Association between Levels of Cortical Excitation/Inhibition and Clinical Response to Theta Burst Stimulation in Individuals with Major Depressive Disorder

by

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Abstract

Introduction. Theta burst stimulation (TBS), a form of repetitive transcranial magnetic stimulation (TMS), is an effective treatment for major depressive disorder (MDD). TBS is thought to modulate cortical excitation and inhibition, which are thought to be implicated in the pathophysiology of MDD. This study assesses excitation/inhibition levels and investigates their potential link with TBS response. Methods. Thirty-seven MDD participants and thirteen healthy controls were recruited. TBS treatment was administered five days/week over 4-6 weeks. At baseline, a magnetic resonance spectroscopy (MRS) scan of the anterior cingulate cortex and TMS to the left motor cortex were performed to probe excitation/inhibition levels. The primary outcome measure was the 17-item Hamilton Rating Scale for Depression (HRSD-17) score. Results. Cortical excitation was lower in MDD participants than in healthy controls, and baseline levels were linked to improvements in mood symptoms. Conclusion. Our results suggest that baseline cortical excitation could help predict TBS therapeutic response.
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1. Introduction

1.1 Major Depressive Disorder: Definition, Pathophysiology, and Treatments

Major depressive disorder (MDD) is one of the most prevalent mental health disorders, affecting 1 in 7 individuals before the age of 24, and approximately 300 million globally (WHO, 2017). MDD is the leading cause of disability in adults between the ages of 15 and 44 (WHO, 2017). It is also associated with a high death rate, with a 1.61 risk score or hazard ratio (HR) when compared with healthy population, along with a very high suicide rate (Pratt et al., 2016). In the last several years, depression rates have increased significantly (Santomauro et al., 2021), amplified by the COVID-19 pandemic (Dubey et al., 2020). According to the Canadian Survey on COVID-19 and Mental Health (SCMH) data from Fall 2020, it was found that 15.2% of the Canadian population had symptoms consistent with MDD (Shields et al., 2021). The prevalence of MDD from the SCMH, based on data from eight Canadian provinces, was 9.6 percentage points higher than the prevalence in the Canadian Community Health Survey (CCHS) -Annual Component from 2015 to 2019- which was conducted before COVID-19 (16.3% vs. 6.7%) (Shields et al., 2021).

MDD is a mental health condition associated with mood dysregulation, cognitive impairment, sleep difficulties, changes in appetite, impairment of social functioning and suicidality. More specifically, the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) characterizes MDD by five or more of the following symptoms: 1) depressed mood, 2) loss of interest or pleasure, 3) change in weight or appetite, 4) insomnia or hypersomnia, 5) psychomotor agitation or retardation, 6) loss of energy or fatigue, 7) worthlessness or guilt, 8) impaired concentration or indecisiveness, 9) thoughts
of death or suicidal ideation or suicide attempt. These symptoms must present during the same 2-week period, must include (1) depressed mood or (2) loss of interest and pleasure, and represent a significant change in functioning. Based on the number and intensity of symptoms, a depressive episode may be categorized as mild, moderate, or severe (DSM-V, 2013). It can also be categorized as a unipolar condition, such as persistent depressive disorder, or a bipolar condition, if associated with manic episodes such as bipolar affective disorder (DSM-V, 2013). Moreover, there is often a correlation between depression and anxiety symptoms (Silverstone & Von Studnitz, 2003). Evidence shows that treatment response is decreased when mental health disorders are combined (Andreasen et al., 2007).

The pathophysiology of MDD is complex and has yet to be fully understood. Neurotransmitter dysfunction, such as reduced serotonin, dopamine, and norepinephrine activity, plays an essential role in the pathophysiology of MDD. This catecholamine dysfunction is known as the monoamine hypothesis and forms the basis of many antidepressants (Hirschfeld, 2000). In addition, it has been shown in a growing number of studies that there is also an alteration in the functioning of inhibitory gamma-aminobutyric acid (GABA) and excitatory glutamate neurons in MDD (Duman et al., 2019). Regarding brain-affected regions in MDD, recent advances in neuroimaging technologies have allowed for the detection of specific brain structures and network alternations. For example, studies have shown the implication of the prefrontal cortex (including the medial, dorsolateral, ventromedial and orbitofrontal portions), the anterior cingulate cortex (ACC), the amygdala, the hippocampus, the insula, the default mode network and the salience network in MDD (Fonseka et al., 2017; Luykx et al., 2012).
Depression may be efficiently treated with antidepressants; however, it can take substantial effort and time (4-8 weeks) to find the correct molecule and dose (Ontario, 2016). Psychotherapy and medication are the initial therapies for MDD; nevertheless, approximately 1/3 of patients are resistant to such treatments. As such, this group faces a disproportionate share of the illness burden, highlighting the significance of development in this domain (Ionescu et al., 2015). Patients who do not respond to antidepressants are categorized as having treatment-resistant depression (TRD). The exact definition of TRD varies, ranging from a failure to respond to one adequate antidepressant trial to a failure to improve clinical symptoms when two distinct pharmacological therapy attempts fail (Gaynes et al., 2020). TRD is thought to be responsible for almost $64 billion of the overall cost of depression (Ionescu et al., 2015). An investigation of antidepressant responsiveness revealed that as many as 34% of individuals with MDD were treatment-resistant, while another 15% improved partially to conventional dosages of antidepressants for 6 weeks or longer (Fava & Davidson, 1996).

Furthermore, recent research found that despite specialist psychiatric treatment, the recurrence risk of MDD 60% after 5 years of remission and 85% over 15 years (Hardeveld et al., 2010). Alternatively, electroconvulsive therapy is also a recognized treatment for MDD. However, it can involve serious side effects like memory impairment (Fitzgibbon et al., 2020). As such, more efficient and acceptable alternative therapy approaches are required. Non-invasive brain stimulation methods, such as repetitive transcranial magnetic stimulation (rTMS), is one option that can improve MDD treatment.
1.2 rTMS as a Treatment for Depression

Cumulative research has shown that TMS can be used as an efficient antidepressant therapy (Godfrey et al., 2021). TMS is a non-invasive tool that creates a magnetic field that can induce an electric field in cortical tissue and stimulate the electrical activity of neurons, resulting in depolarization (Kobayashi & Pascual-Leone, 2003). At sufficiently high intensities, activation of cortical motor regions causes motor-evoked potentials (MEPs) in peripheral muscles controlled by these regions, which may be evaluated by electromyography (EMG) (Rogasch et al., 2014). A single TMS pulse may cause an initial, often transient impact on neural circuit function (lasting up to hundreds of milliseconds) (Allen et al., 2007). On the other hand, repetitive TMS (rTMS) has been shown to change the excitability of the activated region in the brain over time by repeatedly administering stimulation (Maeda et al., 2000b). This repetitive feature is therapeutically advantageous (Buchholtz, 2022), as it is thought to induce activity-dependent changes in the efficacy of synaptic connections between cortical neurons, indicating brain plasticity processes (Fitzgerald et al., 2006).

The impact of rTMS on cortical excitability may be excitatory or inhibitory based on the stimulation frequency (Pascual-Leone et al., 1998). While high-frequency rTMS (HF-rTMS; stimulus rates of 5 Hz or higher) induces an increase in cortical excitability (Maeda et al., 2000a), low-frequency rTMS (LF-rTMS; stimulus frequencies of 1 Hz or less) results in a long-lasting reduction in motor cortex excitability (Chen et al., 1997). Some early neuroimaging studies have shown that people with MDD may have increased cortical excitability in the right dorsolateral prefrontal cortex (DLPFC) and diminished
cortical excitability in the left DLPFC (see Kaskie & Ferrarelli, 2018 for review). These early studies led to the development of rTMS as a treatment for MDD.

1.2.1 Clinical Efficacy of rTMS

Over 15 years of studies have been undertaken on using rTMS for treating individuals with MDD (Fitzgerald & Daskalakis, 2011), with a considerable number of rTMS treatment studies in MDD focusing on the DLPFC (Daskalakis et al., 2008). In terms of targeting the DLPFC, the existence of interhemispheric asymmetry in prefrontal areas like the DLPFC in MDD has made numerous treatment strategies possible (Lefaucheur et al., 2008), using different parameters and protocols. Typical treatments with rTMS involve daily sessions for 4 to 6 weeks, lasting 30 to 45 minutes each day. rTMS, as delivered in treatment protocols and within safety limits, is widely regarded as safe, and very few adverse effects of substantial concern have been recorded despite the growth of clinical studies over the last decade (Rossi et al., 2021). Transient headaches and discomfort at the stimulation site are the most often reported adverse effects (Loo et al., 2008). In the treatment of depression, there are three typical rTMS protocols: HF-rTMS (5–20 Hz) over the left DLPFC and LF-rTMS (1 Hz) over the right DLPFC (George et al., 2000; Klein et al., 1999), and bilateral sequential rTMS stimulation of both DLPFC (LF on the right and HF on the left) (Lefaucheur et al., 2020). Based on recent guidelines, HF-rTMS of the left DLPFC was recommended as the most efficient, with a Level A recommendation (definite efficacy) (Lefaucheur et al., 2020). Furthermore, both LF-rTMS of the right DLPFC and bilateral sequential rTMS were given a Level B of evidence (probable effectiveness)
compared with placebo, although having less statistical power than HF-rTMS of the left DLPFC (Lefaucheur et al., 2020).

Given its recognized efficacy, rTMS is now considered as a first-line treatment for individuals with MDD. However, despite this, response rates (~ 45-55%) remain lower than other treatments, such as electroconvulsive therapy (~ 60-70%) (Fitzgibbon et al., 2020). Exploring rTMS-related stimulation methods that may provide more substantial brain effects may also be beneficial (Chung et al., 2015). In the quest for more effective methods of altering brain activity, investigators have investigated innovative applications of rTMS.

1.3 Theta Burst Stimulation as a New Form of rTMS

One significant advancement in the field of rTMS is the development of a novel paradigm known as theta burst stimulation (TBS) (Huang et al., 2005; Huang & Rothwell, 2004). TBS includes the application of pulses in bursts of three delivered at a high frequency (50 Hz) with an interburst time of 200 ms (5 Hz, which falls within the theta frequency spectrum) (Chung et al., 2015). Continuous (cTBS) and intermittent (iTBS) TBS patterns are two protocols that have opposing effects and are routinely used. In cTBS, 600 pulses (40s) are administered continuously, which decreases cortical excitability up to 1 hr beyond the stimulation period (Huang et al., 2005). In the iTBS protocol, 2 seconds of TBS trains (30 pulses) are performed every 10 seconds for 190 seconds, for a total of 600 pulses. iTBS induces facilitation on motor cortex excitability that persists for at least 15 minutes after stimulation (Huang et al., 2005). TBS protocols have the potential to produce similar effects on cortical excitability and plasticity than regular HF/LF rTMS protocols,
but for much shorter session durations, such as 3 minutes for an iTBS protocol compared with a 20 minutes rTMS session (Lefaucheur et al., 2020).

### 1.3.1 Clinical Efficacy of TBS

Soon after its development, clinical studies assessed the efficacy of TBS in the treatment of MDD. In 2017, Berlim and colleagues conducted a meta-analysis on five placebo-controlled clinical trials (total of 221 MDD participants) that assessed the clinical efficacy of TBS with either iTBS applied to the left DLPFC, cTBS applied to the right DLPFC or bilateral sequential TBS. It was shown that active TBS, as compared to sham TBS, was linked to a substantial decrease in depression symptoms in addition to greater response percentages after an average of 17 sessions (Berlim et al., 2017). When assessing each protocol individually, the meta-analysis showed that bilateral sequential TBS and iTBS applied to the left DLPFC yielded the highest response rates (Berlim et al., 2017). This established the clinical relevance of TBS as a novel treatment for MDD. This also opened the door to eventually replacing LF-rTMS with cTBS administered to the right DLPFC or HF-rTMS with iTBS supplied to the left DLPFC for depression treatment, given the significant reduction in treatment duration with TBS (Chung et al., 2015).

Blumberger and colleagues (2018) conducted the first randomized non-inferiority trial which compared iTBS to HF-rTMS (10 Hz), the standard rTMS treatment for MDD (Blumberger et al., 2018). In the study, 414 individuals were randomly assigned to both treatments. Results showed that iTBS was as effective as regular 10 Hz rTMS in reducing depression symptoms, as measured by changes in the Hamilton Rating Scale for Depression (HAM-D) score. In both clinician-rated and self-reported assessments, non-
inferiority was observed (Blumberger et al., 2018). The study led to the approval of iTBS by the US Food and Drug Administration (FDA) for treating TRD in 2018 (McCalley et al., 2021). In another study, Bulteau and colleagues (2022) conducted a randomized two-arm parallel design to compare the efficacy of iTBS and high-frequency rTMS on a separate sample of individuals with MDD (Bulteau et al., 2022). The Montgomery-Asberg Depression Rating Scale (MADRS) was used to measure response to treatment. Response rates in primary efficacy measures were 33.3% for 10 Hz rTMS and 36.7% for iTBS after missing data correction and were not substantially different between groups. Furthermore, remission rates were 14.8% for 10 Hz rTMS and 18.5% for iTBS, without any difference between groups. Consequently, this research shows that iTBS and 10 Hz rTMS have the same effectiveness in treating MDD, consistent with previous findings (Bulteau et al., 2022).

Since bilateral sequential TBS was also shown to be a promising treatment for MDD, Blumberger and colleagues (2022) conducted a randomized trial to compare the efficacy of bilateral sequential TBS and rTMS. They recruited 172 patients 60 years and older with TRD. Over 4-6 weeks, participants received 20-30 daily sessions. The MADRS score was used as the primary measure from baseline through the end of the treatment. No significant differences were obtained between bilateral rTMS and bilateral iTBS on the reduction of depression symptoms and response rates, suggesting comparable efficacy (Blumberger et al., 2022).

In addition to those large trials, there have been numerous studies since the meta-analysis published in 2017 that assessed the clinical efficacy of TBS in MDD. Li et al. (2018) undertook a neuroimaging study that employed a randomized, double-blind, sham-
controlled design to examine the distinct mechanisms of cTBS and iTBS in treating patients with TRD. The study recruited 56 TRD patients over two years. The findings indicated significant enhancements in the HRSD-17 scores of all active groups after two weeks of TBS treatment compared with the sham-controlled group (Li et al., 2018). Moreover, in an open-label trial, Dhami and colleagues (2019) enrolled a sample of twenty participants aged from 16 to 24 years old diagnosed with MDD. The intervention comprised ten therapy sessions for two weeks, which involved the application of iTBS and cTBS stimulation to the left and right DLPFC, respectively. The primary outcome measure showed a significant decrease in depressive symptoms after TBS treatment in the HRSD-17 scores (Dhami et al., 2019). iTBS shows potential as a novel therapeutic intervention; however, it does not appear to exhibit superior response rates compared to rTMS, contrary to initial assumptions.

1.3.2 Mechanisms of Action of TBS

TBS was developed based on animal studies exploring the induction of plasticity. During exploratory behaviours, the hippocampus of rats produces burst discharges at 4 and 7 Hz (theta range in electroencephalography) which are the basis for the original concept of TBS (Diamond et al., 1988). In the first application of TBS on human subjects, cTBS was used (Huang et al., 2005; Huang & Rothwell, 2004). The fact that cTBS decreased the MEPs amplitude for around 20 min was surprising since, in animal studies, TBS usually increased synaptic effectiveness, causing long-term potentiation (LTP) rather than long-term depression (LTD). However, some experts believe prolonged stimulation may cause LTD if prolonged for sufficient time (Heusler et al., 2000; Larson et al., 1986; Takita et al.,
Therefore, the TBS methodology was modified to provide repeated brief trains like those widely utilized in animal research to induce LTP.

