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THE EFFECT OF ACCELERATED INTERMITTENT THETA BURST STIMULATION ON LEFT DORSOLATERAL PREFRONTAL NEUROMETABOLITES IN MAJOR DEPRESSION AS MEASURED BY PROTON MAGNETIC RESONANCE SPECTROSCOPY

Master's Thesis

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Kiirendatud vahelduva teetapuhang-stimulatsiooni mõju vasaku dorsolateraalse prefrontaalkoore neurometaboliitidele depressioonipatsientidel mõõdetuna prooton-magnetresonantsspektroskoopia abil

Kokkuvõte

Eesmärk: Käesolev magistritöö uurib kiirendatud vahelduva teetapuhang-stimulatsiooni (aiTBS) mõju neurometaboliitidele raviresistentse depressiooniga (RRD) inimestel. Peamine fookus on aiTBSi potentsiaalsel mõjul glutamaatergilisele süsteemile. Lisaks uuritakse aiTBSi turvalisust.

Meetodid: Uuringus osalesid RRD patsiendid ning kontrollrühmana olid kaasatud tervise-kaebusteta isikud. Pooled patsientidest said tegelikku stimulatsioonravi, pooltele rakendati platseebostimulatsiooni. Vasaku dorsolateraalse prefrontaalse ajukoore (DLPFC) neurometaboliitide kontsentratsiooni mõõdeti magnetresonantsspektroskoopia abil.

Tulemused: RRD patsientide aiTBS-i ravi eelse baastaseme võrdlusest kontrollgrupiga ei ilmnenud olulisi erinevusi Glx-i (glutamiin + glutamaat) kontsentratsioonis. aiTBS-raviga ei kaasnenud muutusi Glx-i kontsentratsioonis. Märkimisväärseks tulemuseks oli seos kliinilise paranemise ja Glx-i kontsentratsiooni suurenemise vahel. Kuna tegelikku aiTBS ravi ja platseeboravi saanud isikute ajukoe tervise näitajates täheldati väikseid erinevusi, on aiTBS-i ravi ohutuse tagamiseks vajalikud täiendavad uuringud.

Kokkuvõte: Käesolev magistritöö on esimene uuring, mis vaatleb aiTBS-i mõju glutamaatergilisele süsteemile RRD patsientidel, heites seeläbi valgust ka TMS-i toimemehhanismidele depressiooniravis. Tõsikindlamate tulemuste raporteerimiseks on vaja täiendavaid uuringuid suuremate valimite ja kvaliteetsete andmekogumite abil.

Märksõnad: depressioon, neurometaboliit, glutamaat, ajustimulatsioon, transkraniaalne, prefrontaalne, spektroskoopia,

The Effect of Accelerated Intermittent Theta Burst Stimulation on Left Dorsolateral Prefrontal Neurometabolites in Major Depression as Measured by Proton Magnetic Resonance Spectroscopy

Abstract

Objective: This master's thesis explores the impact of accelerated intermittent theta burst stimulation (aiTBS), a form of repetitive transcranial stimulation (rTMS), on brain metabolites in individuals with treatment-resistant depression (TRD). The main focus is on iTBS's effects on the glutamatergic system, while also examining its potential impact on neuronal integrity.

Methods: Proton magnetic resonance spectroscopy was used to measure neurometabolite concentrations in the left dorsolateral prefrontal cortex (DLPFC) of TRD patients. A control group of healthy individuals was included for comparison. The study was designed as a sham-controlled trial.

Results: Baseline analysis showed no significant differences in Glx (glutamate + glutamine) concentration between TRD patients and healthy controls in the left DLPFC. aiTBS treatment did not lead to substantial changes in Glx concentration. A notable finding was the association between clinical improvement and increased Glx concentration. Minimal differential changes were observed in neuronal integrity markers between real aiTBS and sham groups, highlighting the need for further investigation to ensure treatment safety.

Conclusions: This study sheds light on the effects of aiTBS on the glutamatergic system and the mechanisms underlying TMS's benefits in depression treatment. Further research with larger sample sizes and high-quality datasets is needed to establish definitive conclusions.

Keywords: depression, neurometabolite, glutamate, brain stimulation, transcranial, prefrontal, magnetic resonance spectroscopy

Introduction

This master's thesis explores the impact of accelerated intermittent theta burst stimulation (aiTBS), a form of repetitive transcranial stimulation (rTMS), on brain metabolites in individuals with treatment-resistant depression (TRD). The primary objective of the study is to investigate the effects of aiTBS on the glutamatergic system, while also examining its potential impact on neuronal integrity. In vivo measurements of neurometabolite concentrations are obtained using proton magnetic resonance spectroscopy, specifically focusing on the left dorsolateral prefrontal cortex (DLPFC) region for metabolite assessment.

The rest of the introduction gives a brief overview of the main concepts and methods that are relevant for the study presented in this master's thesis.

Major Depression

Major depression is a seriously disabling mental disorder with a very high prevalence worldwide (Kupfer, Frank, & Phillips, 2012). The characteristic symptoms of depression are depressed mood, loss of interest and enjoyment, inability to experience positive emotions, lack of energy, reduced self-esteem and self-confidence, ideas of guilt and unworthiness, sleep disturbance and changes in appetite, hopelessness regarding the future, ideas or acts of self-harm or suicide and reduced concentration and attention (World Health Organization, 1992). Clinical research shows that in the case of a considerable number of individuals treated for depression, the disorder has a chronic and recurrent course with consequences over the entire lifespan (Hardeveld, Spijker, De Graaf, Nolen, & Beekman, 2010). Furthermore, a significant number of patients receiving antidepressant treatment do not achieve remission or response. Based on the results of STAR*D, the largest depression study done to date, 67% of patients remitted after up to four successive treatment steps, including a switch in antidepressant classes, and augmentation with additional drug or cognitive therapy (Kupfer et al., 2012). This suggests that about a third of depressed patients do not get help from antidepressants. Hence, alternative treatments are urgently needed for TRD patients.

One such option is neuromodulation: various devices and methods are used to alter the electrical activity of the central nervous system (Janicak, Dowd, Rado, & Welch, 2010). Since the brain is a neurochemical organ, its activity can be modulated by both electrical and pharmacological means. Electroconvulsive shock therapy (ECT), a neuromodulation approach that has been in use for 80 years, has several disadvantages (Janicak, Marder, & Pavuluri, 2011). First, lack of access: ECT is not readily available in all geographical regions and it is usually reserved for the

most severely ill patients. Other disadvantages include adverse cognitive effects, considerable relapse rates after a successful acute treatment course and a common negative public image (Janicak et al., 2011). Transcranial magnetic stimulation (TMS) can overcome some of the disadvantages of ECT and there is a large body of studies supporting its being beneficial to patients suffering from an acute episode of major depression (Janicak & Carpenter, 2014).

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that is based on the principle of electromagnetic induction discovered by Faraday in 1831 (Lefaucheur, 2019). When a brief current of very high intensity (several thousand amps) passes through a copper wire coil, a magnetic field is produced that can reach up to about 2 tesla and lasts for about 100 μs (Lefaucheur, 2019). A magnetic field pulse delivered with a TMS coil on the scalp passes through the skull bone unattenuated and generates an electrical current in the superficial layers of the brain (Lefaucheur, 2019). The intensity of the induced current is sufficient to produce action potentials in neurons and, thus, interfere with the ongoing brain activity (Lefaucheur, 2019).

The first TMS machines for clinical use appeared in the mid-1980s (Barker, Anthony T Jalinous & Freeston, 1985). With the large circular coils that were used at first, the area of cortical stimulation was relatively large. In order to produce more focal activation of the brain, double coils with smaller diameters were produced (e.g. figure-of-eight coil). The pulse waveform (monophasic or biphasic) determines the nature of the cortical circuits that are activated in the stimulated area (Lefaucheur, 2019). Biphasic stimulations are thought to be more powerful than the monophasic ones, and the former is the standard pulse waveform used in repetitive transcranial magnetic stimulation (rTMS) (Lefaucheur, 2019). Since TMS activates circuits, the biological changes provoked by TMS may occur at a site distant from the site of application of the stimulation (Lefaucheur, 2019). The intensity of stimulation also impacts the effects of TMS – with increased intensity, the induced electric field reaches deeper into the brain and is able to recruit additional neural networks (Lefaucheur, 2019).

TMS can be administered in single pulses or in a brief series of pulses, called a train (Janicak & Dokucu, 2015). The repetitively applied pulses of rTMS can modulate cortical excitability – increase or decrease it, depending on the parameters of stimulation (Rossi et al., 2009). The therapeutic potential of rTMS comes from the fact that neuromodulatory effects can last longer than the duration of the train of stimulation (Rossi et al., 2009).

