An assessment of 1H decoupling, T1 relaxation times, 1H-31P nuclear Overhauser effects and NAD+. *NMR in Biomedicine*, 34(5):e4169, 2021.

[33] R. Polanía, M. A. Nitsche, and C. C. Ruff. Studying and modifying brain function with non-invasive brain stimulation. *Nature Neuroscience*, 21(2):174–187, 2018.

[34] A. Priori, A. Berardelli, S. Rona, N. Accornero, and M. Manfredi. Polarization of the human motor cortex through the scalp. *NeuroReport*, 9(10):2257, 1998.

[35] S. W. Provencher. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, 30(6):672–679, 1993.

[36] T. Radman, R. L. Ramos, J. C. Brumberg, and M. Bikson. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. *Brain Stimulation*, 2(4):215–228, 2009.

[37] A. Rahman, D. Reato, M. Arlotti, F. Gasca, A. Datta, L. C. Parra, and M. Bikson. Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *The Journal of Physiology*, 591(10):2563–2578, 2013.

[38] J. Ren, A. D. Sherry, and C. R. Malloy. 31P-MRS of healthy human brain: ATP synthesis, metabolite concentrations, pH, and T1 relaxation times. *NMR in Biomedicine*, 28(11):1455–1462, 2015.

[39] A. Rietzler, R. Steiger, S. Mangesius, L.-M. Walchhofer, R. M. Gothe, M. Schocke, E. R. Gizewski, and A. E. Grams. Energy metabolism measured by 31P magnetic resonance spectroscopy in the healthy human brain. *Journal of Neuroradiology*, 49(5):370–379, 2022.

[40] D. B. Salem, P. M. Walker, Y. Bejot, S. L. Aho, B. Tavernier, O. Rouaud, F. Ricolfi, and F. Brunotte. N-acetylaspartate/creatine and choline/creatine ratios in the thalami, insular cortex and white matter as markers of hypertension and cognitive impairment in the elderly. *Hypertension Research*, 31(10):1851–1857, 2008.

[41] T. W. J. Scheenen, D. W. J. Klomp, J. P. Wijnen, and A. Heerschap. Short echo time 1H-MRSI of the human brain at 3T with minimal chemical shift displacement errors using adiabatic refocusing pulses. *Magnetic Resonance in Medicine*, 59(1):1–6, 2008.

[42] J. Songeon, S. Courvoisier, L. Xin, T. Agius, O. Dabrowski, A. Longchamp, F. Lazeyras, and A. Klauser. In vivo magnetic resonance 31P-Spectral Analysis With Neural Networks: 31P-SPAWNN. *Magnetic Resonance in Medicine*, 89(1):40–53, 2023.

[43] C. J. Stagg, S. Bestmann, A. O. Constantinescu, L. Moreno Moreno, C. Allman, R. Mekle, M. Woolrich, J. Near, H. Johansen-Berg, and J. C. Rothwell. Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. *The Journal of Physiology*, 589(23):5845–5855, 2011.

[44] C. J. Stagg, J. G. Best, M. C. Stephenson, J. O'Shea, M. Wylezinska, Z. T. Kincses, P. G. Morris, P. M. Matthews, and H. Johansen-Berg. Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *The Journal of Neuroscience*, 29(16):5202–5206, 2009.

[45] C. J. Stagg and M. A. Nitsche. Physiological basis of transcranial direct current stimulation. *The Neuroscientist*, 17(1):37–53, 2011.

[46] M. Stampanoni Bassi, E. Iezzi, L. Gilio, D. Centonze, and F. Buttari. Synaptic plasticity shapes brain connectivity: implications for network topology. *International Journal of Molecular Sciences*, 20(24):6193, 2019.

[47] J. S. Taylor, D. B. Vigneron, J. Murphy-Boesch, S. J. Nelson, H. B. Kessler, L. Coia, W. Curran, and T. R. Brown. Free magnesium levels in normal human brain and brain tumors: 31P chemical-shift imaging measurements at 1.5 T. *Proceedings of the National Academy of Sciences*, 88(15):6810–6814, 1991.

[48] D. M. J. van de Sande, J. P. Merkofer, S. Amirrajab, M. Veta, R. J. G. van Sloun, M. J. Versluis, J. F. A. Jansen, J. S. van den Brink, and M. Breeuwer. A review of machine learning applications for the proton MR spectroscopy workflow. *Magnetic Resonance in Medicine*, 90(4):1253–1270, 2023.

[49] L. Vanhamme, A. van den Boogaart, and S. Van Huffel. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *Journal of Magnetic Resonance*, 129(1):35–43, 1997.

[50] E. K. Wardzinski, L. Friedrichsen, S. Dannenberger, A. Kistenmacher, U. H. Melchert, K. Jauch-Chara, and K. M. Oltmanns. Double transcranial direct current stimulation of the brain increases cerebral energy levels and systemic glucose tolerance in men. *Journal of Neuroendocrinology*, 31(4):e12688, 2019.

[51] A. J. Woods, A. Antal, M. Bikson, P. S. Boggio, A. R. Brunoni, P. Celnik, L. G. Cohen, F. Fregni, C. S. Herrmann, E. S. Kappenman, H. Knotkova, D. Liebetanz, C. Miniussi, P. C. Miranda, W. Paulus, A. Priori, D. Reato, C. Stagg, N. Wenderoth, and M. A. Nitsche. A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clinical Neurophysiology*, 127(2):1031–1048, 2016.

Part II-A.4: Body’s operational functions