Subsequently, iTBS promoted MEPs effectively (Huang et al., 2005). Although inter-individual variability has been reported in the literature, there is now substantial evidence that cTBS inhibits cortical excitability, while iTBS increases excitability via plasticity-like mechanisms (Huang et al., 2005, 2007; Huang & Rothwell, 2004). Specifically, it is hypothesized that iTBS induces LTP by influencing NMDA receptor-associated Ca2+ influx, while cTBS stimulates the interneural inhibition pathways, resulting in LTD (Huang et al., 2011). In addition, it has been suggested that the neuroplasticity induced by TBS is linked to changes in the balance between excitatory and inhibitory neurotransmission. Specifically, TBS has been shown to modify the activity and levels of GABA and glutamate (Spurny-Dworak et al., 2022; Stagg et al., 2009; Thickbroom, 2007; Vidal-Piñeiro et al., 2015). Interestingly, these neurotransmission systems have been shown to be altered in depression (Duman et al., 2019). In humans, two methods hold promise in assessing GABA and glutamate activity levels, i.e., specific TMS paradigms applied to the motor cortex and magnetic resonance spectroscopy (MRS).

Therefore, it is assumed that TBS targets brain plasticity mechanisms to reduce symptoms of depression by inducing plasticity-like mechanisms and changing the local balance in inhibition and excitation. This, in turn, creates a cascade of changes in interconnected regions, such as the fronto-cingular network, with a specific effect on the ACC that is key to the pathophysiology of MDD. In support of this, studies have shown iTBS-induced changes in GABA and glutamate levels in the ACC using MRS (Iwabuchi et al., 2017). However, the precise mechanisms by which GABA and glutamate contribute
to the neural and therapeutic after-effects of TBS still need to be fully understood, and further research is required to elucidate their roles in this process.

1.4 TMS as a Measure of Cortical Excitation and Inhibition

As mentioned before, alterations in cortical inhibitory pathways, particularly those linked with GABA neurotransmission, may be implicated in the pathophysiology of MDD (Kendell et al., 2005; Möhler, 2012). GABA is the brain’s principal inhibitory neurotransmitter and it is essential for modulating cortical excitability and neuroplasticity (DeFelipe et al., 1986; Schieber & Hibbard, 1993). Overall, the interplay between excitatory and inhibitory neuronal activity determines the degree of cortical excitability.

Glutamate is the principal excitatory neurotransmitter. The modulation of glutamate and GABA metabolism may significantly influence the regulation of cortical excitability (Petroff, 2002). Many lines of evidence suggest that MDD patients exhibit an imbalance between cortical excitability and inhibition (Gabbay et al., 2017; Sanacora et al., 2012), resulting in disturbed GABA/glutamate neurotransmitter balance that regulates brain excitability (Duman et al., 2019).

One method to assess this GABA/glutamate balance non-invasively in humans is via TMS. In addition to its use in the treatment of depression, TMS is recognized as a non-invasive method for investigating excitatory and inhibitory brain systems (Rossini et al., 2015), and has been widely employed to evaluate the excitability of the human motor cortex (Maeda & Pascual-Leone, 2003; Siebner & Rothwell, 2003). TMS, with the single and paired-pulse method, provides ideal spatiotemporal resolution to non-invasively investigate intracortical networks that sustain equilibrium in cortical excitation/inhibition.
Firstly, the single pulse method includes the MEP and resting motor threshold (RMT). The RMT reflects membrane excitability of cortical motor neurons, which is the minimum stimulus intensity necessary to induce a valid MEP in the target muscle across the motor cortex and is measured by EMG and using a figure-of-eight coil (Rossini et al., 2015). The MEP is a consequence of corticospinal tract activity and is measured and quantified using EMG, demonstrating cortical excitability (Rogasch et al., 2014). Surface electrodes are typically used in a bipolar belly-tendon arrangement to record MEPs over the target muscle (Rossini et al., 2015). Secondly, paired-pulse paradigms include intracortical facilitation (ICF), used to assess cortical excitability, and short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI), used to assess cortical inhibition. Paired pulse paradigms are assessed utilizing a conditioning stimulus (CS) followed by the test stimulus (TS) at different intervals (Kujirai et al., 1993). Typically, peak-to-peak MEP amplitudes are compared with those produced by the TS alone as a reference (baseline/control) (Rossini et al., 2015). Figure 1 depicts the typical EMG recording obtained from these measurements, including MEP, SICI, ICF and LICI.

ICF is assumed to reflect cortical excitability, mainly glutamate NMDA receptor-mediated function (Rogasch et al., 2014; Rossini et al., 2015). Most pharmacological research indicates that NMDA receptor antagonists, such as dextromethorphan and memantine, reduce intracortical facilitation (Ziemann et al., 2015). Comparing a suprathreshold TS with a paired-pulse subthreshold CS and suprathreshold TS at 10-15 ms intervals is required for ICF (Rogasch et al., 2014).
SICI is thought to be mediated by the fast ionotropic GABA-A receptors (Ziemann et al., 2015). The post-synaptic location of the ionotropic GABA-A receptor causes a rapid (2–20 ms) hyperpolarization of the postsynaptic neuron (Rogasch et al., 2014). SICI compares the MEP amplitude of a single suprathreshold TS to a paired-pulse condition with a subthreshold CS and a suprathreshold TS at 1–4-ms intervals (Rogasch et al., 2014). SICI is also upregulated in the presence of pharmacological agonists of the GABA-A receptor, such as lorazepam, further confirming a function for GABA (Di Lazzaro et al., 2000; Ziemann et al., 1996, 2015).

LICI is thought to be mediated by the slower metabotropic GABA-B receptors (McDonnell et al., 2006; Paulus et al., 2008). The GABA-B receptor is situated pre- and post-synaptically resulting in delayed (50-500 ms) hyperpolarization of neurons (Rogasch et al., 2014). LICI includes comparing a suprathreshold paired-pulse CS and TS at 50–200 ms intervals with a suprathreshold TS as a consequence of inhibiting the MEP generated by the test stimulus (Ziemann et al., 2015). Pharmacological studies have shown that administering baclofen (an agonist of GABA-B) modulates LICI amplitude, thus suggesting its link with GABA-B receptor activity (McDonnell et al., 2006). In addition, two other drugs, i.e. tiagabine and vigabatrin, have been shown to increase LICI, likely due to the activation of GABA-B in the synaptic cleft due to the increased availability of GABA (Pierantozzi et al., 2004; Werhahn et al., 1999).
Figure 1: TMS measurements of the motor cortex. A) Application of transcranial magnetic stimulation (TMS) to the left motor cortex. B) TMS travels across the corticospinal tract to reach the hand muscles. C) Two electrodes for measuring cortical activity are positioned on the hand muscles. D) Results in motor evoked potential (MEP). E) Short-interval intracortical inhibition (SICI) involves the MEP amplitude of a single test stimulus (TS) and paired-pulse condition with conditioning stimulus (CS) and TS at 2-ms intervals. (F) Long-interval cortical inhibition (LICI) involves paired-pulse CS and TS at 100-ms intervals. (G) Intracortical facilitation (ICF) involves single-pulse TS with paired-pulse CS and TS at 12-ms intervals. Original figure made by Nasim Kiaee with Biorender.com
1.4.1 TMS to Assess Excitation/Inhibition in MDD

According to many studies, motor cortex measures of cortical inhibition and excitation, such as SICI, LICI and ICF, are abnormal in MDD individuals (Kinjo et al., 2021; Radhu et al., 2013). Specifically, in terms of excitation levels, a meta-analysis conducted by Radhu and colleagues in 2013, which involved 115 MDD patients and 130 healthy controls, demonstrated no significant difference in ICF levels between the two groups (Radhu et al., 2013). However, a more recent meta-analysis by Kinjo and colleagues in 2021, comprising nine studies, revealed that individuals with MDD had higher ICF values compared with healthy controls (Kinjo et al., 2021). In contrast, in a study by Lefaucheur et al., in 2008, individuals with MDD exhibited decreased excitability as measured with ICF (Lefaucheur et al., 2008), suggesting conflicting results regarding the direction of the potential alteration of cortical excitability levels. The meta-analysis conducted by Radhu and colleagues in 2013 found no significant difference in RMT between individuals with MDD and the healthy control group (Radhu et al., 2013). However, one of the studies led by Levinson and colleagues in 2010, which was included in the meta-analysis by Radhu and colleagues, showed that RMT was notably higher in MDD participants compared to the healthy control group (Levinson et al., 2010). In support of this finding, Lefaucheur et al. (2008) also showed that the left RMT was higher in 35 participants with MDD compared to 35 healthy controls, suggesting reduced excitability in MDD patients.

Regarding SICI levels, both meta-analyses indicated a decrease in MDD patients compared to healthy controls (Kinjo et al., 2021; Radhu et al., 2013). For instance, in one of the studies in Radhu's meta-analysis, reduced SICI levels were observed in the left
hemisphere of TRD patients compared to the healthy control group at the baseline (Levinson et al., 2010). Bajbouj and their colleagues (2006) also showed that SICI was decreased in 20 individuals with MDD who were not taking any medications, compared to a healthy control group, compatible with the theory that GABAergic alterations are associated with MDD (Bajbouj et al., 2006). Decreased SICI was also observed in a study by Lefaucheur and colleagues in 2008 in the left brain region compared with healthy controls (Lefaucheur et al., 2008). Therefore, according to various publications, decreased SICI seems to be a prevalent sign of MDD. In terms of LICI, levels were not assessed in both meta-analyses due to limited studies. However, one study has shown that adolescents with MDD and suicidal behaviours exhibited LICI impairments compared to healthy controls and MDD adolescents without suicidal behaviours (Lewis et al., 2018). These findings were also observed in a recent study as it was found that SICI and LICI were considerably decreased in depression (Mehta et al., 2021), suggesting deficits in both ionotropic and metabotropic GABA receptors (Fee et al., 2017; Radhu et al., 2013).

However, it is crucial to note that contradictory results in the literature occur relating the SICI, LICI, and ICF measures in MDD. Furthermore, several limitations and dissimilar research approaches exist, including a limited number of participants, variations in participant age range, distinct ISIs employed for each measurement, and diverse assessments utilized for diagnosis. To our knowledge, no research has compared all three TMS measurements in MDD. To get a deeper understanding of the mechanisms underlying MDD and, consequently, to improve TBS protocols, a considerable amount of additional research is required to investigate variations of these measurements between MDD and healthy subjects.
1.5 Magnetic Resonance Spectroscopy as a Measure of GABA/glutamate

Magnetic resonance spectroscopy (MRS), especially spectral-edited MRS, is a technique that can measure the levels of neurotransmitters such as GABA and glutamate in vivo and is utilized to analyze the concentrations of these neurotransmitters (Ashwal et al., 2004; Edden et al., 2014; Frangou & Williams, 1996; Holshouser et al., 2006; Mullins et al., 2014; Ramadan et al., 2013). MRS exploits that protons respond somewhat differently to a magnetic field based on their chemical surroundings. This enables the differentiation of chemical components and the quantification of their quantities, even at low concentrations. The metabolite is assessed in a voxel-defined volume. The nuclear spin of particular nuclei is monitored in the voxel, and its manipulation produces a resonance at a specific frequency, which is indicative of a particular metabolite. The hydrogen proton (H1) is the most often detected nucleus in MRS; however, phosphorus (31P) may also be quantified (Provencher, 1993). The MRS technique makes it possible to investigate a wide variety of substances in vivo in a non-invasive manner. Point-resolved spectroscopy, also known as PRESS (Bottomley, 1987), and stimulated echo acquisition mode, also known as STEAM (Frahm et al., 1987), are the two primary approaches for 1H-MRS. The overlapping of metabolites that are only present at trace levels with other predominantly abundant chemicals is a challenge that might arise in MRS. This is the situation with GABA, which necessitates using a specialized method that enables the differentiation of GABA signals from the more dominant signals of other metabolites by utilizing the established couplings within the GABA molecule, named MEGA-PRESS (Mescher et al., 1998; Mullins et al., 2014).
The metabolite levels assessed via MRS can be presented in ratios or absolute concentrations. A reference signal is necessary to calculate MRS data in a ratio form (Hoch et al., 2017). This reference signal may be an internal one, such as the water signal obtained from an unsuppressed acquisition (Tong et al., 2004; Wang et al., 2014), or it could be as a ratio by another metabolite that appears in the spectrum, such as N-acetyl-aspartate (NAA), creatine (Cr) and choline (Cho), which are often utilized in clinical settings (Hoch et al., 2017).

In some clinical investigations, the quantification of glutamate concentration is represented as Glx, a combined value that includes glutamate and glutamine (Gln). Due to their comparable molecular structures, glutamate and Gln possess similar magnetic resonance spectra, leading to potential contamination of their spectral features by contributions of other substances in the brain, such as GABA, glutathione (GSH), and NAA, despite glutamate having a relatively high concentration. The use of Glx (glutamate+glutamine) has been proposed to prevent spectral misidentification of glutamate and Gln (Ramadan et al., 2013).

1.5.1 MRS to Measure GABA/glutamate Levels in MDD

Deficiencies in inhibitory and excitatory neurotransmitters have been identified in numerous mental health disorders (Egerton et al., 2017; Prévot & Sibille, 2021; Reddy-Thootkur et al., 2020; Savage et al., 2018; Schür et al., 2016). Although multiple regions have been assessed, a large body of literature has focused on the ACC, given its crucial involvement in the pathophysiology of MDD. Research on inhibitory and excitatory neurotransmissions in this area has had conflicting outcomes. One study examined 44
youth with MDD who were not using psychotropic medications and 36 participants who were healthy controls (12-21 years of age). There were no differences in Glx levels between both groups (Gabbay et al., 2017). However, several further studies did reveal lower Glx levels in the ACC of MDD patients (before therapy) compared with healthy controls (Chen et al., 2014; Lener et al., 2017; Luykx et al., 2012; Merkl et al., 2011; Njau et al., 2017; Zhang et al., 2013). In a recent meta-analysis examining 12 trials, Glx was evaluated in the ACC of 232 MDD patients and 226 healthy controls. Lower Glx levels in the ACC of MDD patients measured by MRS compared to healthy controls approached significance \((p = 0.05)\) (Godfrey et al., 2018).

Regarding GABA, the recent meta-analysis by Godfrey et al. (2018) indicated that levels in the ACC of MDD patients were significantly lower than in controls \((p = 0.004)\). They investigated GABA levels by MRS of the ACC in 118 MDD patients and 97 healthy participants across six trials (Godfrey et al., 2018). In other MRS investigations of patients with MDD and young depressed patients, GABA levels in the ACC were shown to be lower than in healthy participants (Bhagwagar et al., 2008; Gabbay et al., 2017; Price et al., 2009). On the other hand, Sanacora and colleagues' study in 2004 took a different approach by examining not only GABA and glutamate separately but also the GABA ratio to glutamate (Sanacora et al., 2004). They discovered that the GABA ratio to glutamate was higher in individuals with depression compared to healthy controls \((p = 0.001)\), which shows a potential alteration in the balance between GABA and glutamate levels in depressed patients, which may have implications for understanding the neurobiology of depression. These studies show that MRS is useful for assessing GABA and glutamate deficits in individuals with MDD.
These findings suggest that GABAergic pathways and glutamatergic function are involved in the pathology of depression (Sanacora et al., 2012). This is further supported by the antidepressant potential of medicines that affect glutamate neurotransmission, such as ketamine and riluzole (Sanacora et al., 2008; Zarate et al., 2006). Therefore, much more research needed to explore biomarkers involved in the pathophysiology of MDD to find more beneficial protocols and treatments for MDD patients to help them. To this date, no studies compare both motor cortex measurements and GABA/glutamate levels of the ACC in MDD patients with a healthy control group.