Several important stimulation parameters can be adjusted while delivering the TMS (Janicak & Dokucu, 2015). The rTMS pulses can be either delivered in a rapid (ie, >1–20 Hz) repetitive fashion, which increases cortical excitability, or in a slow (ie, <1 Hz) repetitive fashion, which has the opposite effect (Janicak & Dokucu, 2015). The intensity of stimulation can be varied in a personalized way to optimize efficacy and minimize adverse effects (Janicak & Dokucu, 2015). Usually, the intensity is chosen based on the individual motor threshold (MT), the intensity of the magnetic field required to activate skeletal muscles when the coil is placed over the subject's primary motor cortex (Janicak & Dokucu, 2015). Coil location is another vital parameter, i.e. over which brain region the coil is placed and which neurons get stimulated (Janicak & Dokucu, 2015). Some other parameters that can be varied are the total number of pulses delivered per session, pulse duration, interpulse interval's duration, the duration of a train of pulses, the length of the intertrain interval, etc (Janicak & Dokucu, 2015). The search for the best combination of parameters for each specific purpose continues.

Theta-burst stimulation (TBS) is a variation of rTMS. It is characterized by the use of a triplet pulse burst at a very high frequency (50-Hz) with a 200-ms interburst interval, typically at 80% of the active MT (Chu et al., 2021). This pattern is mimicking the firing patterns of hippocampal neurons and the mechanisms of long-term potentiation and long-term depression (Janicak & Dokucu, 2015). There are two types of TBS: continuous TBS (cTBS) and intermittent TBS (iTBS). The main protocol of cTBS uses uninterrupted TBS trains for 40 s (600 pulses), while that of iTBS repeats 2s TBS trains every 10s for a total of 20 cycles (600 pulses) (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005).

rTMS has been used to treat a variety of neuropsychiatric disorders, but most often it has been applied to depression. Usually, it is the treatment resistant depressed patients who receive rTMS. Numerous sham-controlled studies have provided consistent evidence for the efficacy of the use of rTMS in depression (Perera et al., 2016). In TRD samples, on average, 30% achieve remission while 60% respond to the rTMS treatment (Carpenter et al., 2012; Sackeim et al., 2020). The mechanism of TMS's action in depression is not well understood (Janicak & Dokucu, 2015). To a large extent, this gap in knowledge is due to the lack of robust pathophysiological theories of depression (Janicak & Dokucu, 2015).

The efficacy of iTBS has been shown to be comparable to the 10Hz rTMS to the left DLPFC (Blumberger et al., 2018). However, iTBS sessions have a significantly shorter duration, which increases the capacity for clinical treatment and also improves patient adherence (Chu et al., 2021).

Classical daily applied rTMS sessions are usually spread over several weeks and the accompanying financial and time burden is problematic for patients (Duprat et al., 2016). Therefore, accelerated stimulation protocols are being increasingly investigated. In those, the number of pulses per session is significantly increased or multiple sessions are delivered the same day, wherefore the whole treatment is completed in a considerably shorter period of time (Baeken et al., 2013; George et al., 2014; Holzer & Padberg, 2010; Sonmez et al., 2019). Accelerated rTMS and aiTBS are well-tolerated and seem to be safe (Caulfield, Fleischmann, George, & McTeague, 2022; Duprat et al., 2016). Yet, there is little knowledge regarding the impact of excitatory/inhibitory neuronal effects of accelerated protocols or potential cellular damage. The intensive nature of such protocols increases the risk of histotoxicity through massive hyperexcitation of neurons, or tissue injury due to ohmic heating of tissue by induced currents (Wassermann, 1998).

Proton magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (^{1}H MRS) is a non-invasive imaging procedure that allows for direct, non-radioactive, in vivo estimation of the concentration of neurochemicals in the human brain (Chen, Dai, Dai, Xu, & Wu, 2014; Near et al., 2021). In contrast to more conventional MR imaging, the spectra of MRS provide physiological and chemical information, rather than anatomy (Bertholdo, Watcharakorn, & Castillo, 2013).

Both MRI and MRS have their roots in nuclear magnetic resonance (NMR), which was first described in 1946 simultaneously by the Nobel Prize winners Edward Purcell and Felix Bloch (Bertholdo et al., 2013). At first, NMR was only used by physicists for the purpose of determining the nuclear magnetic moments of nuclei. In the following decades Lauterbur, Mansfield, and Grannell introduced gradients into the magnetic field, making it possible to determine the location of the emitted signal and the reproduction of the signal on an image. From the mid-1970s onwards, NMR started to be used in vivo and was renamed MR imaging; similarly, NMR spectroscopy used in vivo came to be called MR spectroscopy. The next important milestone were the 1980s, when the first medical scanners became available for clinical use. Since then the MR technique has been refined, most notably by introducing higher field strengths (Bertholdo et al., 2013).

Although a variety of nuclei may be used to obtain MR spectra, for clinical applications of the human brain, protons (^{1}H) are mostly used due to their high sensitivity and abundance (Bertholdo et al., 2013). The proton MR spectrum is altered in most of the neurologic disorders

and accumulating knowledge points to alterations in psychiatric disorders (Bertholdo et al., 2013; Bustillo, 2013).

The MRS technique makes use of the chemical shift properties of atoms (Bertholdo et al., 2013). The nuclei in a tissue exposed to an external magnetic field, resonate at Larmor frequency. Interactions with the surrounding molecules cause slight changes in the spin frequencies of atoms – this phenomenon is called chemical shift. The value of the chemical shift (expressed in parts per million (ppm)) gives information about the molecular group carrying ^{1}H (Bertholdo et al., 2013).

The main objective of ^{1}H-MRS is to detect weak signals from metabolites. This can be done at different magnetic field strengths, but higher field strength brings along advantages like higher signal-to-noise ratio (SNR), better resolution and shorter acquisition times (Bertholdo et al., 2013).

Most broadly, MRS can be divided into two types – single-voxel spectroscopy (SVS) and magnetic resonance spectroscopy imaging or chemical shift imaging (Bertholdo et al., 2013). In the case of SVS the signal is obtained from a predefined single voxel placed in the volume of interest (VOI). In the case of MR spectroscopy imaging many voxels are acquired simultaneously providing information about the spatial distribution of metabolites. Typically, either pointed-resolved spectroscopy (PRESS) or stimulated echo acquisition mode (STEAM) is used for the acquisition of ^{1}H-MRS spectra. PRESS is the most used technique in SVS. The press sequence consists of a 90º pulse followed by two 180º pulses, each of which is applied at the same time as a different field gradient. ^{1}H-MRS can be acquired using different echo times (TE). With short TE (typically 20-40 milliseconds) the acquired spectrum includes more metabolite peaks, however, overlap of the peaks is more common than with long TE. Spectra acquired with long TE (135-288 milliseconds) are simpler with fewer sharp peaks (Bertholdo et al., 2013).

Water is the most abundant metabolite in the brain and as such its signal in the MR spectrum is much higher (100,000 times greater) than that of other metabolites, which have a low concentration in brain tissues (Bertholdo et al., 2013). Water-suppression techniques are used to avoid the high peak from water to be superimposed on the signal from other metabolites (Bertholdo et al., 2013).

Once the MRS data is acquired, it needs to be properly postprocessed to obtain spectra that allow for reliable interpretations. In what follows, a brief description is given of those metabolite peaks that are directly relevant for this master's thesis.

N-acetylaspartate (NAA) peak assigned at 2.02 ppm is the highest peak in the normal brain (Bertholdo et al., 2013). Synthesised in the mitochondria of neurons, NAA is subsequently transported into the neuronal cytoplasm and along axons. NAA is exclusively found in the nervous system – both in the central (CNS) and the peripheral nervous system; it can be detected both in grey and in white matter. NAA is viewed as a marker of neuronal and axonal integrity, viability and density: decreased concentration or the absence of the NAA peak are a sign of neuronal degradation and loss (Bertholdo et al., 2013). For example, in neurodegenerative and vascular disease NAA is reduced, while being absent from brain tumour tissue (Bustillo, 2013). However, NAA's function remains unclear – it has been hypothesized to be an osmolyte and an acetate donor involved in myelination, but it is clearly not a neurotransmitter or neuromodulator (Bustillo, 2013).

Creatine (Cr) peak, which represents a combination of molecules of creatine and phospho-creatine, is assigned at 3.02 ppm (Bertholdo et al., 2013). The role of creatine and phospho-creatine is in energy metabolism (Bustillo, 2013). Considered a stable metabolite with a relatively constant concentration, it is used as an internal reference to calculate metabolite ratios (Bertholdo et al., 2013). Regional and individual variability in Cr concentrations do exist, but interpretation of these differences is poorly understood (Bertholdo et al., 2013; Bustillo, 2013).