1.6 Predictors of Response to TBS

As mentioned earlier, despite promising findings, all MDD patients will not respond to brain stimulation. In a recent large iTBS trial, response rates of 49% and remission rates of 32% were reported, which seem consistent with the current literature (Blumberger et al., 2018). This is challenging because there is currently no way to predict who will respond to TBS treatment; thus, treatment selection is based on trial and error. This is why estimating who would likely respond to treatment is crucial. Therefore, clinicians will have more confidence in suggesting TBS to MDD patients by defining biological markers that can act as predictors.

Furthermore, it is essential to note that inter-individual variability seems to be connected to response to TBS, irrespective of stimulation site or population. For instance, when stimulating the motor cortex in healthy controls, it was shown that approximately 50% of participants show the expected modulation of brain activity following TBS (Corp et al., 2020; Hordacre et al., 2016). Nevertheless, it is essential to note that variability is
also observed for other types of antidepressant treatments. Approximately 35% of individuals who receive ECT do not respond to the treatment, and only about half will achieve remission (Rosenquist et al., 2018). Moreover, in terms of response to antidepressant drugs, as discussed earlier, less than 40% of patients achieve remission after their first effort at medication; therefore, pharmacological therapy for depression is often a process of trial and error (Rush, Trivedi, et al., 2006), and up to one-third of individuals getting pharmacological intervention seem to exhibit drug resistance (Rush, Kraemer, et al., 2006).

1.6.1 TMS to Study Predictors of Response

As discussed earlier, many studies have shown that cortex excitability and inhibition in MDD patients are disrupted, as assessed via TMS measures such as SICI, LICI and ICF. It is noteworthy that TBS can influence these values of the motor cortex (Huang et al., 2005; Jacobs et al., 2014; Suppa et al., 2008). Importantly, TMS measures are interesting potential biomarkers as TMS of the motor cortex must be conducted in all clinical studies to determine the motor threshold that will be used to select the intensity of TBS or rTMS treatment. As such, implementing TMS measures of cortex excitation/inhibition would be highly feasible in a clinical setting.

To our knowledge, only one study explored if motor cortex measurements such as SICI, LICI and ICF can predict response to rTMS treatment (Fitzgerald et al., 2004), and none explored this for TBS. In the study of Fitzgerald et al., in 2004, cortical excitability (ICF) and inhibition (SICI and cortical silent period) were assessed in MDD patients using TMS prior to a trial of rTMS of the DLPFC. Sixty individuals with TRD participated in
this research; 46 were medicated during the experiment (antidepressants, mood stabilizers and antipsychotics). The authors reported that increased inhibition in the left motor cortex, measured by the cortical silent period (CSP), predicted a worse response to rTMS therapy (Fitzgerald et al., 2004). Regarding predictor biomarkers to other antidepressant treatments, researchers have identified substantial differences in cortical inhibition between responders and non-responders to ECT treatment using TMS. According to a study conducted in 2016 by Voineskos and colleagues, baseline cortical inhibition of the motor cortex could predict response to ECT with a sensitivity of 80% and specificity of 60% in a sample of 25 TRD patients (Voineskos et al., 2016). Predictors of response to other antidepressant treatments were also studied by Sun and their colleagues in 2016 and 2018 (Sun et al., 2016, 2018). They found the predictor role of cortical inhibition for responding to magnetic seizure therapy (MST). The correlation between remission of suicidal ideation, as measured by the Beck Scale for Suicidal Ideation (SSI), and the degree of LICI in the frontal and central midline region, measured via electroencephalography, was significant. LICI in the frontal brain had a 90% sensitivity and 89% specificity for predicting remission of suicidal thoughts (Sun et al., 2016). Furthermore, they reported that a change in the DLPFC LICI accurately predicted the cessation of suicidal thoughts with 85.7% sensitivity and 100% specificity in a group of 20 TRD patients receiving MST (Sun et al., 2018). Altogether, these studies highlight the potential role of cortical inhibition in predicting response to TBS treatment.
1.6.2 MRS to Study Predictors of Response

In recent years, MRI has been increasingly used to help target rTMS and TBS treatment and has thus become routine in clinical settings. Implementing another MRI sequence, like MRS, would also be feasible to help predict treatment. To our knowledge, no studies have evaluated GABA/glutamate levels in the ACC as predictors of response to TBS or rTMS treatment in MDD patients. However, GABA and glutamate levels were assessed in other brain regions with MRS and their potential to predict rTMS antidepressant response. Through these studies, some findings indicated that glutamate levels, measured in the DLPFC, were lower at baseline in rTMS responders than non-responders (Luborzewski et al., 2007; Yang et al., 2014). Therefore, a lower glutamate level at baseline predicted better response to rTMS.

In contrast, Baeken et al. (2017) showed a trend of higher baseline Glx in the left DLPFC predicting response to rTMS (Baeken et al., 2017). Similarly, higher pre-treatment left DLPFC Glx predicted more considerable improvement after rTMS in another study (Bhattacharyya et al., 2021). On the other hand, in their naturalistic open-label study, Godfrey et al. (2021) indicated no significant correlations between baseline Glx measured from the left DLPFC and response to rTMS (Godfrey et al., 2021). Regarding GABA levels as a predictor of treatment response, previous studies have revealed that baseline GABA concentrations in the DLPFC do not predict clinical results substantially (Baeken et al., 2017; Bhattacharyya et al., 2021; Godfrey et al., 2021). With regards to the ratio of Glx to GABA, to our knowledge, no study to date assessed the relationship with rTMS treatment. However, one study evaluated if the ratio of Glx to GABA levels in the ACC at baseline
predicts response to antidepressant medication in MDD and found no significant relationship (Narayan et al., 2022).

As previously stated, there is a need to investigate further the role of ACC metabolic levels as a potential predictor of response to TBS. Using other imaging modalities, previous studies have shown the potential to predict antidepressant response using activity in the ACC. For example, a previous study showed that pregenual ACC function during emotional stimulation is a predictor of ketamine's antidepressant effect. In particular, higher pgACC activity during the presentation of emotional cues in one task was linked with a better clinical response 24 hours after ketamine treatment (Weigand et al., 2022). Several studies have utilized fMRI to explore the predictive role of ACC in MDD patients. One study investigated 330 patients with TRD and used two protocols, comprising 10 Hz rTMS or iTBS, focusing on functional connectivity as a predictor of response. Results indicated that treatment response could be predicted by lower functional connectivity from the left DLPFC to the left ACC (Dunlop et al., 2017). Similarly, Ning et al. (2022) showed that treatment response to rTMS could be predicted by measuring the dorsal ACC and lateral PFC connectivity at baseline (Ning et al., 2022). These studies highlight the need to explore further how the ACC can help predict treatment response to TBS.

1.7 Rationale for this Study

It is challenging to unravel the underlying pathophysiology of depression and its therapy due to the variability of depression's characteristic symptoms (Moriguchi et al., 2019). At present, the precise neural mechanisms underlying MDD in the brain remain unclear. However, as has been previously discussed, using TMS and MRS to compare
cortical excitability and inhibition levels between individuals with MDD and healthy controls has the potential to shed light on the pathophysiology of depression, thereby informing the development of more effective treatment protocols. To date, no studies have simultaneously evaluated these two biomarkers in MDD patients and compared the results to those of a healthy control group.

TBS has demonstrated positive outcomes in treating MDD patients. This can be potentially attributed to its effects on specific neurotransmitters, such as glutamate and GABA, and motor cortex inhibition/excitation levels, including SICI, LICI, and ICF, which play a significant role in the pathophysiology of MDD. Additionally, the role of the ACC in depression has been established. By identifying predictors of response to TBS treatment in MDD patients, specifically the levels of GABA and glutamate in the ACC and cortex inhibition/excitation, baseline biomarkers can be established for determining which individuals are more likely to respond to TBS treatment. No studies to date have evaluated the combined use of motor cortex measurements and GABA/glutamate levels in the ACC as predictors of response to TBS treatment.

2. Objectives and Hypotheses

The main objective of this study is to identify neurobiological predictors of TBS response using data from an ongoing naturalistic, open-label, randomized investigation. This research will employ MRS to determine baseline GABA and glutamate concentrations in the ACC. In addition, we will use TMS to investigate baseline cortical inhibition and excitation in MDD patients. To further investigate the pathophysiology of MDD, we will also compare these neurobiological markers between healthy controls and MDD patients.
Objective 1. We aim to compare baseline levels of GABA, glutamate and GABA/Glx in the ACC and baseline amounts of SICI, LICI, ICF, and RMT between healthy controls and MDD patients. Hypothesis 1.1 We hypothesize that GABA and glutamate levels in the ACC will be lower in individuals with MDD than healthy controls (Bhagwagar et al., 2008; Chen et al., 2014; Gabbay et al., 2017; Lener et al., 2017; Luykx et al., 2012; Price et al., 2009). Since few studies investigated GABA/Glx ratios, the comparison is exploratory. Hypothesis 1.2 We hypothesize that baseline levels of SICI and LICI in the motor cortex in MDD will be reduced in individuals with MDD compared to healthy controls (Bajbouj et al., 2006; Fitzgerald et al., 2004; Levinson et al., 2010; Radhu et al., 2013). Hypothesis 1.3 Despite some conflicting findings, we hypothesize lower baseline levels of ICF and higher baseline levels of RMT by MRS levels of glutamate, in individuals with MDD compared with healthy controls (Lefaucheur et al., 2008).

Objective 2. As a second objective, we will investigate if the baseline levels of GABA, glutamate and GABA/Glx in the ACC and motor cortex inhibition and excitation are linked to the therapeutic response, that is, the reduction of symptoms post-acute TBS treatment in comparison with baseline scores. The primary outcome will be the scores on the 17-item Hamilton Rating Scale for Depression (HRSD-17). In contrast, secondary outcomes will be the Montgomery-Asberg Depression Rating Scale (MADRS), 16-item Quick Inventory of Depressive Symptoms-Self Report (QIDS-SR 16), Beck Anxiety Inventory (BAI), Beck Scale for Suicidal Ideation (BSS), and sub-clusters items in HRSD-17 including mood, anxiety, insomnia and somatic symptoms (Kaster et al., 2023). Hypothesis 2.1 We hypothesize that the ACC’s baseline levels of glutamate and GABA
will significantly correlate with symptom improvements following TBS. Based on previous studies, we expect that higher levels of glutamate in the ACC (Baeken et al., 2017; Bhattacharyya et al., 2021) and higher levels of GABA in the ACC (Bhagwagar et al., 2008; Gabbay et al., 2017; Price et al., 2009) will correlate with the magnitude of change in HRSD-17 scores and of change in scores for secondary outcomes. Since few studies investigated GABA/Glx ratios, the correlation is exploratory. **Hypothesis 2.2** We predict that baseline levels of cortical inhibition will significantly correlate with symptom improvements following TBS. Based on previous studies (Bajbouj et al., 2006; Fitzgerald et al., 2004; Levinson et al., 2010), we expect that higher levels of SICI and LICI will correlate with the magnitude of change in HRSD-17 scores and scores of secondary outcome measures. **Hypothesis 2.3** We predict that baseline levels of cortical excitation, such as ICF and RMT, will correlate with the magnitude of change in HRSD-17 scores and potentially with scores of secondary outcome measures. Due to the absence of previous studies examining this relationship, the direction of the correlation is unknown.

**Objective 3.** Lastly, we will investigate if levels of GABA, glutamate, GABA/Glx, and motor cortex inhibition and excitation differ between responders and non-responders to TBS treatment and if they can predict the response (i.e., responders versus non-responders). **Hypothesis 3.1** We hypothesize that GABA and glutamate in the ACC will be higher in responders than non-responders and will predict response to treatment (Baeken et al., 2017; Bhattacharyya et al., 2021). Since few studies investigated GABA/Glx ratios, the objective is exploratory. **Hypothesis 3.2** We hypothesize that SICI and LICI baseline levels will predict response to treatment and that levels will be higher in responders than
non-responders (Sun et al., 2016). **Hypothesis 3.3** The objective is exploratory due to the limited number of studies examining RMT and ICF in relation to response.

3. Methodology

3.1 Participants

The MDD sample included 37 individuals with a predominant diagnosis of major depressive episode who have been enrolled in a naturalistic trial comparing the efficacy of uni- and bi-lateral TBS in the treatment of MDD (NCT04142996) at the Royal Institute of Mental Health Research (affiliated with the University of Ottawa) (IMHR; REB # 2019017). For the control group, 13 healthy volunteers were recruited as part of an ongoing project comparing the impact of unilateral and bilateral TBS on prefrontal activity (REB # 2018033). They were recruited through advertisements at the Royal Ottawa Mental Health Centre, medical clinics in the Ottawa and Gatineau area and web-based media.

For inclusion in the study, MDD participants must fulfill all the following criteria: 1) voluntary and competent to consent to study, 2) female or male aged 18 years old or older, 3) can speak and read English and/or French, 4) primary and/or predominant diagnosis of MDE without psychotic features (confirmed by a Mini-International Neuropsychiatric Interview), 5) depressive symptoms had not improved after ≥ 1 adequate dose of antidepressant trial in the current depressive episode, 6) moderate symptoms in the current depressive episode as indexed by a score of at least 13 (in this current sample) at the HRSD-17, 7) had been referred to rTMS treatment by their treating physician, and took a free and informed decision to follow this treatment, 8) were able to adhere to treatment schedule, 9) had received a stable medication (including prescribed cannabis) or psychotherapy regiment for at least four weeks prior to entering the trial, 10) had an
education-adjusted score of \( \geq 24 \) at the Mini-Mental State Evaluation (MMSE) if are aged \( \geq 65 \). MDD Participants were not be eligible to take part in this research if they: 1) had current or past (< 3 months) substance (excluding caffeine or nicotine) or alcohol abuse/dependence, as defined in DSM-5 criteria, 2) had current use of illegal substances or cannabis (unless medical use), 3) had a concomitant major unstable medical or neurologic illness (e.g. uncontrolled diabetes or renal dysfunction), 4) had a known organic cause to the depressive symptoms (e.g. thyroid dysfunctions), as ruled out by the referring physician, 5) had acute suicidality or threat to life from self-neglect, 6) were pregnant or breastfeeding, or thinking of becoming pregnant during the course of treatment (pregnancy was assessed by a urine test), 7) had a specific contraindication for TMS, such as a history of epilepsy or seizure, traumatic brain injury, metallic head implant, pacemaker, 8) were unwilling to maintain the current antidepressant regiment for the four weeks prior to and for the duration of the study 9) had failed a course of ECT within the current depressive episode (due to the lower likelihood of response to rTMS), 10) had any other condition that, in the opinion of the investigators, would adversely affect the participant’s ability to complete the study. As this is a naturalistic context, we accepted persons with co-morbid mental health disorders if the major depressive episode was the primary and/or predominant diagnosis. In addition, individuals were excluded if they were reluctant to continue their existing antidepressant protocol or taking more than 1 mg of lorazepam or a comparable drug.

For the healthy control group, participants were included in the study if they: 1) were voluntarily and competent to consent to the study, 2) were male or female, 3) were aged between 18 and 65, 4) could speak and read English. They were excluded from the
study if they: 1) had a lifetime history of psychiatric, mental health, and/or neurologic disorder, 2) had a substance or alcohol abuse/dependence in the past six months, 3) had a significant neurodevelopmental, cardiovascular, renal, hepatic, hematological, gastrointestinal, pulmonary, or metabolic-endocrine disorder, 4) were pregnant or breastfeeding, 5) had a specific contraindication for TMS (e.g. personal history of epilepsy or convulsion, metallic head implant, pacemaker), 6) had used illegal substances of abuse in the past month as assessed with interviews and individuals who had a positive urine test for illegal substances and non-prescribed drugs, 7) had any other condition that in the opinion of the principal investigator could create a hazard to the participant safety, endanger the study procedures or interfere with the interpretation of study results, 8) were currently taking any benzodiazepines or psychoactive drugs.

3.2 Procedure - MDD Component of the Study

The participants’ treating physician filled out a referral form and forwarded it to the study team through dedicated fax or email. Once received, a research team member performed a short phone interview to determine eligibility. A research team member collected demographic data from participants (i.e., age, sex, highest level of education, ethnicity, maternal language, employment and marital status, household information, and medication use). If deemed eligible at the initial screening, participants underwent a baseline clinical baseline assessment to further determine eligibility and a consultation with one of the psychiatrists on the rTMS clinical platform team. Participants underwent an MRI of the whole brain (including 1H-MRS) and a TMS session for motor threshold assessments and cortical excitability/inhibition measures if eligible.
Eligible participants were randomly assigned to receive either bilateral sequential or unilateral TBS in a 1:1 ratio using the study randomizer software (https://www.studyrandomizer.com). A permutated block was used to randomize participants into the treatment groups. Participant stratification was based on the following variables: 1) age (< 65 or ≥ 65 years old), 2) depression (unipolar or bipolar), 3) comorbid generalized anxiety (yes or no), and 4) biological sex (male or female). Upon entering these participant characteristics, the software provided a number (e.g., 0 or 1) corresponding to a treatment group of which experimenters and participants were unaware. During the first four weeks, the TBS therapy was provided five days weekly (Monday through Friday). Participants who did not reach remission (i.e., non-remitters) after the initial treatment phase as per the HRSD-17 (a score of ≤ 7) underwent two additional weeks of therapy (a total six weeks of complete treatment). Participants who did not respond to therapy (over 50% reduction in pre-treatment HRSD-17 score) after two further weeks of treatment were dismissed from the trial, and the study psychiatrist made treatment recommendations to their referring physician. Participants who demonstrated remission at week 4 or response/remission at week 6 underwent a six-month maintenance phase in which the frequency of treatment was reduced, depending on their symptomatology. During the first four weeks of this phase, participants were contacted (by phone or video call) once weekly for clinical outcomes, and TBS therapy was provided twice weekly. In the subsequent eight weeks (weeks 9-16), treatment frequency was reduced further, and the HRSD-17 score, which was assessed at various times remotely, determined the number of treatment sessions for that week; participants may receive 0, 1, or 2 treatment sessions based on their Hamilton score. Of note, the maintenance phase was not further detailed as only data from the
treatment phase (weeks 4 to 6) was included in this study. The study timeline is outlined in Figure 2.

Figure 2: Study design and timeline of assessments and treatment schedule.

### 3.2.1 TBS Treatment

Treatment was delivered at the IMHR’s Neuromodulation Research Clinic. The TBS settings used in this investigation were in accordance with previously stated recommendations (Rossi et al., 2021). TBS consisted of 50-Hz triple-pulse bursts delivered at a frequency of 5 Hz utilizing a MagPro X100-MagOption stimulator device (Magventure, Farum, Denmark). iTBS was sent using a B70 figure-of-eight cooled-coil, whereas cTBS was transmitted by an active/sham B65 figure-of-eight cooled-coil (Magventure, Farum, Denmark). Unilateral TBS comprised one active train of excitatory-inducing iTBS over the left DLPFC for 190 seconds, followed by 40 seconds of sham cTBS over the right DLPFC. Bilateral sequential TBS included one active train of inhibitory-inducing cTBS administered over the right DLPFC for 40 seconds, followed by one active
train of excitatory-inducing iTBS applied over the left DLPFC for 190 seconds. The stimulation intensity for both iTBS and cTBS was 80% of the AMT as determined by single-pulse TMS. MRI-assisted neuronavigation enabled accurate coil placement and DLPFC target selection for each participant. A Brainsight stereotaxic neuronavigation system (Rogue Research Inc., Montreal) was used to ensure stable coil positioning. An optical camera was used to register the participant’s position in space by placing a small headband containing an optical tracer. Anatomical landmarks were identified on the participant’s scalp to fit the head morphology with the anatomical MRI obtained at baseline. The TMS coil was tilted 45 degrees from the midline centered against the location of the left and right DLPFC (MNI-152 stereotactic coordinates: x=-38, y=44, z=26) (Blumberger et al., 2018).

Both participants and researchers conducting interviews and TBS treatment were unaware of the treatment conditions. All participants received bilateral sequential TBS, but in the unilateral group, the stimulation on the right side was a placebo. In contrast, the stimulation on the right side was active in the bilateral group. All participants received 4 minutes of daily TBS. The B65-type stimulation coil used in the study has two sides: active stimulation and sham stimulation. An electronic sensor recorded the orientation of the coil. To keep the technicians unaware of the treatment, the patient's ID code was entered into the device, which then selected the active or sham coil for stimulation. The electronic sensor in the coil checked the orientation and only started the treatment if it matched the patient's ID. The intensity of stimulation was set by hand by trained personnel based on baseline values. Both the active and sham stimulation produced identical auditory and sensory stimulation, and the sham stimulation had a built-in electrical stimulator to
reproduce the sensation of actual stimulation. The sham stimulation made the same sound as the actual stimulation. Figure 3 illustrates coil positioning and a pattern for cTBS and iTBS.

Figure 3: Inhibitory and excitatory stimulation of TBS. The inhibitory-inducing continuous theta burst stimulation (cTBS) in the right DLPFC and the excitatory-inducing intermittent theta burst stimulation (iTBS) in the left DLPFC. Original image made by Arthur. R. Chaves with Autodesk, Sketchbook.

3.2.2 Clinical Assessments

Clinical assessments were conducted at several time points during the treatment phase. See Table 1 for measures and procedure timeline included in the current study and Appendix 1 for all supplementary measures. The assessments were either self-reports or clinical interviews performed by a trained study staff member. At baseline, weeks 2, 4 and 6, a full clinical assessment was conducted, including a consultation with a study psychiatrist. Self-report questionnaires were filled out weekly to record symptoms. The
following standardized questionnaires or scales were selected to provide a comprehensive understanding of depressive-related symptoms and served as treatment response outcomes.

**16-item Quick Inventory of Depressive Symptoms-Self-report (QIDS-SR-16).** The QIDS-SR-16 is a self-report measure designed to evaluate the severity of depression symptoms during the past week. There are 16 questions that measure nine symptom domains: depressed mood, concentration, self-criticism, suicidal thoughts, interest, energy/fatigue, sleep difficulties, decrease/increase in appetite/weight, and psychomotor agitation/delay. The score varies from 0 to 27, with lower scores indicating less severe depression (Rush et al., 2003). For secondary analyses, response to treatment was defined as a ≥ 50% reduction in pre-treatment symptoms severity as measured by the mean QIDS-SR$_{16}$ score after 4 and/or 6 weeks of treatment. Remission was defined as a QIDS-SR score ≤ 6 after 4 and/or 6 weeks of treatment.

**Mini International Neuropsychiatric Interview for DMS-5 (MINI, Version 7.0.0).** The MINI is a structured diagnostic interview conducted at the baseline with a scoring “yes” or “no”, providing a tool for assessing the presence of various psychological disorders based on the DSM-V (DSM-V, 2013; Lecrubier et al., 1997). This clinical evaluation aimed to verify the occurrence of Major Depressive Episodes in the study participant during the baseline clinical interview. If the participant did not exhibit symptoms of this disorder, they were excluded from further participation in the study. Additionally, the assessment facilitated the examination of any concurrent disorders, such as anxiety disorder, post-traumatic stress disorder, or other related conditions.

**Hamilton Rating Scale for Depression 17-item (HRSD-17).** HRSD-17 is a structured clinician-administered questionnaire to rate the severity and frequency of
depression, which has 17 items using Likert scales on a 3-point Likert-type scale (0 = No difficulty; 2 = Significant difficulties) or 5 points (0 = Absent; 2= Moderate; 4 = Severe). The score range is between 0-53 (0-7, no depression; 8-13, mild depression; 14-18, moderate depression; 19-22, severe depression; ≥ 23, very severe depression). It is possible to evaluate 17 features of depression using this measurement scale, including mood, sleep, agitation, suicidal ideation, etc. (Snaith, 1977). Response to treatment was primarily defined as a ≥ 50% reduction in pre-treatment symptom severity as measured by the HRSD-17 score after 4 and 6 weeks of treatment. Treatment remission was defined as an HRSD-17 score ≤ 7 after 4 and/or 6 weeks of treatment. These definitions of treatment response and remission are based on recent literature assessing the effects of rTMS in individuals with depression (Kennedy et al., 2009; Lefaucheur et al., 2020; Milev et al., 2016; Rossini et al., 2015). For our study, we considered participants as responders to treatment who experienced a reduction of 50% or more in the baseline symptoms severity as our primary outcome.

Montgomery–Asberg Depression Rating Scale (MADRS). MADRS is a structured clinician-administered questionnaire with 10 items utilizing the Likert scale to rate the severity of depression alongside the HRSD-17. The score range in MADRS is between 0-60, with 0-6 representing no symptoms, 7-19 indicating mild depression, 20-34 indicating moderate depression, and 35-60 demonstrating severe depression. For secondary analyses, response to treatment was defined as a ≥ 50% reduction in pre-treatment symptoms severity as measured by the mean MADRS score after 4 and/or 6 weeks of treatment. Remission was defined as a MADRS score ≤ 12 after 4 and/or 6 weeks of treatment.
Beck Anxiety Inventory (BAI). BAI is a self-report questionnaire used to evaluate the extent of anxiety symptoms experienced by an individual over the past seven days. It comprises 21 items rated on a 4-point Likert scale, ranging from 0 (Not at all) to 3 (A lot), designed to measure nervousness, difficulty relaxing, and fear of losing control, among other indicators of anxiety (Beck et al., 1988). This self-administered questionnaire was administered throughout the study, including the initial evaluation, after every five treatment sessions, and at the end of the fourth and sixth weeks of treatment.

Beck Scale for Suicidal Ideation (BSS). BSS is a self-report questionnaire that enables the assessment and quantification of suicidal thoughts experienced over the last seven days. This clinical measurement instrument consists of a self-reported questionnaire comprising 19 items rated on a 2-point Likert scale, with response options varying based on the evaluated domains (Beck, 1986). The BSS was administered regularly throughout the study, including at the initial evaluation, after every five treatment sessions, and after the fourth and sixth weeks of treatment.

Furthermore, to conduct an analysis examining the correlation between baseline all the measurements and changes in sub-cluster symptoms assessed by HRSD-17, we computed the total scores for specific sub-scales using the following question numbers: 1, 2, 3, 7, 8, and 9 for the mood sub-cluster; 7, 8, 10, 11, and 15 for the anxiety sub-scale; 4, 5, and 6 for insomnia symptoms; and 12, 13, 14, and 16 for somatic sub-cluster symptoms (Kaster et al., 2023). The scores for each sub-scale were obtained by summing the individual scores of the respective questions. Assessments are detailed in Table 1.
**Table 1. Timeline of Clinical Assessments – Randomized Treatment**

<table>
<thead>
<tr>
<th>Clinical Assessment or Procedure</th>
<th>Screening visit</th>
<th>First session</th>
<th>Each session</th>
<th>End of week 1 and 5</th>
<th>End of week 2</th>
<th>End of Week 3</th>
<th>End of week 4 and 6</th>
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<td></td>
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<td></td>
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<tr>
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<tr>
<td>HRSD-17</td>
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<td></td>
<td>X</td>
<td>X</td>
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<tr>
<td>MADRS</td>
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<tr>
<td><strong>During treatment</strong></td>
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<td></td>
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</tr>
<tr>
<td>Administered by trained RA/graduate student</td>
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<td>QIDS-SR16</td>
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<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>BAI</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td></td>
</tr>
<tr>
<td>BSS</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
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<tr>
<td>MRI</td>
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<td></td>
</tr>
<tr>
<td>Physician consultation</td>
<td>X(^1) As needed</td>
<td>As needed</td>
<td>As needed</td>
<td>As needed</td>
<td>As needed</td>
<td>As needed</td>
<td>X</td>
</tr>
</tbody>
</table>

MINI = Mini International Neuropsychiatric Interview  
MADRS = Montgomery–Asberg Depression Rating Scale  
HRSD-17 = Hamilton Rating Scale for Depression 17-item  
QIDS-SR16 = 16-item Quick Inventory of Depressive Symptoms – self report  
BAI = Beck Anxiety Inventory  
BSS = Beck Scale for Suicidal Ideation  
MRI = Magnetic Resonance Imaging

\(^1\) Physician consultation will be after the initial telephone screening, and before the actual screening visit. It can also be planned as needed during the course of the study

### 3.2.3 MRI Acquisition

A single MRI session was performed at baseline. The 45-minute session was conducted at the Royal’s Brain Imaging Centre. The MRI scan was performed using a 3T Siemens mMR Integrated Whole-Body PET/MR Scanner and 32-channel head coil (Ceresensa Inc.). First, a T1-weighted 3D magnetization prepared rapid acquisition gradient echo (MEMPRAGE) scan was acquired (TR = 2500ms, TE1 = 1.69ms, TE2 = 3.55ms, TE3 = 5.41ms, TE4 = 7.27ms, flip angle = 7°, voxels = 1 x 1 x 1 mm; 1 mm slice thickness; 176 slices; 256mm field of view [FOV], 5.48 min). Individual anatomical images were utilized to target TBS to the DLPFC. This scan was followed by two runs of
BOLD-EPI (TR = 2300ms, 27 mm3 isotropic voxel-size, 183 volumes/run, 8 minutes per run) to collect resting state images, which was not used in the current project.

Lastly, a single voxel 1H-MRS scan was collected using the GABA-edited MEGA-PRESS sequence (Mescher et al., 1996, 1998) with the following parameters: TR = 2000ms, TE = 68ms, acquisition bandwidth = 2000 Hz, pulse placement edit-on/edit-off = 1.9/7.5 ppm, number of excitations edit-on/edit-off = 64/64 for a total of 128 averages. The voxel was positioned over the bilateral ACC (35x20x20 mm) using the T1 images and according to previous literature (Narayan et al., 2022). The voxel of interest (VOI) was situated above the anterior half of the corpus callosum. The inferior boundary of the VOI was aligned with the anterior half of the corpus callosum. Additionally, the anterior edge should be congruent with the tangent to the knee of the corpus callosum. The VOI was required to be positioned at the midpoint of the cingulate gyrus (Wu et al., 2022). Two MEGA-PRESS acquisitions edited with GABA were acquired (4.36min each), and two water-unsuppressed reference scans (0.48min each). Figure 4 demonstrates the voxel placement (above) and a sample of 1H-MRS spectra (below).
Figure 4: MRS voxel placement over ACC and example spectra of Glx and GABA peaks. The panel was obtained from the anterior cingulate cortex (ACC), with location displayed in sagittal (left), axial (centre), and coronal (right) pictures (above). A sample 1H-MRS of the difference spectra shows peaks of Glx and GABA in the ACC, fitted by LCModel (below).