Glutamate + glutamine (Glx) peaks are assigned at 2.05 to 2.50 ppm (Bertholdo et al., 2013). The complex overlapping peaks from glutamate, glutamine and gamma-aminobutyric acid are difficult to separate at 1.5 T, but they can be measured at higher field strengths (Bustillo, 2013). Besides being the principal excitatory neurotransmitter in the CNS, the amino acid glutamate also plays a role in the redox cycle (Bertholdo et al., 2013). Only a small part of the glutamate signal measured by MRS comes from the synapses (Bustillo, 2013). Glutamine is mainly synthesized in the glia from synaptic glutamate and has been used as an index of the glutamatergic neurotransmission (Bustillo, 2013).

Gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the CNS, can be quantified at higher field strengths employing special editing techniques (Bustillo, 2013).

Neurochemistry and brain regions in depression

In both human and animal studies of depression, altered brain levels of amino acid neurotransmitters glutamate and GABA have been found (Lener et al., 2017). According to the glutamate hypothesis of depression, glutamatergic system is a primary mediator of psychiatric pathology – glutamate neurons and synapses by far outnumber all other neurotransmitter systems in the brain and glutamate synaptic transmission largely mediates both cognition and emotion (Sanacora, Treccani, & Popoli, 2012). Hence, the brain can be viewed as a largely glutamatergic excitatory machine, regulated by a GABAergic inhibitory component and modulated by a much smaller number of neurons releasing various other neurotransmitters (including monoamines) (Sanacora et al., 2012). It has been suggested that the underlying cause of many of the differences observed in the amino acid neurotransmitter content between depressed and healthy control subjects may be due to glial pathology (Rajkowska & Stockmeier, 2013; Sanacora et al., 2012; Sanacora, Zarate, Krystal, & Manji, 2008).

Numerous neuroimaging studies have reported regional volumetric changes and structural abnormalities in the brain areas associated with stress-responsiveness and emotional/cognitive processing in patients with mood disorders (Campbell & MacQueen, 2006; Konarski et al., 2008; Lorenzetti, Allen, Fornito, & Yücel, 2009). In depressed patients, large volume reductions have been detected in frontal regions including the anterior cingulate and orbitofrontal cortex with smaller reductions in the prefrontal cortex (PFC) and moderate reductions in the hippocampus and striatum (Koolschijn, Van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009). Many of the aforementioned regions overlap with those brain regions demonstrated to have significant reductions in glial cell numbers and density, and neuronal atrophy in patients with mood disorders (Rajkowska, 2002).

Abnormalities in excitatory and inhibitory neurotransmission and neuroplasticity may lead to maladaptive changes in functional connectivity in large brain networks (Lener et al., 2017). Functional brain imaging studies show an increase in the activity of the default mode network (DMN) in depressed patients, accompanied by decreases in the salience (SAL) and central executive networks (CEN) (Evans et al., 2018; Greicius et al., 2007; Kaiser, Andrews-Hanna, Wager, & Pizzagalli, 2015). DMN is responsible for resting-state introspection and rumination, SAL processes salient information from external sources and CEN is responsible for working memory and attention (Menon, 2011). The above-mentioned changes in the activity level of these networks are consistent with increased rumination and introspection and decreased engagement with external inputs in depressed patients (Evans et al., 2018; Greicius et al., 2007;

Kaiser et al., 2015). DMN including subregions of PFC where structural alterations have been observed in depression, one could speculate that sustained long-term increases in DMN activity may lead to excitotoxic effects and atrophy of glutamate principle neurons in the brain region (Duman, Sanacora, & Krystal, 2019).

Since Cousins & Harper (1996) first reported an association between mood and ^{1}H-MRS determined glutamate related neurochemical measures, a remarkable number of studies have been published examining this relationship in a variety of brain regions (Arnone, Mumuni, Jauhar, Condon, & Cavanagh, 2015; Godfrey, Gardner, Kwon, Chea, & Muthukumaraswamy, 2018; Kondo et al., 2011; Luykx et al., 2012; Yksel & Öngür, 2010). Since it is technically challenging to separate glutamate from glutamine at conventional MR field strengths, the two are often reported together as a composite measure called Glx (Godlewska, Near, & Cowen, 2015). Glx reflects predominantly glutamate content but also contains glutamine and GABA components (Sanacora et al., 2012). Findings of the MRS studies are inconsistent, with some studies reporting increases in glutamatergic metabolites in patients with depression as compared to healthy controls (Godlewska, Masaki, Sharpley, Cowen, & Emir, 2018), others reporting decreases (Baeken, Lefaucheur, & Van Schuerbeek, 2017; Chen et al., 2014; Michael, Erfurth, et al., 2003) and yet others reporting no difference (Gabbay et al., 2017). Recent meta-analyses also present varying results regarding the differences in depressed patients in comparison to healthy controls. One of the meta-analyses found that during a depressive episode, patients had lower glutamate levels within the anterior cingulate cortex (ACC) (Luykx et al., 2012). Another reported that Glx levels were decreased in the prefrontal cortex (PFC) in depressed patients, whereas no significant difference in glutamate levels was detected between the two groups (Arnone et al., 2015). Another found lower levels of GABA in depressed patients and a trend towards reduction of Glx in the prefrontal region (Godfrey et al., 2018). The most recent meta-analysis reported significant decreases in Glx in the medial frontal cortex (Moriguchi et al., 2019). The inconsistent results can be explained by differences in brain regions studied, the placement of regions of interest (ROIs), MRS methodologies, stage or severity of illness of the subjects, and the use of medications (e.g., antidepressant treatments) (Moriguchi et al., 2019).

Regarding changes accompanying treatment and symptom reduction in depressed patients, MRS studies have also reported varying results. For example, Chen et al. (2014) found that the levels of N-acetylaspartate (NAA), Glx and myo-inositol (MI), which had been significantly lower at baseline in the bilateral ACC in MDD patients as compared to controls, were normalized after antidepressant treatment with selective serotonin reuptake inhibitor (SSRI).

No differences in the aforementioned metabolites were detected in the bilateral DLPFC between unmedicated MDD patients and healthy controls, and no changes in metabolite levels in these regions were found to accompany treatment.

Godlewska, Near, & Cowen (2015), on the other hand, measured GABA, glutamate and glutathione (GSH) in a voxel placed in the occipital cortex. Patients underwent a 6-week treatment with an SSRI (escitalopram); although the majority of the patients showed significant clinical improvement, there were no alterations in the concentrations of GABA, glutamate or GSH.

The effect of electroconvulsive therapy (ECT) on neurometabolites has also been studied with MRS. For example, Michael et al. (2003) reported that ECT normalized reduced prefrontal Glx in patients with treatment-resistant depression.

As regards the effect of TMS on brain chemicals, the majority of previous neurostimulation studies have examined HF-rTMS of the left DLPFC, which is the classical paradigm for depression therapy. Some of the studies showed an increased glutamatergic transmission after rTMS (Croarkin et al., 2016; Luborzewski et al., 2007; Michael, Gösling, et al., 2003; Yang et al., 2014), while others found primarily GABA involvement (Dubin et al., 2016; Stagg et al., 2009; Vidal-Piñeiro et al., 2015).

As for accelerated rTMS protocols, the only MRS study looking at the effect on neurometabolites published so far is by Baeken, Lefaucheur, & Van Schuerbeek (2017). They studied the impact of accelerated high frequency rTMS (aHF-rTMS) on brain neurochemicals in treatment-resistant depression (TRD). They measured the neurochemical concentrations in the left DLPFC, which was the area stimulated, and connected areas – the right DLPFC and rostral ACC. As compared to healthy controls, patients with (TRD) had lower glutamatergic concentrations (sum absolute concentrations glutamate and glutamine) in the left DLPFC. aHF-rTMS applied to the left DLPFC did not significantly alter neurochemical concentrations in the three predefined brain regions. However, a strong correlation was found between clinical improvement and GABA concentration change in the left DLPFC. They concluded that aHF-rTMS could potentially play a role in the increase of the GABAergic neurotransmission in the left DLPFC of those patients that improved clinically and did not affect the neural integrity (Baeken et al., 2017).

Research questions of this study

This master's thesis focuses on measuring the changes in two composite neurometabolites: Glx (glutamate + glutamine) and tNAA (N-acetylaspartate + N-acetylaspartylglutamate). The main questions that the study is aiming to answer are as follows: 1) Is there a difference at baseline between TRD patients and healthy controls in the concentration of Glx in the left DLPFC? 2) Does the concentration of Glx in the left DLPFC of TRD patients change after a treatment course with aiTBS? 3) Is there an association between clinical improvement and the change in the concentrations of Glx in the left DLPFC of TRD patients? 4) Does the concentration of tNAA in the left DLPFC of TRD patients change after the treatment course with aiTBS, implying neuronal damage? The hypotheses formulated based on these questions are as follows:

H1: Compared to healthy controls, TRD patients at baseline exhibit lower concentrations of Glx in the left DLPFC.