3.2.4 TMS Assessment

**Thresholds and Neuronavigation.** Participant MRI scans were imported into the neuronavigation software (Brainsight, Rogue Research Inc, Montreal, QC) to facilitate visualization of the primary motor cortex and guide coil placement during the TMS session.
(i.e., neuronavigated MRI-assisted TMS). To elicit MEPs in the contralateral first dorsal interosseous (FDI) muscle, a MagPro X100-MagOption Stimulator with a 70 mm figure-of-eight coil (MagVenture, Denmark) was used for single-pulse TMS. Biphasic pulses were utilized to identify the treatment’s active thresholds, while monophasic pulses were used to identify resting thresholds for TMS biomarkers. The neuronavigation-integrated electromyography (EMG) system was used to gather MEPs (Brainsight, Rogue Research). MEPs were collected using surface electrodes (Kendall, Cardinal Health, Waukegan, USA) positioned over the belly of the contralateral FDI muscles. The reference and ground electrodes were put in the index finger's ipsilateral interphalangeal joint and on any prominent ipsilateral bone (e.g., olecranon, medial/lateral epicondyle, styloid process), respectively.

First, suprathreshold stimulations were administered systematically at several scalp regions across the subjects' left and right motor areas using a pre-defined grid in the neuronavigation software. The stimulated site with the highest averaged peak-to-peak MEP amplitude was classified as the FDI hotspot and was the location of the next TMS stimulations/experiments. The relative frequency estimate approach was then used to calculate the individuals' resting and active motor thresholds (RMT and AMT, respectively) (for detailed protocol, see; (Rossini et al., 2015)). In summary, RMT and AMT were respectively determined by the least amount of stimulation intensity required to elicit five out of ten 50µV amplitude MEPs during total relaxation and 200µV amplitude MEPs when subjects performed 10% of their maximum pinch contraction. RMT and AMT were used to normalize subsequent studies between subjects and to personalize the stimulation intensity for the TBS treatment (e.g., percentage of RMT and AMT).
TMS biomarkers indexing corticospinal excitation and inhibition, such as MEP amplitudes, ICF and short- and long-intracortical inhibition (SICI, LICI, respectively), were collected. Baseline MEP amplitudes were collected with single-pulse TMS at a stimulation intensity of 130% RMT. ICF, SICI and LICI were assessed with paired-pulse TMS, where the first conditioning stimulus precedes a test stimulus separated by an interstimulus interval (ISI). For ICF, the conditioning stimulus, delivered at 80% of RMT, was followed by a test stimulus at 130% of RMT, with 12 milliseconds of ISI. For SICI, the conditioning stimulus, delivered at 80% of RMT, was followed by a test stimulus, delivered at 130% RMT, at an ISI of 2 milliseconds. For LICI, both the conditioning and test stimulus were delivered at 130% of the RMT, and the ISI was 100 milliseconds. 20 pulses were delivered every 5-7 seconds for each of the four measurements while EMG responses were recorded.

3.3 Procedure - Healthy Control Component of the Study

Each participant underwent a phone interview (approximately 20 minutes), one virtual session (approximately 30 min), and 4 in-person testing sessions lasting 1 to 3 hours. The total duration of sessions for healthy participants was approximately 7.5 hours over three to four weeks. The first session included filling out informed consent, administering questionnaires to rule out depressive symptoms (Beck Depression Inventory, BDI) and a clinical interview (MINI) to rule out any current or past mental health disorders. For the second session, an MRI was conducted at the Royal’s Brain Imaging Centre using the same protocol as in the MDD component of the study. For the third session, motor threshold assessments and cortical excitability/inhibition measures were conducted again using the
same protocol as the MDD component of the study. Moreover, the fourth and fifth sessions included electroencephalography (EEG) recordings pre and post one uni- and bi-lateral TBS session. In the current project, only data from sessions 1 to 3 were used.

3.4 Analyses

3.4.1 1H-MRS Processing and Metabolite Quantification

Post-processing of MRS signals was conducted using Matlab scripts (MathWorks, USA) and LCModel (Provencher, 1993). The LCModel software was used to quantify the concentrations of GABA and Glx in the anterior cingulate cortex (ACC). Using the LCModel, two approaches, namely "EDIT OFF" and subtraction spectra, were examined to determine the best linear fit between the observed spectrum and a combination of model spectra (Provencher, 1993). This process was used to estimate the optimal combination of model spectra for accurate quantification of GABA and Glx concentrations in the ACC.

GABA and glutamate values were expressed as a ratio of water (a reference peak collected concurrently to minimize inter-subject variability). Individual resonances having Cramer-Rao Lower Bounds (CRLB) greater than 20% were omitted from further investigation. Moreover, data was excluded from spectra that exhibited noticeable artifacts, such as large head motions, lipid contamination (prominent peak near 1 ppm) and poorly shimmed data. Post-processing included phase correction and frequency drift correction of the individual sub-spectra utilizing residual water as a reference, followed by the average of the phase- and frequency-corrected spectra. The fitting was conducted in the spectral range of 1.0 to 4.2 ppm. A residual water signal fluctuation was used to identify motion retrospectively (Bhattacharyya et al., 2013). The final edited spectrum was obtained by
subtracting the OFF-resonance spectrum from the ON-resonance spectrum. The signal peak at 3 ppm was identified as the GABA peak. The 3.8 ppm doublet peak was identified as the Glx peak (Godfrey, 2021). GABA levels were obtained from difference spectra, while Glx levels were obtained from spectra acquired with the "EDIT OFF" technique.

### 3.4.2 TMS Data Analysis

Brainsight was used to visually inspect all TMS data for noise, abnormal responses, and TMS artifacts, as well as to ensure that stimulation was being performed at the proper location. Any response peak that contained any of the previously mentioned errors was excluded. The amplitude of the EMG signals was exported from Brainsight to Excel. For each TMS sample, a background check for prior muscle contractions was conducted in Excel. Samples were excluded if muscle contraction greater than 3 SD from the mean was quantified prior to the TMS pulse. This was conducted as contractions can alter EMG signals by lowering the threshold for inducing EMG activity (Rossini et al., 2015).

Peak-to-peak MEP amplitudes (µV) were averaged across all trials for each TMS measure. Using SICI and ICF, the mean conditioned MEP amplitude (i.e., SICI or ICF) was divided by the mean unconditioned MEP amplitude (single-pulse TMS) to quantify cortical inhibition and excitation, respectively. To quantify cortical inhibition with LICI, a ratio of the mean conditioned to the mean unconditioned MEP amplitude between the two LICI pulses was computed (i.e., LICI pulse 2/LICI pulse 1). Ratios greater than one represented facilitation, and ratios less than one represented inhibition (Rossini et al., 2015).
3.4.3 Statistical Analysis

All analyses were conducted on the open-source R statistical software Version 4.2.1 (R core Team, Vienna, Austria, and the R Studio packages rstatix and car; Kassambara, 2023). Statistical procedures were two-tailed, with a significance set at < 0.05. It is important to note that all statistical analyses were conducted while blinded to the TBS condition that is unilateral or bilateral sequential. As such, clinical responses were recorded for both conditions together. Additionally, any extreme outliers below -3 or above 3 SD were removed from the TMS and MRS data. Normality was assessed for each baseline TMS and MRS measurement while computing one-way ANOVAs and ANCOVA using the Shapiro-Wilk test. When data distribution did not reach normality, scores were converted to their natural logarithm.

3.4.3.1 Objective One: Comparison Baseline Levels of GABA, Glutamate and GABA/Glx in the ACC and Baseline Amounts of SICI, LICI, ICF and RMT between Healthy Controls and MDD Patients

One-way analyses of covariance (ANCOVA) were computed to compare the baseline levels of GABA, glutamate and GABA/Glx in the ACC, as well as the baseline measures of SICI, LICI, ICF and RMT between individuals with MDD and healthy control participants. The Age variable was considered as a covariate to control for its potential influence on comparing the two groups.
3.4.3.2 Objective Two: Investigating Associations Between Baseline Levels of GABA, Glutamate and GABA/Glx in the ACC and Motor Cortex Inhibition/Excitation with Therapeutic Response to TBS in MDD Patients

Relationships between baseline cortical inhibition/excitation levels and clinical outcome measures were investigated using Pearson’s correlation coefficients. Specifically, correlations were computed between GABA, glutamate and GABA/Glx levels assessed with MRS and motor cortex TMS measurements, and the magnitude of change in the clinical outcomes was computed as a subtraction of the post-treatment score (week 4 for remitters or week 6 for other participants) from the pre-treatment score. The relationships were examined with HRSD-17 change in the total score as the primary outcome. For secondary outcomes, the MADRS, QIDS-SR-16, BAI and BSS change in total scores were used, as well as the four HRDS-17 sub-clusters defined by Kaster and collaborators (2023) to assess possible relationships with specific depressive symptoms categories. Bonferroni corrections were applied for multiple comparisons.

3.4.3.3 Objective Three: Investigating Prediction of Baseline GABA, Glutamate and GABA/Glx Levels, and Motor Cortex Inhibition/Excitation for Response to Treatment and Difference between Responders and Non-Responders to TBS Treatment

A logistic regression model was computed to identify potential predictors if a significant correlation was found with the primary outcome measure. Additionally, if a possible prediction was identified, receiving operating characteristic curves (ROC) were performed to predict response to treatment (e.g., “responders” and “non-responders” and
HRSD-17) with the neurobiological outcomes (MRS, TMS) entered as predicting variables, and to plot the sensitivity and specificity of predictive variables.

To evaluate possible differences between responders and non-responders, GABA/glutamate levels in the ACC and TMS baseline measurements, such that SICI, LICI and ICF, were compared between both groups using one-way ANOVAs.

4. Results

4.1 Demographic Information

The study included a total of 37 participants in the clinical group, comprising 17 females and 20 males, with an average age of 47 (SD = 14.71). Some participants were excluded from undergoing MRI due to either experiencing claustrophobia or being unable to tolerate the conditions within the MRI session. Nevertheless, all individuals who did TBS sessions completed the motor cortex measurement session. Therefore, for the TMS analysis, 36 MDD participants were included, and for the MRS analysis, 31 MDD participants were included. The healthy control group consisted of 13 individuals, 7 females and 6 males, with an average age of 28 (SD = 12.17). Detailed demographic information is included in Table 2. In the clinical sample, while all participants were in a predominant major depressive episode, 33 (89%) were diagnosed with MDD, and 4 (11%) had bipolar disorder. The most frequent psychiatric comorbidity was generalized anxiety disorder.

Additionally, 30% of MDD participants had no comorbid mental health disorders. During TBS treatment, all MDD participants continued to use medication. However, their medication regimens remained unchanged for 4 weeks before initiating TBS treatment. Most participants (72%) in the study were taking antidepressant medications. In addition
to antidepressant medications, other participants in the study were also prescribed low doses of antipsychotic and atypical antipsychotic medications, mood stabilizers, stimulants, and benzodiazepines.

Of all participants who completed TBS treatment, 22 participants (69%) were classified as responders to TBS treatment, meaning they showed a reduction of at least 50%, either of 4 weeks or 6 weeks treatment period, in their pre-treatment HRSD-17 score. On the other hand, 11 participants (31%) were classified as non-responders, as they did not meet the criteria of a ≥ 50% reduction in their pre-treatment HRSD-17 score. See Table 3 for mean scores of all assessments before and after TBS treatment. Furthermore, Table 4 provides information regarding the therapeutic response to treatment among the participants.

Table 2

Demographic, Diagnosis and Medical Information for Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N (MDD Group)</th>
<th>N (Healthy Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological Sex</td>
<td></td>
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</tr>
<tr>
<td>Female</td>
<td>17 (46%)</td>
<td>7 (53.5%)</td>
</tr>
<tr>
<td>Male</td>
<td>20 (54%)</td>
<td>6 (46.5%)</td>
</tr>
<tr>
<td>Mean Age (range in years)</td>
<td>47 (21-77)</td>
<td>28 (19-45)</td>
</tr>
<tr>
<td>Primary Diagnosis</td>
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<tr>
<td>MDD</td>
<td>33 (89%)</td>
<td>-</td>
</tr>
<tr>
<td>BP</td>
<td>4 (11%)</td>
<td>-</td>
</tr>
<tr>
<td>Comorbid Diagnosis</td>
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<td></td>
</tr>
<tr>
<td>GAD</td>
<td>16 (21%)</td>
<td>-</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Count</td>
<td>Percentage</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------</td>
<td>------------</td>
</tr>
<tr>
<td>Panic Disorder</td>
<td>10</td>
<td>(13%)</td>
</tr>
<tr>
<td>Social Anxiety</td>
<td>10</td>
<td>(13%)</td>
</tr>
<tr>
<td>PTSD</td>
<td>8</td>
<td>(10.5%)</td>
</tr>
<tr>
<td>ADHD</td>
<td>7</td>
<td>(9%)</td>
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<tr>
<td>Other Diagnosis</td>
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<td>(19.5%)</td>
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<tr>
<td>Agoraphobia</td>
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<td>(47%)</td>
</tr>
<tr>
<td>OCD</td>
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<td>(33%)</td>
</tr>
<tr>
<td>Eating disorder</td>
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<td>(14%)</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>1</td>
<td>(6%)</td>
</tr>
<tr>
<td>NON</td>
<td>11</td>
<td>(14%)</td>
</tr>
</tbody>
</table>

**Medication Category**

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<thead>
<tr>
<th>Category</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antidepressants</td>
<td>31</td>
<td>(86%)</td>
</tr>
<tr>
<td>SNRI</td>
<td>14</td>
<td>(39%)</td>
</tr>
<tr>
<td>SSRI</td>
<td>13</td>
<td>(36%)</td>
</tr>
<tr>
<td>NDRI</td>
<td>6</td>
<td>(17%)</td>
</tr>
<tr>
<td>Serotonin Modulator</td>
<td>8</td>
<td>(22%)</td>
</tr>
<tr>
<td>Tetracyclic</td>
<td>2</td>
<td>(5.5%)</td>
</tr>
<tr>
<td>Tricyclic</td>
<td>4</td>
<td>(11%)</td>
</tr>
<tr>
<td>Atypical Antipsychotic</td>
<td>16</td>
<td>(44%)</td>
</tr>
<tr>
<td>Stimulants</td>
<td>5</td>
<td>(14%)</td>
</tr>
<tr>
<td>Mood Stabilizer</td>
<td>2</td>
<td>(5.5%)</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>5</td>
<td>(14%)</td>
</tr>
</tbody>
</table>

Table 3

Pre-post TBS Mean Scores for All Assessments

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>HRSD-17</th>
<th>MADRS</th>
<th>QIDS-SR</th>
<th>BAI</th>
<th>BSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-TBS</td>
<td>19.27 (4.25)</td>
<td>27.42 (7.00)</td>
<td>15.06 (4.40)</td>
<td>16.51 (12.17)</td>
<td>5.24 (6.84)</td>
<td></td>
</tr>
<tr>
<td>Post-TBS (Week 4 or 6)</td>
<td>8.96 (5.91)</td>
<td>12.57 (9.45)</td>
<td>7.96 (4.83)</td>
<td>6.90 (8.67)</td>
<td>2.5 (5.48)</td>
<td></td>
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<tr>
<td>Change Score (Post-Pre TBS)</td>
<td>-10.30 (5.51)</td>
<td>-14.84 (8.41)</td>
<td>-7.09 (5.63)</td>
<td>-9.60 (9.69)</td>
<td>-2.81 (5.51)</td>
<td></td>
</tr>
</tbody>
</table>

Note. SD = Standard Deviation. HRSD-17 = Hamilton Rating Scale for Depression 17-item. MADRS = Montgomery–Asberg Depression Rating Scale. QIDS-SR16 = 16-item Quick Inventory of Depressive Symptoms. BAI = Beck Anxiety Inventory. BSS = Beck Scale for Suicidal Ideation. TBS = Theta Burst Stimulation.
Table 4

Therapeutic Response to TBS

<table>
<thead>
<tr>
<th>Responders</th>
<th>Non-responders</th>
<th>Remitters</th>
<th>Non-remitters</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 (69%)</td>
<td>11 (31%)</td>
<td>17 (51.5%)</td>
<td>16 (48.5%)</td>
</tr>
</tbody>
</table>

Responders after 4 weeks

<table>
<thead>
<tr>
<th>9 (41%)</th>
</tr>
</thead>
</table>

Responders after 6 weeks

<table>
<thead>
<tr>
<th>13 (59%)</th>
</tr>
</thead>
</table>

Note. N = 33 participants completed TBS treatment. Responders = reduction in HRSD-17 ≥ 50%. Remitters = reduction in HRSD-17 ≥ 7. HRSD-17 = Hamilton Rating Scale for Depression 17-item. TBS = Theta Burst Stimulation.