H2: Following a treatment course with aiTBS, the concentration of Glx in the left DLPFC of TRD patients increases.

H3: A negative association exists between the concentration of Glx in the left DLPFC and clinical depression scores. As clinical scores decrease, the concentration of Glx increases. This association is stronger in patients who receive real aiTBS as opposed to sham aiTBS.

H4: The concentration of tNAA remains unchanged after the aiTBS treatment. There is no difference in tNAA concentration between pre-treatment and post-treatment measurements.

Methods

Participants

The study, which is part of a larger ongoing project investigating different neurophysiological and clinical markers of aiTBS in TRD (ClinicalTrials.gov. National Library of Medicine (U.S.), 2017-2023), was approved by the ethics committee of the University Hospital (UZGent). All subjects gave their written informed consent. For the current ^{1}H MR spectroscopy study, forty-six scanned patients were included (Female:Male (F:M) = 22:24, age = 41.09 y, SD = 13.39). Never-depressed[1] healthy volunteers were included as the control group (n = 49, F:M = 33:16, age = 37.20 y, SD = 10.37). All participants were native Dutch speakers.

[1] No history of or current neurological or psychiatric problems

All TRD patients were diagnosed with unipolar depression and were selected to participate in the study by a certified psychiatrist, who administered (semi-)structured clinical interviews (17-item Hamilton Rating Scale for Depression (HDRS; Hamilton, 1960) and Mini International Neuropsychiatric Interview (MINI, Sheehan et al., 1998)). All of them were at least stage 2 treatment resistant (at least two unsuccessful treatment trials with an antidepressant, up to nine failed trials). The patients went through a medication washout period of at least two weeks before entering the study and were free from any antidepressant, neuroleptic and mood stabilizer for at least two weeks before entering the treatment protocol.

The following were considered as exclusion criteria for participation: psychotic features, bipolarity, dysthymia, active substance abuse or dependence within a year prior to inclusion, any neurological condition, current or past history of epilepsy, pregnancy or without effective anticonception for the duration of the trial, ECT non-responder, no response to more than 9 antidepressants, neurosurgical interventions, known allergic reaction to radiotracers or associated compounds, any implanted electronic device susceptible for magnetic field radiation (e.g. pacemaker), any implanted metal device in the head region.

aiTBS treatment protocol

Patients were treated with a total of 20 accelerated intermittent Theta Burst Stimulation (aiTBS) sessions over the left DLPFC. The treatment was spread over 4 consecutive days. On each stimulation day, a given patient received 5 sessions with a between-session delay of 15 minutes. Each session consisted of 100 cycli of thetaburst trains of 2s, separated by an inter-train-interval of 6 seconds (the total of 3000 pulses per session). The TMS impulses were administered at a stimulation intensity of 110% of the motor threshold.

Patients were randomized to receive either the real aiTBS (n = 24, F:M = 13:11, age = 42.58 y, sd = 14.32) or sham treatment (n = 22, F:M = 9:13, age = 39.45 y, sd = 12.42). However, the sham group received real aiTBS treatment 10 days after the completion of the sham treatment.

The DLPFC was located using Brainsight neuronavigation software. Individual neuroanatomical MRI data was used and the left DLPFC was visually identified based on the subject's own gyral morphology. The iTBS stimulation was applied using a Magstim Rapid2 Plus1 magnetic stimulator (Magstim Company Limited, Wales, UK) connected to a 70 mm “figure-of-eight” shaped coil. To administer sham stimulation, a specially designed sham coil was utilized, aimed at closely mimicking the characteristics of the real coil.

^{1}H MR spectroscopy scans were performed at baseline (D_0) and 10 days after the end of the aiTBS or sham treatment (~D_{14}). Changes in depression severity were rated with the HDRS by a clinician at baseline (D_0), 3 days after aiTBS or sham (~D_7) and 10 days after aiTBS or sham (~D_{14}). At the same time points, patients also filled in the Beck Depression Inventory (BDI-II; Beck, Steer, & Brown, 1996).

^{1}H MR spectroscopy

The ^{1}H MR spectroscopy scans were conducted on a Siemens 3T Magnetom Prisma Fit scanner (syngo MR E11, Siemens), using a 64-channel SENSE head coil.

All sessions started with acquiring a 3D, T1-weighted, MPRAGE sequence to position the MRS volume. The ^{1}H MR spectroscopy measurement was done with a PRESS sequence (volume size = 15 × 15 × 15 mm (left DLPFC), TR/TE = 2.000/35 ms, 128 averages).

The ^{1}H MR spectroscopy data (.ima metabolite files with all dimensions precombined by the scanner software) was processed with the Osprey MRS software version 2.3.0 (Oeltzschner et al., 2020) to get the metabolite estimates. A MATLAB (version R2021b; The MathWorks, 2021) script was used to collect the data from the catalogue system and batch process using the Osprey toolbox.

Two MRS scans were excluded from further analysis due to technical issues that became evident during visual inspection, indicating errors in the acquisition process. The quality of the MRS spectra exhibited significant variation. Figure 1 illustrates a good quality MRS spectrum.

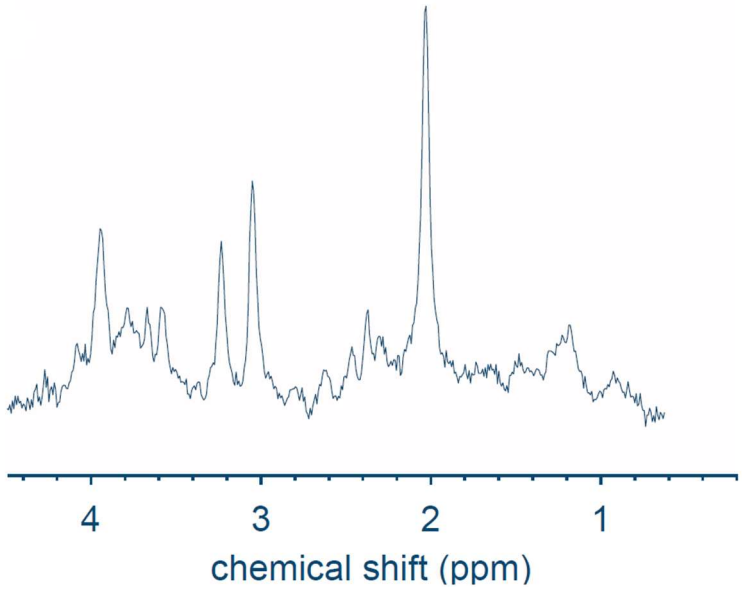

Figure 1. A magnetic resonance spectroscopy spectrum

Figure 2 depicts the stage of MRS data processing where the individual metabolite peaks, such as Glu, Gln, NAA, NAAG, Cr, and PCr, are identified and quantified. For the study objectives, the relevant focus was on these peaks. In the statistical analysis, metabolite ratios rather than absolute concentrations were utilized, with the reference signal derived from the combined total creatine (Cr + PCr). A metabolite ratio calculated relative to the total creatine (tCr) signal in magnetic resonance spectroscopy (MRS) data analysis is typically referred to as "tCr ratio". The tCr ratio is calculated by dividing the signal intensity or concentration of a specific metabolite of interest by the signal intensity or concentration of tCr. It is commonly used in MRS studies as a normalization method to account for variations in total creatine levels and provide relative quantification of metabolite concentrations (Near et al., 2021).

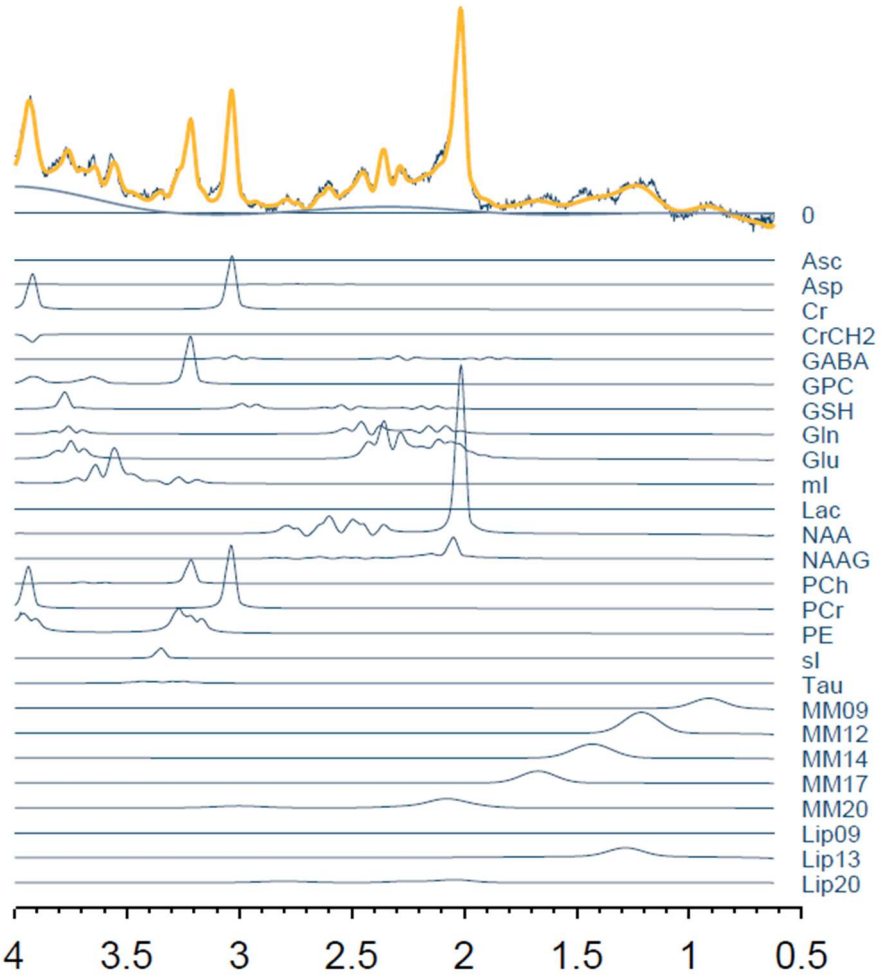

Figure 2. Peak Fitting: the individual metabolite peaks are identified and quantified.

Statistical analysis

The statistical analysis was performed using R version 4.1.2 (R Core Team, 2021). R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) were used for linear mixed model analyses. R packages ggrain (Allen et al., 2019) and ggplot (Wickham, 2016) were used for the graphics.

The significance level was set to $p < 0.05$ for all analyses. The *a priori* hypotheses (see above) were tested using linear models and linear mixed models. Model comparison approach (Philips, 2018) was used in order to build parsimonious models: more complex models were built from simpler models by adding a single independent variable at a time and testing[2] whether the more complex model offered a significantly better fit to the data. For the analysis corresponding to each hypothesis, models were compared that either included or excluded age and/or gender as covariates (to eliminate confounding).

Hypotheses H1 and H3 were addressed with linear models, whilst H2 and H4 were addressed with linear mixed models (LMMs). LMMs are especially well suited for repeated measures analysis, since they assume *missing at random (MAR)*, which means that an individual participant's data can still be used in a repeated measures analysis even if some data points are missing. After identifying the best model through model comparison, calculations were performed to obtain the model estimates. Prior to interpreting the results, a thorough examination was conducted to ensure that the selected model fulfilled the assumptions of the analysis method employed.

The analysis related to H3 was based on changes in pre- to posttreatment Glx concentrations and changes in HDRS and BDI scores. The change in Glx was calculated by subtracting the pretreatment measurement from the posttreatment measurement. For both HDRS and BDI, percentage change scores were calculated based on the following formula ((S2-S1)/S1)*100, where S1 is a patient's pretreatment score and S2 their posttreatment score.

A patient's pretreatment Glx/tCr ratio was estimated to be 3.01, which was more than three standard deviations higher than the sample mean. Based on the available literature, a Glx/tCr ratio above three in the DLPFC would be considered very high and potentially implausible. However, the exact thresholds for what would be viewed an impossible value, vary across studies, and depend on specific methodologies, populations, and experimental conditions. In

[2] R's anova() function was used for model comparison

general, Glx/tCr ratios in the brain are reported to be within the range of 0.2 to 1.6 (Bhattacharyya, 2021; Kegeles, 2012, Yildirim et al, 2019). Therefore, it is likely that the Glx/tCr ratio 3.01 resulted from an estimation error. Since the datapoint was also identified as a potential outlier when assessing the statistical models used in this thesis, this patient's pretreatment Glx concentration was not included in the analyses. The same patient's posttreatment Glx/tCr fell into the normal range and was included in the mixed model analysis related to H2. Both the pre- and posttreatment tNAA/tCr estimates of the same patient were similar to the sample mean and, as such, there was no reason to exclude those from the relevant analysis (H4).

The lowest value of tNAA/tCr (0.75), measured posttreatment for a patient, deviated significantly from the tNAA/tCr values of the rest of the participants and was highlighted as a potential outlier by multiple diagnostic model assessment methods and, as such, was excluded from the analysis relating to H4. Since the previous literature either reported changes in the tNAA/tCr ratio or the significance of group differences rather than the raw values measured specifically in the DLPFC, it is not possible to comment on the biological implausibility of the value.

Certain data were lost at earlier stages during the course of the study due to participant attrition or incomplete MR scans, due to reasons such as claustrophobia. Additionally, two spectra had to be excluded due to technical issues, as previously described. As a result, the available data varied for different analyses. Specifically, only the pretreatment MRS data was available for five patients, while only the posttreatment data was available for one patient. Furthermore, for testing H3, the inclusion of clinical scores was necessary, but a few scores were missing. Additionally, the statistical methods utilized in the study employed different approaches for handling missing values. Consequently, the analysis was performed using the following number of participants for each study hypothesis: H1 – data from 44 patients and 49 controls; H2 – data from 46 patients (with only pretreatment data for five patients and only posttreatment data for two patients); H3 – data from 40 patients in the analysis with HDRS and data from 39 patients in the analysis with BDI; H4 – data from 46 patients (with only pretreatment data for six patients and only posttreatment data for one patient).

Results

Glx concentration: patients vs controls

H1 was tested using a linear regression model to predict Glx concentration. Model comparison showed that the model with *Status* (patient vs control) and *Age* as predictors represented the data the best.

Being a patient was associated with lower Glx concentration in the left DLPFC, but the effect is not statistically significant. On the other hand, Age was found to be a statistically significant predictor with a negative effect on Glx concentration. Figure 3 illustrates the distribution of the Glx concentration in the two groups. The overall model explains 12% of the variance in the Glx concentration in the left DLPFC of the participants. The model is statistically significant, but the proportion of variance explained is weak (R^2 = 0.12, $F(2, 90) = 6.23$, $p = 0.003$, *adj.* R^2 = 0.10). The model coefficient estimates are presented in Table 1.

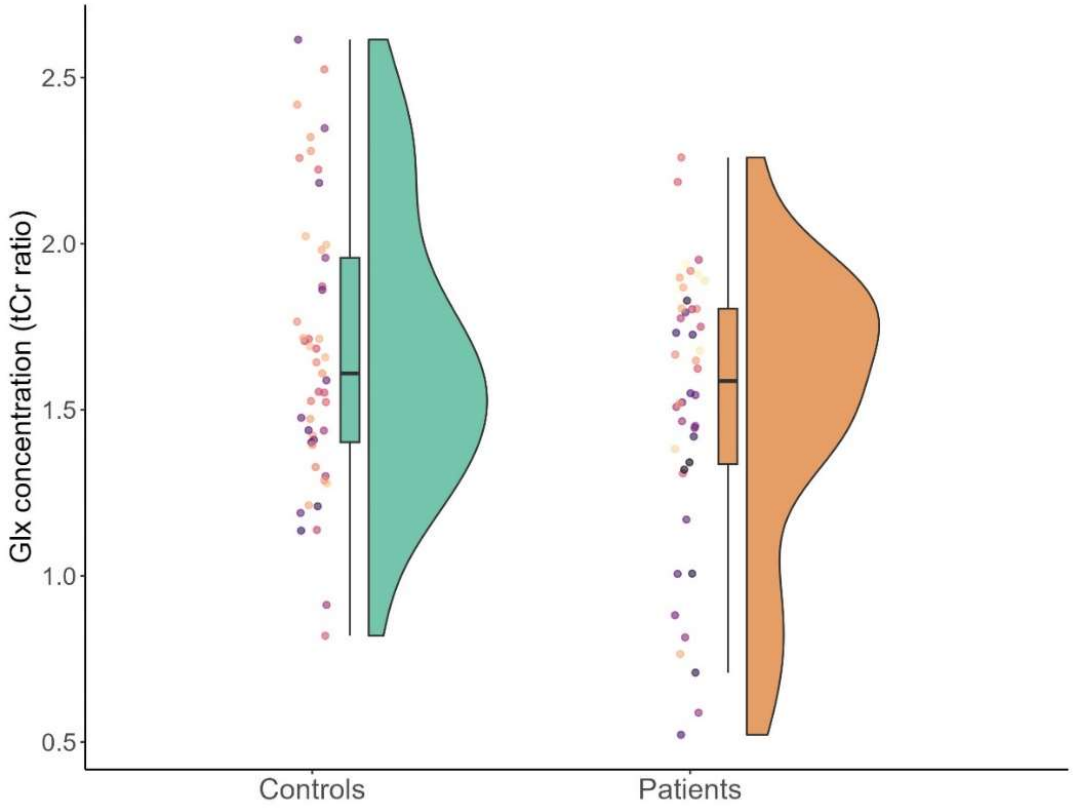

Figure 3. Glx concentration (tCr ratio) in the left DLPFC of patients and controls. The colour of the data points indicates participants' age: darker colour denotes greater age.