4.2 Objective One: Comparison of Baseline Levels of GABA, Glutamate, GABA/Glx in the ACC and Baseline Amounts of SICI, LICI, ICF and RMT between Healthy Controls and MDD Patients

Firstly, one-way ANOVAs were conducted to compare all baseline measures of GABA, glutamate and the ratio of GABA to Glx (GABA/Glx) in the ACC between MDD and healthy controls, without controlling for age. The findings indicated a statistically significant difference in baseline levels of Glx ($F(1,39) = 14.14, p = 0.001, etasq = 0.266$) and GABA/Glx ($F(1,39) = 4.508, p = 0.04, etasq = 0.104$), between the group diagnosed with MDD and the healthy control group. However, no significant difference or trend was observed regarding GABA levels when comparing the MDD group to the healthy control group ($F(1,38) = 0.000127, p = 0.991, etasq = 0$). Subsequently, an ANCOVA analysis was then computed with age as a covariate, a variable that might impact the findings given the different age means between both groups, to account for its potential influence.
ANCOVA results revealed a trending significant difference in baseline Glx levels between MDD and healthy groups ($F(1,38) = 3.352$, $p = 0.075$, $etasq = 0.054$, $d = 0.78$). ANCOVA analysis revealed no other significant difference in baseline GABA levels ($F(1,37) = 0.411$, $p = 0.526$, $etasq = 0.011$, $d = 0.28$), and GABA/Glx levels ($F(1,38) = 0.402$, $p = 0.530$, $etasq = 0.008$, $d = -0.27$) between MDD and healthy groups.

Means, estimated means and standard deviations for baseline levels of GABA, glutamate and GABA/Glx in the ACC achieved through MRS in the MDD group and healthy control group are found in Table 5. Figure 5 and Figure 6 display boxplots and plots based on estimated means of trending and non-significant differences for baseline levels of GABA, glutamate and GABA/Glx between MDD and healthy controls.

**Table 5**

*Baseline Means and Estimated Means of GABA, Glx and GABA/Glx in the ACC*

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Estimated Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDD</td>
<td>HC</td>
</tr>
<tr>
<td>GABA</td>
<td>1.24 (0.171)</td>
<td>1.24 (0.087)</td>
</tr>
<tr>
<td>Glx</td>
<td>6.18 (0.415)</td>
<td>6.70 (0.334)</td>
</tr>
<tr>
<td>GABA/Glx</td>
<td>0.202 (0.03)</td>
<td>0.181 (0.017)</td>
</tr>
</tbody>
</table>
Figure 5: Boxplots of baseline Glx, GABA and GABA/Glx levels in the ACC. Box plots show the median (solid line), interquartile range (shaded region), and 95% confidence interval (whiskers) of MDD individuals and healthy controls for baseline A) Glx levels ($p = 0.001$), B) GABA levels ($p = 0.991$), and C) GABA/Glx levels ($p = 0.04$). ** $p < 0.05$. Glx = Glutamate + Glutamine. MDD = Major Depressive disorder. HC = Healthy Controls.
Figure 6: Plots of baseline Glx, GABA and GABA/Glx levels in the ACC. Plots displaying means adjusted for age (emmean, represented by the dot) and standard deviations (error bars) of MDD individuals and healthy controls for baseline A) Glx ($p = 0.079$), B) GABA ($p = 0.504$), and C) GABA/Glx ($p = 0.557$). * Shows trending differences. Glx = Glutamate + Glutamine. MDD = Major Depressive disorder. HC = Healthy Controls.

As mentioned, baseline scores of SICI, LICI and RMT were transformed to their natural logarithms for motor cortex measurements. The ANOVA analysis indicated no significant or trending difference for ICF ($F(1,43) = 1.821, p = 0.184, etasq = 0.041$), SICI
Subsequently, an ANCOVA analysis considering the age variable as a covariate was performed and the results revealed a significant difference in baseline ICF between MDD and healthy controls \((F(1,42) = 5.157, p = 0.028, eta^2 = 0.107, d = 0.91)\). There was no other significant or trending difference between two groups in terms of baseline SICI \((F(1,46) = 0.069, p = 0.794, eta^2 = 0.001, d = -0.1)\), LICI \((F(1,45) = 1.323, p = 0.256, eta^2 = 0.028, d = -0.45)\), and RMT \((F(1,45) = 0.200, p = 0.657, eta^2 = 0.004, d = -0.17)\) in ANCOVA.

Table 6 indicates means, means logarithms and estimated means for baseline ICF, SICI, LICI and RMT in depressed groups and healthy controls. See Figure 7 and 8 for boxplots and plots based on estimated means for significant and non-significant differences between MDD and healthy control groups.
Table 6

Means and Estimated Means for Baseline Motor Measures in MDD and HC

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) for ICF</th>
<th>Estimated Mean (SD) for ICF</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean Logarithm for SICI, LICI, RMT</td>
<td>Estimated Mean Logarithm for SICI, LICI, RMT</td>
</tr>
<tr>
<td>MDD</td>
<td>MDD</td>
<td>HC</td>
</tr>
<tr>
<td>ICF</td>
<td>1.2 (0.551)</td>
<td>1.47 (0.705)</td>
</tr>
<tr>
<td>SICI</td>
<td>-1.02 (0.794)</td>
<td>-1.39 (0.672)</td>
</tr>
<tr>
<td>LICI</td>
<td>-1.7 (1.28)</td>
<td>-1.85 (1.38)</td>
</tr>
<tr>
<td>RMT</td>
<td>3.94 (0.213)</td>
<td>3.86 (0.171)</td>
</tr>
</tbody>
</table>

NOTE. MDD = Major Depressive Disorder. HC = Healthy Controls. ICF = Intracortical Facilitation. SICI = Short-Interval Intracortical Inhibition. LICI = Long-Interval Intracortical Inhibition. RMT = Resting Motor Threshold.
**Figure 7**: Boxplots of baseline ICF, SICI, LICI and RMT. Box plots show the median (solid line), interquartile range (shaded region), and 95% confidence interval (whiskers) of MDD individuals and healthy controls for baseline A) ICF ($p = 0.184$), B) SICI ($p = 0.14$), C) LICI ($p = 0.721$), and D) RMT ($p = 0.247$). ICF = Intracortical Facilitation. SICI = Short-Interval Intracortical Inhibition. LICI = Long-Interval Intracortical Inhibition. RMT = Resting Motor Threshold. MDD = Major Depressive disorder. HC = Healthy Controls.
Figure 8: Plots of baseline ICF, SICI, LICI and RMT. Plots displaying means adjusted for age (emmean, represented by the dot) and standard deviations (error bars) of MDD individuals and healthy controls for baseline A) ICF ($p = 0.029$), B) SICI ($p = 0.806$), C) LICI ($p = 0.250$), and D) RMT ($p = 0.655$). ** shows a significant difference. ICF = Intracortical Facilitation. SICI = Short-Interval Intracortical Inhibition. LICI = Long-Interval Intracortical Inhibition. RMT = Resting Motor Threshold. MDD = Major Depressive disorder. HC = Healthy Controls.
4.3 Objective Two: Investigating Associations Between Baseline Levels of GABA, Glutamate and GABA/Glx in the ACC and Motor Cortex Inhibition/Excitation with Therapeutic Response to TBS in MDD Patients

Pearson correlations were employed to examine the associations between baseline levels of GABA, Glx, GABA/Glx, and changes in scores across various assessment questionnaires, including the HRSD-17, MADRS, QIDS-SR 16, BAI, and BSS. A Bonferroni correction was applied to account for multiple comparisons; thus, a \( p < 0.01 \) was considered significant. Regarding the primary outcomes of interest, no statistically significant or trending relationships between the baseline levels of GABA, Glx, GABA/Glx, and change in HRSD-17 scores were obtained (all \( p > 0.01 \)). However, concerning the secondary outcomes, a significant positive correlation was observed between the baseline levels of GABA and the change scores in the BAI (\( r = 0.61, p = 0.000705 \)). Furthermore, a significant positive correlation was found between the baseline level of GABA/Glx and the change in scores in the BAI (\( r = 0.53, p = 0.0048 \)). No other significant or trending associations were observed between the baseline levels of neurotransmitters and the change scores in all assessments.

Figure 9 displays scatter plots for the significant correlations and correlation matrix for all baseline levels of GABA, Glx, GABA/Glx and assessments score changes.
**Figure 9**: Correlation between baseline metabolites and change scores in all assessments. A) Correlation matrix for baseline levels of GABA, Glx, GABA/Glx and score changes in HRSD-17, MADRS, QIDS-SR16, BAI, and BSS. In the matrix, each square indicates correlation coefficients. Cross squares show non-significant correlations. Blue and orange colours are indicators of positive and negative correlations, respectively. B) Scatter plot for positive significant correlations between baseline level of GABA in the ACC and BAI change score. C) Scatter plot for significantly correlated between baseline levels of GABA/Glx in the ACC and BAI change score. Glx = Glutamate + Glutamine. HRSD-17 = Hamilton Rating Scale for Depression 17-item. MADRS = Montgomery–Asberg Depression Rating Scale. QIDS-SR16 = 16-item Quick Inventory of Depressive Symptoms. BAI = Beck Anxiety Inventory. BSS = Beck Scale for Suicidal Ideation.
Pearson correlation analyses were performed to examine the relationship between baseline levels of motor cortex inhibition and excitation, specifically ICF, SICI, LICI and RMT, and scores changes in all assessments. According to multiple comparisons, a Bonferroni correction was applied, leading to a significance level of \( p < 0.01 \) for determining statistical significance. The results indicated no statistically significant correlation between baseline ICF, SICI, LICI, RMT and the change in the main outcome measure, i.e., HRSD-17 scores (all \( p > 0.01 \)). However, for secondary outcome measures, negative trending relationships were observed between baseline LICI, and score change in BSS (\( r = -0.37, p = 0.039 \)), as well as baseline RMT and score change in BSS (\( r = -0.4, p = 0.023 \)). Figure 10 indicates trend correlations and correlation matrix graph for all measures and assessments.
Figure 10: Correlation between baseline motor measurements and change scores in all assessments. A) Correlation matrix graph for all baseline measurements in the motor cortex includes ICF, SICI, LICI and score changes in HRSD-17, MADRS, QIDS-SR 16, BAI, and BSS. In the matrix, each square indicates correlation coefficients. Cross squares show non-significant correlations. Blue and orange colours indicate positive and negative correlations, respectively. B) Scatter plot for a trending correlation between baseline LICI and change score in BSS. C) Scatter plot for a trending correlation between baseline RMT and change score in BSS. ICF = Intracortical Facilitation. SICI = Short-Interval Intracortical Inhibition. LICI = Long-Interval Intracortical Inhibition. HRSD-17 = Hamilton Rating Scale for Depression 17-item. MADRS = Montgomery–Asberg Depression Rating Scale. QIDS-SR16 = 16-item Quick Inventory of Depressive Symptoms. BAI = Beck Anxiety Inventory. BSS = Beck Scale for Suicidal Ideation.
Furthermore, we analyzed the associations among baseline measurements and change score in sub-clusters within the HRSD-17 scale, which include mood, anxiety, insomnia, and somatic symptoms (Kaster et al., 2023). This analysis was performed using Pearson correlation. Because of the exploratory nature of this analysis, a Bonferroni correction was not applied, and a significance level of $p < 0.05$ was used to determine statistical significance. The results of the Pearson correlation revealed a positive significant correlation between score change in mood sub-cluster of HRSD-17 and baseline Glx ($r = 0.4, p = 0.039$). No other significant correlation existed between baseline measures and score changes in sub-clusters (all $p > 0.05$). See Figure 11 for a significant correlation graph and matrix for all other measures and sub-cluster symptoms in HRSD-17.

![Correlation matrix and scatter plot](image)

**Figure 11:** Correlation between baseline metabolites and change scores in sub-clusters of HRSD-17. A) Correlation matrix graph for baseline levels of GABA, Glx, GABA/Glx, and score changes in sub-clusters of HRSD-17 includes anxiety, mood, insomnia, and somatic symptoms. In the matrix, each square indicates correlation coefficients. Cross squares show non-significant correlations. Blue and orange colours indicate positive and negative correlations, respectively. B) Scatter plot highlights the significant correlation between
baseline level of Glx in the ACC and change of score in the mood-sub-cluster. Glx = Glutamate + Glutamine. HRSD-17 = Hamilton Rating Scale for Depression 17-item.

4.4 Objective Three. Investigating Prediction of Baseline GABA and Glutamate Levels, and Motor Cortex Inhibition/Excitation for Response to Treatment and Difference between Responders and Non-Responders to TBS Treatment

Due to the absence of any observed correlation between the baseline measurements and the response to treatment as assessed by the HRSD-17, the regression analysis and ROC were not conducted to investigate the predictive value of these baseline measures on treatment response. A one-way ANOVA analysis was performed to explore potential differences between individuals who responded to the treatment and those who did not. The significance level was considered as p < 0.05. It is worth mentioning that, as previously stated, response to treatment in this study was defined as a reduction of 50% or more in the HRSD-17 scores. To maintain the assumption of normality in the ANOVA analysis, the baseline scores of SICI, LICI, and RMT were transformed into their natural logarithms.

Table 7 indicates the means and standard deviations of baseline GABA, Glx and GABA/Glx in the ACC in Responders and Non-responders to TBS treatment. ANOVA analysis revealed no significant or trend difference for all baseline GABA (\(F(1,25) = 0.217, p = 0.646, \text{ges} = 0.009\)), Glx (\(F(1,25) = 0.009, p = 0.924, \text{ges} = 0.000373\)), and GABA/Glx (\(F(1,25) = 0.202, p = 0.657, \text{ges} = 0.008\)) between responders and non-responders to therapeutic response to TBS.

Figure 12 shows the boxplots for comparing baseline levels of GABA, Glx and GABA/Glx in the ACC between responders and non-responders.
Table 7

Means and Standard Deviations of Baseline Measures for Responders and Non-responders

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Responders</td>
<td>Non-responders</td>
<td></td>
</tr>
<tr>
<td>GABA</td>
<td>1.243 (0.166)</td>
<td>1.273 (0.146)</td>
<td></td>
</tr>
<tr>
<td>Glx</td>
<td>6.196 (0.343)</td>
<td>6.215 (0.671)</td>
<td></td>
</tr>
<tr>
<td>GABA/Glx</td>
<td>0.201 (0.029)</td>
<td>0.206 (0.029)</td>
<td></td>
</tr>
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</table>

Figure 12: Boxplots of baseline metabolite levels in responders and non-responders. Significance level = p < 0.05
A) Boxplot of baseline GABA, no significant difference between responders and non-responders to therapeutic response to TBS treatment. B) Boxplot of baseline glutamate, no significant difference between responders and non-responders. C) Boxplot of baseline GABA/Glx, no significant difference between
responders and non-responders. Box plots show median (solid line), interquartile range (shaded region), and 95% confidence interval (whiskers). TBS = Theta Burst Stimulation.