Table 1

Regression coefficients for predicting Glx concentration in patients and controls.

Parameter	*b*	*95% CI (b)*	*β*	*95% CI (β)*	*t(90)*	*p*
(Intercept)	2.06	[1.78, 2.35]	0.12	[-0.15, 0.39]	14.45	<.001
Status [pat]	-0.11	[-0.28, 0.06]	-0.26	[-0.65, 0.14]	-1.28	.205
Age	-0.01	[-0.02, -0.00]	-0.30	[-0.50, -0.10]	-3.02	.003

95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution approximation.

Glx concentration change from pre- to posttreatment in the real and sham group

H2 was tested using linear mixed models. Since the main question of interest was whether real TMS had a different impact on Glx concentration in the left DLPFC as compared to sham, the formula included an interaction term *Condition* * *Time*. *Condition* refers to treatment condition – real or sham, and *Time* signifies either pre- or posttreatment measurement. Model comparison showed that the data was best described by a mixed model including *Age* as a covariate, but not *Gender*. The model included a random intercept, i.e. a subject random effect to capture interindividual variation in Glx levels. The model was fitted using maximum likelihood estimation and the "lmer" function from the "lme4" package in R.

The model's total explanatory power is substantial, explaining 34% of the variance in Glx concentration (conditional R^2 = 0.34) and the part related to the fixed effects alone is 15% (marginal R^2 = 0.15).

The model's fixed intercept corresponds to the mean of the sham group's pretreatment measurement at *Age*=0. The effects of *Condition*, *Time* and the interaction *Condition* * *Time* on Glx concentration were small or very small not significantly contributing to the model. *Age* was the only significant predictor of Glx, with a small effect size influencing the concentration in a negative direction. Random effects analysis indicated that the subjects' "personal" intercepts accounted for a considerable amount of the variance (s^2 = 0.031, *SD* = 0.176, $\chi^2(1)$= 1.99, p = .158) in the Glx levels. The pre- to posttreatment change in Glx levels of the patients in both treatment conditions is illustrated in Figure 4.

Table 2

Mixed model coefficients for predicting Glx using Condition, Time, Age and Subject

Parameter	*Estimate*	*95% CI (b)*	*β*	*95% CI (β)*	*t(78)*	*p*
Fixed effects	*b*					
(Intercept)	2.00	[1.69, 2.31]	0.12	[-0.29, 0.53]	12.71	<.001
Condition [real]	-0.05	[-0.27, 0.18]	-0.11	[-0.67, 0.44]	-0.41	.682
Time [post]	-0.00	[-0.21, 0.21]	-0.00	[-0.51, 0.51]	-0.01	.991
Age	-0.01	[-0.02, 0.00]	-0.36	[-0.58, -0.14]	-3.30	.001
Condition [real] * Time [post]	-0.10	[-0.39, 0.19]	-0.24	[-0.95, 0.47]	-0.67	.505
Random effects	*SD*					
Subject	0.18					
Residual	0.33					

Linear mixed model fit by maximum likelihood. t-tests use Satterthwaite's method.

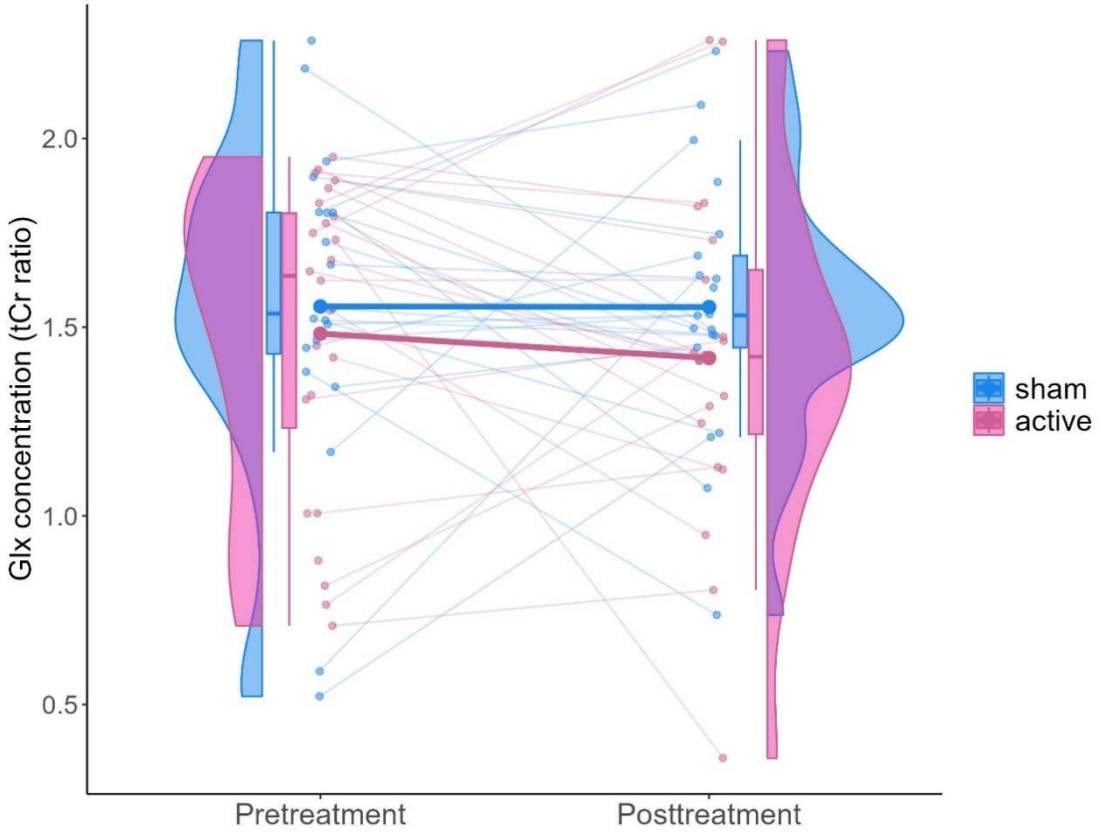

Figure 4. Glx concentration change from pre- to posttreatment in the real/active and sham group. The small dots represent individual participants and the narrow lines between the dots indicate the individual trajectories of Glx concentration change for each patient. The larger dots illustrate group means and the wider lines an average change in Glx.

Changes in Glx and clinical improvement

H3 makes two claims: first, that changes in Glx and depression scores are negatively associated, and second, this association is stronger for patients receiving real rather than sham iTBS. Therefore, linear models predicting the change in Glx (*ΔGlx*) were fitted in two steps. In the first step, the percentage change in the depression score (either *ΔHDRS or ΔBDI*) was the only predictor. In the second step, *Condition* and the interaction term, either *ΔHDRS*Condition* or *ΔBDI*Condition,* were used as additional predictors.

The model with only *ΔHDRS* as a predictor explains a proportion of variance in *ΔGlx* that is statistically not significant and very weak (R^2 = 0.009, $F(1, 38) = 0.35$, p = .560, *adj.* R^2 = -0.02). The effect of *ΔHDRS* was statistically non-significant and of negligible size (β = -0.10, p = .56).

Including *Condition and ΔHDRS*Condition* in the model in addition to *ΔHDRS,* improves the model's explanatory power, but the model still explains only a weak proportion of variance (7%) in *ΔGlx* and remians statistically non-significant (R^2 = 0.07, F(3, 36) = 0.88, p = 0.463, *adj.* R^2 = -0.01). All the predictors in the model are statistically non-significant: *ΔHDRS* (β = 0.03, p = 0.868), *Condition* (β = -0.25, p = .163) and *ΔHDRS*Condition* (β = -0.48, p = .184).

The model with only *ΔBDI* explains 13% of variance in *ΔGlx* and is statistically significant (R^2 = 0.13, F(1, 37) = 5.48, p = 0.025, *adj.* R^2 = 0.11). The model with *ΔBDI, Condition* and *ΔHDRS*Condition* is not statistically significant, but explains a moderate proportion (14%) of variance (R^2 = 0.14, F(3, 35) = 1.95, p = 0.140, *adj.* R^2 = 0.07). The coefficients of both models can be seen in Table 3 and the association between *ΔBDI* and *ΔGlx* is illustrated in Figure 5.

Table 3

Regression coefficients for predicting *Δ*Glx with *ΔBDI, Condition* and *ΔBDI*Condition.*

Parameter	R^2	*b*	*95% CI (b)*	*B*	*95% CI (β)*	*t(90)*	*p*
Step 1	0.13						.025
(Intercept)		-0.15	[-0.31, 0.02]	0.00	[-0.31, 0.31]	-1.80	.075
ΔBDI		-0.01	[-0.01, 0.00]	-0.36	[-0.67, -0.05]	-2.34	.025
Step 2	0.14						.140
(Intercept)		-0.09	[-0.32, 0.14]	0.11	[-0.33, 0.55]	-0.80	.429
ΔBDI		-0.01	[-0.02, 0.00]	-0.36	[-0.79, 0.07]	-1.69	.100
Condition [real]		-0.12	[-0.46, 0.22]	-0.23	[-0.86, 0.39]	-0.73	.472
ΔHDRS * Condition		-0.00	[-0.01, 0.01]	-0.01	[-0.65, 0.63]	-0.04	.969

95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution approximation.

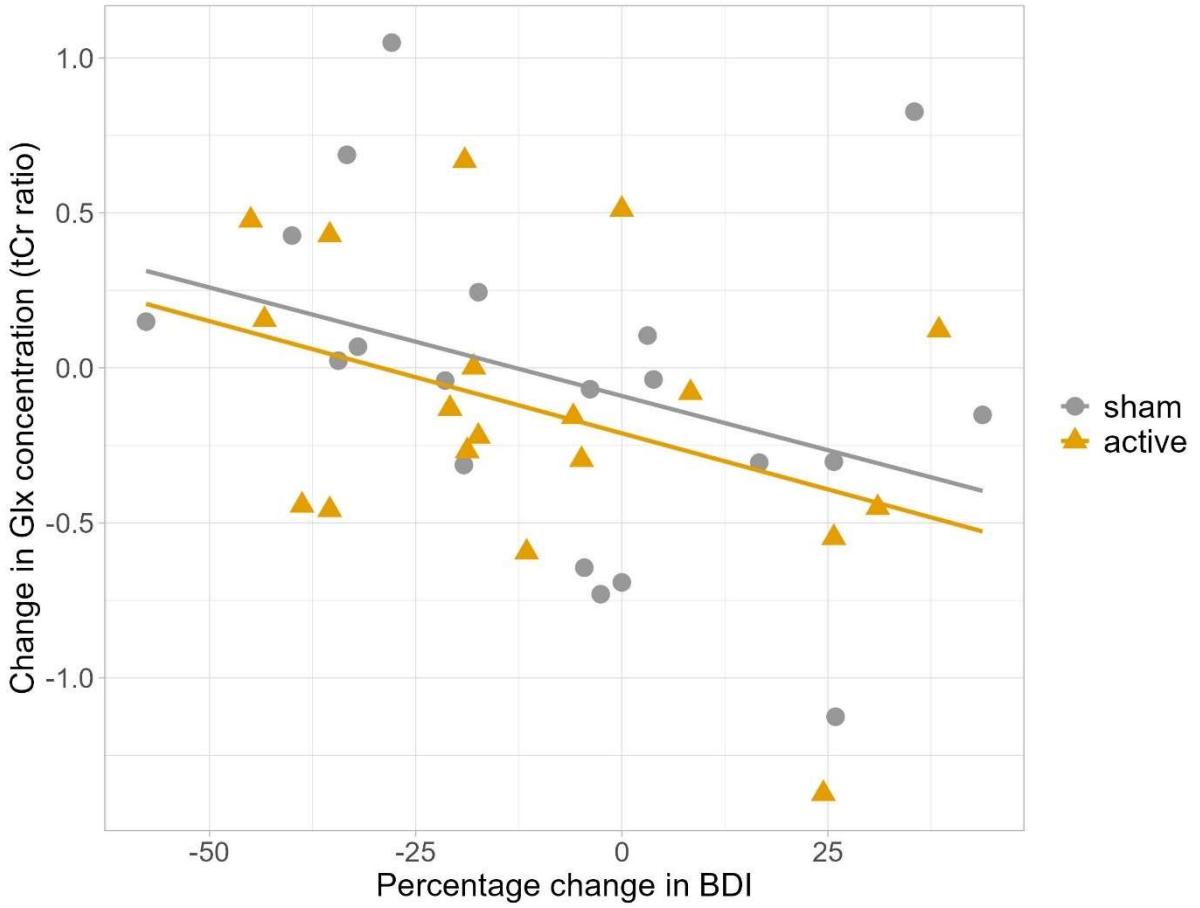

Figure 5. Association between Glx concentration and BDI scores in the real/active and sham group

tNAA concentration stability pre- to posttreatment

H4 was tested using linear mixed models. The principal question was whether real and sham TMS had a different effect on the tNAA concentration in the left DLPFC of patients. Hence, the primary focus of interest lay in the interaction term between *Condition* and *Time*. Model comparison showed that the data was best described by a mixed model including *Age* as a covariate. The model included a random intercept for *Subject* to account for the differences between the participants. The model was fitted using maximum likelihood estimation and the "lmer" function from the "lme4" package in R.

The overall model has a substantial explanatory power, explaining 45% of the variance in tNAA concentration (conditional R^2 = 0.45) and the part related to the fixed effects alone is 16% (marginal R^2 = 0.16). The model's fixed intercept corresponds to the mean of the sham group's pretreatment measurement at *Age* = 0. The main effect of *Condition* is small and not statistically

significant. *Time, Age* and the interaction *Condition * Time* are statistically significant predictors of tNAA levels. The effect size of *Age* is small, but the influence of both Time and *Condition * Time* correspond to medium effect size. While in the sham group the tNAA increases from pre- to posttreatment, in the real TMS group the opposite holds. Figure 6 illustrates the pre- to posttreatment change in tNAA levels of the patients in both treatment conditions. Random effects analysis indicated that the *Subject* intercepts accounted for a significant amount of the variance (s^2 = 0.001, *SD* = 0.09, $\chi^2(1) = 4.99$, $p = .025$) in Glx levels.

Table 4

Mixed model coefficients for predicting tNAA using Condition, Time, Age and Subject

Parameter	*Estimate*	*95% CI (b)*	*β*	*95% CI (β)*	*t(78)*	*P*
Fixed effects	*b*					
(Intercept)	1.46	[1.33, 1.59]	-0.18	[-0.58, 0.22]	22.14	<.001
Condition [real]	0.03	[-0.06, 0.13]	0.20	[-0.34, 0.75]	0.75	.458
Time [post]	0.09	[0.01, 0.17]	0.53	[0.06, 0.99]	2.25	.029
Age	-0.00	[-0.01, 0.00]	-0.34	[-0.56, -0.11]	-2.93	.005
Condition [real] * Time [post]	-0.12	[-0.23, -0.02]	-0.74	[-1.39, -0.10]	-2.28	.027
Random effects	*SD*					
Subject	0.09					
Residual	0.12					

Linear mixed model fit by maximum likelihood. t-tests use Satterthwaite's method.