Table 8 shows the means, mean logarithms, and standard deviations of baseline ICF, SICI, LICI and RMT in responders and non-responders. There was no significant or trend difference between responders and non-responders to TBS in terms of baseline ICF ($F(1,27) = 0.046, p = 0.831, ges = 0.002$), SICI ($F(1,30) = 0.319, p = 0.577, ges = 0.011$), LICI ($F(1,30) = 0.436, p = 0.514, ges = 0.014$), and RMT ($F(1,30) = 2.139, p = 0.154, ges = 0.067$).

See Figure 13 for boxplots of all baseline measures in responders and non-responders to therapeutic response to TBS treatment.

**Table 8**

*Mean, Mean logarithm and Standard Deviation of Baseline Motor measurements*

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Non-responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean for ICF (SD)</td>
<td>1.17 (0.495)</td>
<td>1.23 (0.791)</td>
</tr>
<tr>
<td>Mean Logarithm for SICI, LICI, RMT (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Responders</strong></td>
<td><strong>Non-responders</strong></td>
<td></td>
</tr>
<tr>
<td>ICF</td>
<td>-0.906 (0.804)</td>
<td>-1.08 (0.767)</td>
</tr>
<tr>
<td>SICI</td>
<td>-1.57 (1.31)</td>
<td>-1.90 (1.17)</td>
</tr>
<tr>
<td>LICI</td>
<td>3.975 (0.218)</td>
<td>3.861 (0.13)</td>
</tr>
</tbody>
</table>

*Note. ICF = Intracortical Facilitation. SICI = Short-interval Intracortical Inhibition. LICI = Long-interval Intracortical Inhibition. RMT = Resting Motor Threshold.*
Figure 13: Boxplots of baseline motor measurements in responders and non-responders. Significance Level $= p < 0.05$ A) Boxplot of baseline ICF, no significant difference between responders and non-responders to therapeutic response to TBS treatment. B) Boxplot of baseline SICI, no significant difference between responders and non-responders. C) Boxplot of baseline LICI, no significant difference between responders and non-responders. D) Boxplot of baseline RMT, no significant difference between responders and non-responders. Box plots show median (solid line), interquartile range (shaded region), and 95% confidence interval (whiskers). ICF = Intracortical Facilitation. SICI = Short-Interval Intracortical Inhibition. LICI = Long-Interval Intracortical Inhibition. RMT = Resting Motor Threshold. TBS = Theta Burst Stimulation.

5. Discussion

This current research investigates the association between baseline neurobiological markers and TBS therapeutic response in individuals diagnosed with MDD. This study also aims to identify potential predictive biomarkers associated with TBS response in
individuals with MDD and determine the potential difference between responders and non-responders to TBS treatment. Additionally, it aims to gain further insights into the underlying pathophysiological mechanisms of depression by comparing baseline biomarkers between the MDD group and a control group comprising healthy individuals. To accomplish these objectives, this study utilizes MRS to quantify the levels of GABA and glutamate in the ACC, while TMS is employed to assess motor cortical inhibition and excitation.

This research demonstrated a difference in the levels of glutamate in the ACC between individuals with MDD and the healthy control group. In addition, these two groups exhibited different levels of motor cortex excitation at baseline. Moreover, a significant relationship was obtained between the baseline levels of GABA and glutamate in the ACC, and the changes in scores on the BAI and subscales of mood in the HRSD-17, respectively, in patients with MDD. Further, there were significant associations observed between the baseline levels of cortical inhibition/excitation and changes in scores related to suicidal ideation among individuals with depression. However, the direction of the relationships varied depending on the specific measurement being considered.

5.1 Comparison of Baseline Biomarkers Between MDD and HC

In terms of MRS measures of excitability, our study indicated a trending difference in the baseline levels of Glx in the ACC between individuals with MDD and the healthy control group. Specifically, our results indicated that individuals diagnosed with MDD exhibit lower baseline levels of Glx in the ACC compared to the healthy control group. This finding suggests that impaired glutamate functioning may be one of the underlying
mechanisms of MDD. Our current finding aligns with previous research demonstrating a notably lower level of glutamate levels among individuals diagnosed with MDD. Specifically, this finding aligns with results from a recent meta-analysis examining findings from 12 studies in which Glx was evaluated in the ACC of 232 MDD patients and 226 healthy controls. Consistent with our findings, they reported a trend ($p = 0.05$) for lower levels of Glx in the ACC of MDD patients (Godfrey et al., 2018), suggestive of hypoexcitability. The concentrations of Glx measured by MRS reflect the global brain pool of glutamate and glutamine, predominantly reflecting intracellular levels (Erecińska & Silver, 1990; Lehmann et al., 1983). Our results thus indicate abnormally low intracellular pools of Glx. While this could be related to dysfunctions in glutamate signalling or excitation and inhibition balance in the ACC, previous authors have suggested that altered Glx levels may be linked to abnormal glial cell levels and function in the ACC of individuals with MDD (Cotter et al., 2002; Cotter et al., 2001; Hasler et al., 2007). However, future studies linking MRS measures with glial cell function are needed to elucidate this potential relationship.

Interestingly, some studies that showed altered levels of glutamate in the ACC did not report any abnormal levels in the prefrontal cortex, which indicates that the ACC may be particularly key to the pathophysiology of MDD (Chen et al., 2014; Luykx et al., 2012; Merkl et al., 2011). As such, our MRS finding provides additional support for the considerable involvement of this brain region in the underlying mechanisms of MDD. This is in line with research on structural and functional aspects, providing evidence of reduced activity and volume reduction in the ACC during depressive states (Drevets et al., 1997). Many functional brain imaging studies, including fMRI, single-photon emission
tomography (SPECT), and PET, indicate that among all the brain regions investigated, the ACC consistently exhibits functional differences in individuals with depression compared to control subjects (Fitzgerald et al., 2008). For instance, a structural imaging meta-analysis revealed that MDD patients display significant volume reductions, particularly in the ACC (Koolschijn et al., 2009). Nevertheless, it is essential to approach functional interpretations derived from MRS data cautiously because MRS does not directly measure neurotransmission or receptor activity but provides information about the relative concentrations of extracellular and intracellular metabolites within the region of interest (Stagg et al., 2011).

With regards to TMS measures of excitation, our results also indicated lower excitability, indexed with ICF levels, in MDD individuals compared with healthy controls and no difference in RMT levels. The ICF finding is in line with our hypothesis and our MRS findings, as well as results from the study by Lefaucheur et al. (2008), who observed a reduced level of ICF in 35 individuals with MDD compared to healthy controls (Lefaucheur et al., 2008). Lefaucheur and colleagues highlighted the idea that supports a link between major depression and hypoexcitability in the left frontal brain area. This includes both the inhibitory and excitatory pathways. Therefore, our finding suggests that cortical excitation is reduced in motor areas of individuals with MDD, which aligns with our previous results of lower levels of Glx. However, it is crucial to interpret the consistency between these two results cautiously due to the difference in the brain regions assessed. While glutamate levels were measured in the ACC, ICF is related to the motor cortex. Although the motor cortex is not considered to have a primary role in the pathophysiology of depression (Lefaucheur et al., 2008), numerous studies have
demonstrated that TMS measures, although applied to the motor cortex, are linked to both functionality and symptoms within and beyond motor regions (Rossini et al., 2015). Consequently, TMS evaluations are widely employed in research endeavours to comprehend the CNS-induced changes caused by different interventions. Moreover, they serve as valuable biomarkers for disease, symptom progression, and recovery in brains affected by lesions (Rossini et al., 2015).

ICF is thought to link cortical excitability and circuits mediated by glutamate NMDA receptors in M1 (Ziemann et al., 2015). Furthermore, ketamine, known as a glutamate NMDA-receptor antagonist, has shown promise in the treatment of TRD patients (McGirr et al., 2015), which supports that individuals diagnosed with depression may have notable reductions in the expression levels of specific NMDA-receptors subunits within the frontal cortex (Feyissa et al., 2009). Therefore, it is plausible that NMDA-receptors dysfunction is reflected by lower levels of ICF and that the fast-acting anti-depressive effect of ketamine may be linked to an increase of brain excitability (Sarawagi et al., 2021), which would counteract the hypoexcitability highlighted in this study by both ICF and Glx levels.

With regards to our MRS measures of GABAergic inhibition, our results did not show any trending or significant differences between individuals with MDD and the healthy control group, contrary to what we had expected based on our initial hypotheses and previous findings. Furthermore, in the MDD group, the GABA to Glx ratio in the ACC did not show any significant difference compared to the healthy control group in the current study. This finding contrasts with the results of a study by Sanacora and colleagues in 2004, which reported higher GABA/Glx ratio levels in patients with MDD compared to healthy
individuals (Sanacora et al., 2004). Our findings also contrast with results from a meta-analysis by Godfrey and colleagues (2018) that showed that baseline GABA levels in the ACC were lower in MDD participants in four studies \( (p = 0.004) \) (Gabbay et al., 2017; Gabbay et al., 2012; Price et al., 2009; Wang et al., 2016). Nevertheless, there were some differences between these studies and our research, particularly with regards to participants’ characteristics and study methodology. For example, in some studies, participants were not taking any medication for their condition (Gabbay et al., 2017; Price et al., 2009), or were post-menopausal women who had not been diagnosed with severe depression (Wang et al., 2016).

Additionally, the MRS method used to acquire the GABA-edited 1H-MRS data was a J-edited spin-echo method in the Gabbay study compared with MEGA-PRESS in ours (Gabbay et al., 2017), which could potentially influence the reported results for GABA levels in the ACC. Each method has strengths and limitations, and variations in the acquisition technique might lead to varying measurements and outcomes. Therefore, the disparity in MRS methodologies should be considered when interpreting and comparing the results from both studies.

Notably, certain studies have reported findings consistent with our research, showing no significant difference in baseline GABA levels in the ACC between individuals with MDD and the healthy control group (Walter et al., 2009). Instead, they found differences in glutamine levels within the same brain region. Even though it is important to mention that glutamine typically acts as a precursor to GABA, our study and Walter and colleagues' study (2009) may challenge the hypothesis of a direct correlation between GABA and glutamate levels. Even though our study and several other studies have
indicated no significant difference in baseline GABA levels between MDD and healthy control groups, it does not imply that GABA plays no role in the underlying mechanisms of depression. It may be attributed to other factors such as methodology, region of interest and heterogeneity of depression, and the small sample size of the healthy control group.

Regarding TMS measures of cortical inhibition levels (i.e., SICI and LICI), our study does not indicate any significant or trending differences between individuals with MDD and healthy controls. Although in line with our MRS findings, this result contradicts our initial hypotheses and previous research findings. For instance, results from two meta-analyses suggested reduced SICI levels (Kinjo et al., 2021; Radhu et al., 2013). However, studies included in these meta-analyses generally had a small sample size. They included high heterogeneity of the clinical groups in different research articles, such as variations in medication usage. These factors can introduce confounding variables that may influence the outcomes and make direct comparisons challenging. For example, participants in Bajbouj and colleagues (2006), which were included in the meta-analyses, did not take any antidepressant medication (Bajbouj et al., 2006), while our study have included individuals on medication.

In contrast, other studies have reported findings in line with our results. For example, Concerto et al. (2013) found no variation in SICI between healthy participants and drug-resistant MDD patients (Concerto et al., 2013). Another study by Lewis and colleagues (2018), which included adolescents, also showed no difference in baseline SICI and LICI between individuals with MDD and healthy controls (Lewis et al., 2018). Based on these collective findings, it is plausible to suggest that medications may affect GABA receptor activity in patients with MDD. Consequently, the baseline levels of SICI and LICI,
associated with GABA-A and GABA-B receptors, may not differ significantly from those of healthy individuals, as they may be normalized through medication to the level of a healthy condition. A larger sample will allow us to further assess this question by controlling for the type of medication, for example. Altogether, this highlights the complexity of investigating cortical inhibition or excitation in the context of depression and underscores the need for further research to clarify these discrepancies.

5.2 Baseline Biomarkers and Association with Response to TBS Treatment

Our research investigated the relationship between baseline Glx, GABA, GABA/Glx levels and the response to TBS treatment, as measured by the reduction in HRSD-17 scores. Our findings did not reveal any significant or trending correlation among these factors, contrasting our hypotheses (Baeken et al., 2017; Bhattacharyya et al., 2021). However, to further assess this relationship, symptom clusters of the HRSD-17, including mood, anxiety, insomnia, and somatic symptoms (Kaster et al., 2023), were considered. Kaster and colleagues (2023) demonstrated that in TRD patients' treatment, the application of rTMS on the left DLPFC led to notably more enhancements in mood, somatic symptoms, and insomnia compared to the improvements observed in anxiety-related symptoms. Various investigations exploring the effects of brain stimulation techniques, such as ECT and transcranial direct current stimulation (tDCS), have reported similar findings. These studies have shown that core mood and dysphoric symptoms exhibit more favourable responses to treatment than other symptom clusters. In other words, interventions targeting brain regions associated with mood and dysphoria have demonstrated superior outcomes compared to addressing other symptom domains (Goerigk
et al., 2021; Wade et al., 2020). Therefore, by employing total depression scores to assess treatment response, the ability to detect different responses among individual symptom clusters may be compromised (Fried & Nesse, 2015). Taken together, in the current study, the relationship of all baseline biomarkers with response to TBS treatment by 4 sub-clusters of HRSD-17 were analyzed. To our knowledge, our research is the first study that considered the baseline levels of GABA and glutamate in the ACC, along with their correlation with the response to TBS therapy and with HRSD-17 subscales.

Our results showed a significant correlation between the baseline Glx levels in the ACC and the changes in the mood sub-cluster of HRSD-17 in MDD patients. Specifically, we observed that individuals with lower baseline Glx levels in the ACC experienced more improvements in their mood symptoms. Our data suggests that baseline Glx levels in the ACC may impact response to treatment, specifically that individuals with lower baseline Glx levels are more prone to experience improvement in their mood symptoms. Although contrary to our hypothesis, our findings align with certain studies; however, these studies examined the relationship using total scores rather than individual subscales. In the study by Godfrey and colleagues (2021), individuals with lower baseline levels of Glx in the right M1 showed more antidepressant response to rTMS assessed with MADRS scores (Godfrey et al., 2021). Luborzewski and colleagues' study (2007) revealed that patients who responded positively to rTMS treatment had notably lower glutamate levels in the left DLPFC compared to the non-responder group. Additionally, after treatment, the responders exhibited increased glutamate levels (Luborzewski et al., 2007).

On the other hand, Baeken and colleagues (2017) showed that elevated baseline levels of Glx in the left DLPFC were associated with a more favourable response to
accelerated rTMS therapy using the Beck Depression Inventory (BDI-II) (Baeken et al., 2017). Nevertheless, these baseline levels did not predict treatment response despite this finding. Of note, all patients with TRD included in the study were not receiving antidepressant medications during the research period, and their diagnosis was focused on melancholic unipolar major depression. Bhattacharyya and colleagues (2021) also found that higher Glx levels in the DLPFC were associated with better response to the rTMS treatment, with a predictive role for the effectiveness of rTMS (Bhattacharyya et al., 2021).