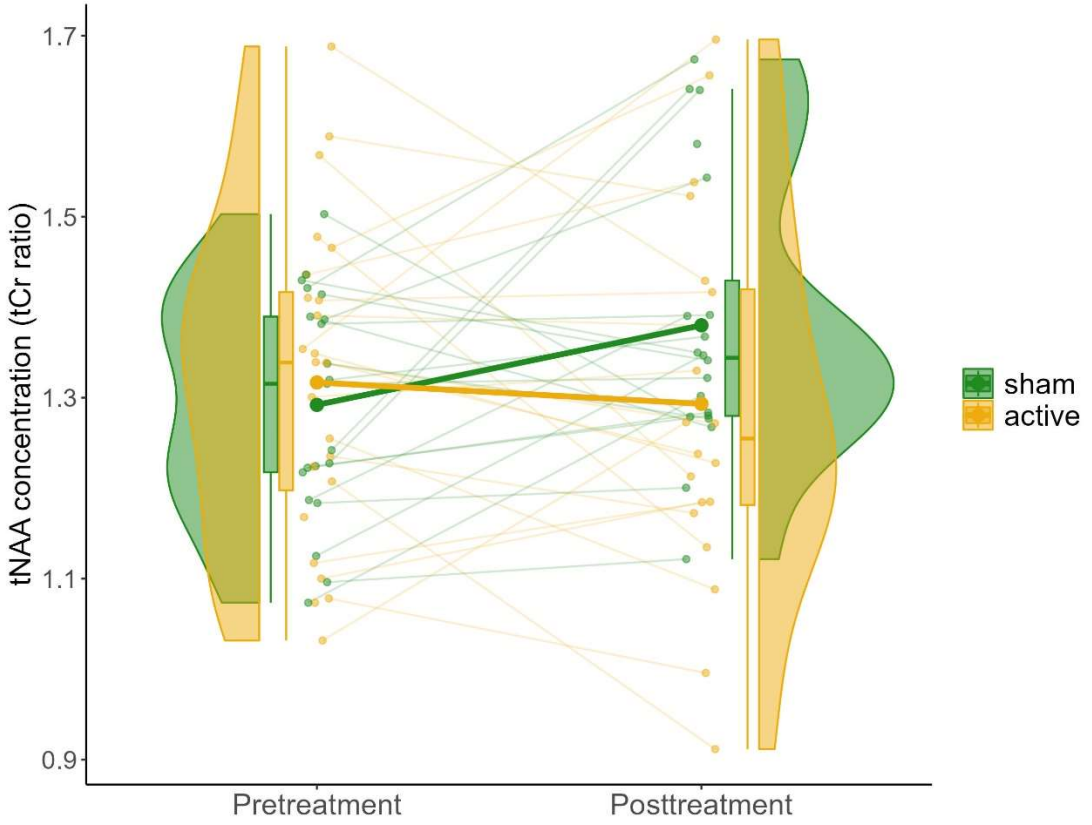

Figure 6. tNAA concentration change from pre- to posttreatment in the real/active and sham group. The small dots represent individual participants and the narrow lines between the dots indicate the individual trajectories of tNAA concentration change for each patient. The larger dots illustrate group means and the wider lines an average change in tNAA.

Discussion

This study is the first to use ^{1}H MR spectroscopy to explore the effects of accelerated iTBS treatment on Glx concentrations and cellular integrity (tNAA/tCr) in TRD patients. The baseline comparison between TRD patients and controls revealed that, within the study sample, TRD patients exhibited lower Glx concentration in the left DLPFC compared to the controls. However, the observed difference did not reach statistical significance, indicating that it may not be generalizable beyond this specific sample. Notably, considering the lower boundary of the confidence interval, the true difference could be up to 2.5 times larger than what was observed in the current study sample. Considering solely the statistical significance of the finding, this result aligns with previous studies that also reported no significant differences between patients and controls, albeit focusing on different cortical regions (Gabbay et al., 2017). However, this finding is not in conflict with studies that have identified lower Glx concentrations specifically in the left DLPFC or other cortical regions of depressed patients

(Baeken, Lefaucheur, & Van Schuerbeek, 2017; Michael, Erfurth, et al., 2003; Chen et al., 2014; Luykx et al., 2012; Arnone et al., 2015; Moriguchi et al., 2019). The involvement of the left DLPFC in the pathophysiology and treatment of MDD is well-established (Niciu et al., 2014). Studies have consistently shown reduced left prefrontal activity in clinically depressed individuals (Bruder et al., 2017), which would align well with lower Glx levels in the left DLPFC of TRD patients.

In the study sample, a slight decrease in Glx levels was observed in the real TMS group compared to the sham group in the pre- to post-treatment measurements. It is important to note, however, that the model may have been influenced by abnormally low post-treatment Glx concentrations in specific patients, which may have been inaccurately estimated due to poor quality of the MRS data. Yet, upon comparing the median Glx values before and after treatment in the real TMS group (refer to Figure 4) the change observed in the study sample also appears to be inclined towards a decrease. Regardless, the change observed in the sample did not reach statistical significance. Many other studies have reported no significant changes in glutamate or Glx concentration following antidepressant, ECT or TMS treatment (Godlewska et al., 2015; Baeken et al, 2017, Bhattacharyya, 2021), although some have indicated increases in concentration levels (Chen et al., 2014; Michael et al., 2003; Croarkin et al., 2016).

A significant negative association was observed between the percentage change in BDI scores from pre- to post-treatment and the corresponding change in Glx concentration: as clinical depression scores decreased, Glx concentration increased. In other words, there is a concurrent improvement in clinical symptoms and increase in Glx levels. This association was consistent for both the real and sham treatment groups. Interestingly, no significant association was found between the percentage change in HDRS scores and the change in Glx concentration. This difference in association may be attributed to the distinct nature of the BDI and HDRS scales. It is worth noting that the BDI relies on self-report from individuals experiencing the symptoms, while the HDRS involves a clinician's assessment based on an interview. The HDRS provides a more comprehensive and standardized evaluation of a wider range of depressive symptoms, including the clinician's observations of the patient's behaviour and affect during the interview. Notably, a previous study by Baeken and colleagues (2017) reported a similar association between clinical improvement and increases in GABA concentration in the left DLPFC.

Finally, a significant interaction effect was observed between the real TMS and sham groups in the measurements taken before and after the treatment. The real TMS group displayed a slight decrease in tNAA concentration, while the sham group showed an increase. Considering that

NAA is commonly regarded as a marker of neuronal and axonal integrity, caution must be exercised when interpreting these results, prioritizing patient safety. Decreased concentration or the absence of the NAA peak indicates neuronal degradation and loss (Bertholdo et al., 2013, Bustillo, 2013). However, even in patients with the lowest posttreatment tNAA concentration, the peak is far from absent from the MRS spectrum. The clinical significance of the small decrease identified through regression analysis remains unclear. Furthermore, it should be noted that the MRS spectra corresponding to the lowest tNAA values also exhibit low quality, which may introduce confounding influences.

One of the key limitations of this study is the variability in the quality of the acquired MRS scans. Yet, determining the threshold for excluding data based on its quality is a challenging task. Relying on arbitrary cut-offs derived from available quality metrics or subjective visual inspection can introduce bias in the selection of datasets to keep or discard. While the consensus among methods for clinical MR acknowledges the difficulty in establishing universal guidelines for acceptable MRS data quality due to diverse metabolite profiles and acquisition protocols, a suggested cutoff value of 0.1 ppm for spectral linewidth has been proposed (Wilson et al., 2019). However, applying this cutoff to the dataset in this study would have resulted in the loss of 15% of the acquired spectra. Therefore, a decision was made to include the entire dataset in the statistical analysis, knowing that this approach introduces noise and may obscure patterns. Further investigation is warranted to address the data quality issues and perform the same analyses on higher quality data.

Another limitation of this study is the relatively small sample size. While the overall sample size is substantial, the division of patients into the real TMS and sham groups results in approximately 20 participants per group. This sample size may not be sufficient to detect small effects as statistically significant. Larger sample sizes are generally needed to enhance the ability to detect subtle effects, improve the generalizability of findings, and increase the reliability of the results. It is worth noting, however, that many neuroimaging studies have considerably smaller sample sizes.

One of the major advantages of the current ^{1}H MR spectroscopy study is the utilization of neuronavigated accelerated intermittent theta burst stimulation in a well-defined sample of treatment-resistant antidepressant-free depression patients, particularly considering the potential interference of concomitant antidepressant use on the study results (Godlewska et al., 2015).

Conclusions

This master's thesis examined the effects of accelerated intermittent theta burst stimulation (aiTBS), a form of repetitive transcranial stimulation (rTMS), on brain metabolites in individuals with treatment-resistant depression (TRD). The primary focus of the study was to investigate the impact of aiTBS on the glutamatergic system, while also exploring its potential effects on neuronal integrity. In vivo measurements of neurometabolite concentrations were obtained using proton magnetic resonance spectroscopy, with a specific emphasis on assessing metabolites in the left dorsolateral prefrontal cortex (DLPFC) region.

In addition to individuals with TRD, this study also included a control group consisting of never-depressed healthy individuals. The study was designed as a sham-controlled trial. Statistical analysis did not reveal any significant differences in glutamate (Glx) concentration in the left DLPFC between TRD patients and healthy controls. Similarly, no substantial changes in Glx concentration were observed as a result of the TMS treatment. However, a noteworthy finding was the association between the change in clinical depression scores and the corresponding change in Glx concentration, suggesting that an increase in Glx concentration accompanied clinical improvement. Regarding the combined measure of N-acetylaspartate and N-acetylaspartylglutamate (tNAA), differential changes were observed in patients receiving real TMS treatment compared to the sham group. Although the reduction in tNAA concentration after real TMS treatment was minimal, it warrants careful investigation as a decrease in NAA is commonly associated with neuronal damage. Given the variable quality of the MRS data in this study, future analyses should be conducted using high-quality datasets. While the results of this study are not definitive, they represent a significant step towards comprehending the impact of aiTBS on the glutamatergic system and elucidating the mechanisms underlying the beneficial effects of TMS in the treatment of depression.

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