While the disparity between these studies and ours may be linked to methodological factors, our findings suggest that the ACC may be specifically associated with improved mood symptoms following TBS. As previously stated, ACC plays a crucial role in the pathophysiology of depression and has been identified as a key region involved in emotional processing during stimulation (Weigand et al., 2022). It is known to regulate mood and motivation and is often linked to functional and structural irregularities in individuals affected by depression. These irregularities have been connected to symptoms such as anhedonia, amotivation, apathy, rumination, and negative thoughts commonly observed in depressed patients (Dunlop et al., 2017; Levar et al., 2017; Northoff & Sibille, 2014a). Furthermore, certain studies have indicated increased glutamine/glutamate ratios in the ACC with rTMS therapy (Croarkin et al., 2016), and some reported a rise in Glx/tNAA levels in the targeted stimulation area (i.e., left DLPFC) with iTBS (Spurny-Dworak et al., 2022). Numerous studies highlighted the significance of glutamate and its receptors in mediating the physiological response to TBS in the human brain (Huang et al., 2007; Ishikawa et al., 2007; Li et al., 2019). Taken together, our research findings emphasize the role of glutamate in the ACC in relation to improved mood, regardless of its
direction with response to TBS treatment. This raises inquiries regarding the potential clinical implications, such as whether baseline glutamate levels could serve as predictive indicators for treatment response and influence the decision-making process when considering the use of glutamatergic or non-glutamatergic medications, or even the use of brain stimulation therapy that is involved in the glutamatergic system.

In addition, this study found no significant correlation between baseline GABA and GABA/Glx levels in the ACC and depression symptom improvement. To our knowledge, there has been no evidence of this connection in prior studies regarding GABA/Glx level. Interestingly, in terms of baseline GABA levels, certain studies have shown no association between GABA levels and the response to treatment or reductions in assessment scores, regardless of the brain region under investigation. However, they did observe a similar correlation with glutamate or Glx levels for rTMS therapy (Baeken et al., 2017; Bhattacharyya et al., 2021; Godfrey et al., 2021), aligning with the findings of our study. The absence of significant correlations between GABA levels and treatment response is not specific to rTMS or TBS therapies; this trend has also been observed in other treatment modalities. For example, in a study evaluating ketamine infusion therapy for MDD, there was no apparent link between the baseline prefrontal cortex GABA level and the improvement in MDD symptoms (Salvadore et al., 2012). In addition, our study found no significant correlations between baseline Glx and baseline GABA levels in the ACC. Consequently, it is plausible to suggest that the baseline GABA levels may not be a sensitive biomarker of MDD symptoms reduction, regardless of the treatment approach employed. Of note, in terms of baseline GABA/Glx with response to TBS treatment, there was one study which considered the baseline levels of Glx/GABA in the ACC to assess the
correlation between them; however, they also did not show a significant correlation with response to antidepressant medications (Narayan et al., 2022), consistent with our findings.

Despite the lack of a significant association between GABA and treatment response in relation to depressive symptoms in all mentioned studies and this current study, it is essential to consider the potential role of GABA in influencing treatment outcomes for comorbid symptoms. Specifically, our study observed a noteworthy correlation between the baseline GABA level and the GABA/Glx ratio in the ACC and the magnitude of anxiety symptoms improvement following TBS treatment. These findings suggest that individuals with lower baseline GABA and GABA/Glx levels experienced reduced anxiety scores as measured by the BAI, which was one of our secondary outcomes. This may suggest a role of GABA in mediating anxiety symptoms. Pharmacological studies have consistently demonstrated that medications which affect anxiety and depression, such as benzodiazepine, exert their effects by modulating the GABA neurotransmitter system (Möhler, 2012).

Furthermore, long-term stress in animal studies has demonstrated its association with decreased presynaptic GABA release and a decline in postsynaptic GABA receptors in the frontal cortex (Smith & Vale, 2006), which shows the role of GABA in anxiety mechanisms. As mentioned earlier, TBS aims to decrease depression symptoms by influencing brain plasticity through plasticity-like mechanisms and altering the balance between inhibition and excitation in the brain. GABA plays a crucial role in regulating cortical excitability and neuroplasticity (DeFelipe et al., 1986; Schieber & Hibbard, 1993). Consequently, it is plausible to consider that TBS may alleviate anxiety symptoms that often coexist with depression by affecting the GABAergic system. Furthermore, GABA
plays a significant role in regulating the brain's response to stress, which is a crucial vulnerability factor in mood disorders. Additionally, GABA is involved in the control of hippocampal neurogenesis, a process linked to successful antidepressant treatment (Luscher et al., 2011; Northoff & Sibille, 2014b). Therefore, TBS may not only impact depression symptoms but also have a potential role in managing stress, contributing to the efficacy of the antidepressant treatment.

Regarding the initial levels of TMS measurements, specifically SICI, LICI, and ICF, our research did not identify any substantial associations between these values and the response to TBS treatment, as assessed by the HRSD-17 scores, in contrast to our hypotheses. This is inconsistent with two previous studies that suggested a relationship between baseline levels of cortical inhibition and response to rTMS or ECT. For instance, a study conducted in 2004 by Fitzgerald et al. showed that increased inhibition, as indicated by CSP levels, was linked to a poorer response to rTMS treatment, measured by MADRS (Fitzgerald et al., 2004). Voineskos et al. (2016) also reported a noteworthy association between cortical inhibition levels at baseline (CSP) and response to ECT treatment (Voineskos et al., 2016), which indicated that cortical inhibition may have a potential role in predicting response to ECT treatment in depression. We did not collect CSP measurements in the current study, which may explain the differential results. However, we did measure LICI, which is also thought to also index GABA-B-ergic activity. Of note, our study is the first to assess the relationship between these two baseline biomarkers (i.e., SICI, LICI) and the response to TBS treatment in MDD patients.

Nevertheless, we observed a significant relationship between the baseline levels of LICI, RMT, and the change score in the BSS assessment after TBS sessions. This trend
indicated that lower baseline cortical inhibition (i.e., LICI) and excitation levels (i.e., RMT) were associated with a positive outcome regarding suicidal thinking, which is one of the symptoms of depression. Consequently, reduced baseline cortical inhibition and excitation were linked to reduced suicidal ideations, highlighting that baseline inhibition and excitation levels may have a role in suicidal ideation changes. This finding was consistent with Fitzgerald and colleagues’ study (2004), in which lower levels of inhibition correlated with better response to treatment. Moreover, the research conducted by Lewis and colleagues (2018) discovered that adolescents with suicidal tendencies had impairments in LICI when compared to a group of healthy individuals and patients who did not display suicidal behaviour (Lewis et al., 2018). Our study aligns with these findings and may indicate that there could be a connection between LICI or GABA-B receptor activity and the symptoms of suicidality in individuals with depression.

Further, cortical inhibitory impairments in individuals with suicidal tendencies could hold significant implications for advancing treatments aimed at reducing the risk of suicide (Lewis et al., 2018). Sun and colleagues (2016) reported that the baseline levels of LICI in the DLPFC, assessed through TMS-EEG, were associated with alterations in suicidal ideation symptoms in TRD patients following MST (Sun et al., 2016), which was replicated in a subsequent study by the same group (Sun et al., 2018). In contrast, the current body of research does not include studies that specifically explored the relationship between RMT and suicidal thinking. However, one study did reveal that patients with TRD exhibited higher left RMT, indicating reduced membrane excitability compared to healthy controls (Levinson et al., 2010). Therefore, the current study suggests that baseline levels of LICI and RMT may play a role in predicting the response to the intervention regarding
suicidal thinking, highlighting the potential importance of inhibitory and excitatory cortical processes in the context of suicidal ideation within the domain of depression. However, despite the valuable insights gained from existing studies, further research is necessary to acquire a more comprehensive understanding of these links.

5.3 Comparison of Baseline Measurements in Responders and Non-responders

In this study, no significant differences were observed in the baseline levels of any measurements between individuals who responded positively to TBS treatment and those who did not. As previously discussed, we did not find a significant correlation between the baseline levels of cortical inhibition and excitation and the response to TBS, assessed using HRSD-17, which can explain the absence of a significant distinction between responders and non-responders. Notably, consistent with our study, Merkl and colleagues (2011) did not observe any significant differences between responders and non-responders to ECT treatment regarding baseline metabolite concentrations in the ACC and DLPFC, despite finding significant differences between the MDD group and healthy controls (Merkl et al., 2011). Although this may suggest the lack of a predicting value of cortical excitation and inhibition levels in the ACC and motor cortex for TBS therapeutic response, our finding may be also due to the small sample size. A larger sample size would be needed to obtain more robust and reliable results for this particular analysis and allow to conduct prediction analysis using regressions. In addition, a larger sample size will allow us to further divide the group according to other factors, such as partial response and remission, which may also provide important insights into potential predictors of therapeutic efficacy.
5.4 Strengths and Limitations

One of the notable strengths of the present study is its utilization of both MRS and TMS to assess the levels of cortical excitation/inhibition before initiating the TBS treatments. It is worth highlighting that this study uniquely incorporates the sequential application of these two methods to measure baseline biomarkers in a single investigation. This integrated methodology may offer a more comprehensive view of the studied conditions' neurobiological underpinnings. In addition, this study is a component of a large, naturalistic double-blinded clinical trial, resulting in a diverse group of participants with MDD that reflects the varied population of depressed individuals. The inclusion of various participants in our clinical group enriches its composition and accurately represents a genuine cohort of individuals with MDD, even though it may lead to high heterogeneity among the MDD participants. However, as the number of participants increases, the issue of heterogeneity is expected to diminish. Our study also includes many clinical assessments, allowing us to assess the therapeutic effect of TBS on symptoms of depression and comorbidities.

Despite heterogeneity being a strength, it also posed a limitation due to variations in participants' psychotropic medication regimens and comorbid mental health disorders. Although the trial closely monitored the medication regimen in participants and disallowed any alterations during treatment or at least 4 weeks before the study began, the current study does not consider the potential impact of medication dosage and use on the biomarkers because of the small sample. As mentioned earlier, our study's naturalistic design made it impossible to alter participant conditions, contributing to the observed heterogeneity. MDD is characterized by a significant degree of heterogeneity, presenting
nearly 1500 possible combinations of symptoms that can lead to a diagnosis of MDD (Yang et al., 2018). The DSM-5 includes specifiers for MDD, such as "with anxious distress" or "with mixed features," but these categories do not consider different cultures, characteristics, interindividual differences or many other varieties. It might be a subset of patients experiencing MDD with an imbalance in GABA and glutamate. Moreover, these imbalances could vary based on the specific brain region and the stage of the illness (Draganov et al., 2020; Njau et al., 2017). This study is also limited by sample size. A more significant number of participants would have been beneficial to conduct a more robust statistical analysis. An important consideration in our current analyses is the absence of control for baseline severity scores when calculating Pearson's correlations among the change scores of clinical outcomes and our baseline excitation and inhibition measures. Consequently, we advise interpreting our current correlational findings with caution. This caution stems from the recognition that baseline severity scores represent a confounding variable, as they exhibit a direct association with the observed change scores. It is essential to acknowledge that this limitation will be addressed and rectified in the final analyses of our clinical trial, ensuring a more robust and accurate assessment of the relationships between our variables.

There are some limitations to using MEGA-PRESS, used in our study for GABA and glutamate quantification in MRS, including inherent difficulties in resolving GABA at 3T because of low concentration and overlapping macromolecules. Moreover, MEGA-PRESS does not allow the separation of the glutamate and glutamine peaks (Godfrey et al., 2021), resulting in a composite measurement of Glx. Nevertheless, some studies have demonstrated that the Glx peak reliably indicates glutamate levels in vivo (Dhamala et al.,
Another limitation of the current study is that the two groups (MDD and healthy controls) were not initially matched regarding sex and age. However, to account for these differences, we controlled for age and treated it as a covariate factor in our analysis. This helps to mitigate the potential confounding effects of age on the results and allows for a more accurate comparison between the two groups. As previously mentioned, our study lacked post-treatment measurements, precisely the absence of TMS and MRS sessions after TBS treatment. Consequently, we could not compare biomarkers before and after TBS treatment sessions, which would have provided valuable insights into the mechanism of action of TBS and its impact on cortical excitation and inhibition. These post-treatment measurements would have allowed for a more comprehensive understanding of the treatment's effects and influence on brain activity and function.

5.5 Future Directions

Moving forward with our research, the next phase involves recruiting more participants within the MDD group to reach a target of 256 individuals. Additionally, we aim to increase the size of the healthy control group, which currently has fewer participants than the MDD group, and to match participants for age and sex. This strategic approach is intended to enhance the statistical power of our study significantly. By incorporating a larger pool of participants, we anticipate that the correlation will become more apparent and meaningful, showing valuable insights into our research. Subsequently, we can conduct prediction analyses to identify potential predictors for the response to TBS treatment. This step is essential for better understanding the factors that influence treatment outcomes, resulting in more personalized and effective therapeutic interventions. Another
important undertaking would be to study predictive biomarkers appropriate for treating left-sided iTBS and bilateral sequential TBS separately. Unfortunately, due to the ongoing clinical trial, a large double-blinded study designed to compare the treatment protocols, we could not differentiate between the TBS protocols (unilateral and bilateral). The final sample will allow for comparisons of both paradigms' potential shared and combined predictors.

Additional markers that could be potential predictors for TBS treatment in future studies include other TMS measures of cortical inhibition, such as the cortical silent period, that is not collected in the current study. For instance, in the study conducted by Fitzgerald and colleagues in 2004, they uncovered a significant role of CSP in predicting the response to rTMS sessions among participants diagnosed with depression (Fitzgerald et al., 2004). In addition, future analyses of the current sample could include other brain metabolites that were collected as part of the MRS sequence, such as NAA, which was previously shown to be lower in the ACC of individuals with MDD compared to healthy controls (Chen et al., 2014; Merkl et al., 2011) and showed to increase after iTBS (Zavorotnyy et al., 2020), highlighting its potential role in TBS mechanisms. Exploring other brain regions, such as the specific stimulation site for iTBS or cTBS (left and right DLPFC), would also allow the study of predictors in those areas. However, our current study has focused on investigating the ACC region.

Another aspect that needs exploration in the future involves the evaluation and measurement of the biomarkers discussed in this study post-TBS treatment. This approach would enable us to compare the markers before and after the treatment, thereby facilitating the discovery of additional mechanisms of action associated with TBS treatment and its
impact on biomarkers. Additionally, investigating the changes in biomarkers may serve as a predictive component, offering valuable insights into treatment outcomes.

5.6 Conclusion

The current study revealed that patients with MDD exhibited reduced levels of cortical excitation, as evidenced by lower glutamate levels in the ACC and ICF levels in the motor area when compared to the healthy control group. This finding provides additional insight into the complex underlying mechanisms of MDD. The findings from this study also revealed a correlation between lower baseline levels of glutamate and a reduction in mood-related symptoms, using the mood sub-cluster of the HRSD-17, following 4 to 6 weeks of TBS treatment. This suggests that individuals with MDD with lower baseline excitation levels are potentially more likely to experience decreased mood symptoms following TBS treatment. Such information is crucial for clinicians as it could aid them in making informed decisions regarding treatment selection for TBS sessions.

Furthermore, the study demonstrated that lower baseline levels of GABA and the GABA/Glx ratio were associated with reduced anxiety symptoms. This suggests that MDD patients with decreased inhibition levels may be more likely to experience reduced anxiety symptoms after TBS treatment. Finally, the study also found that lower inhibition measured by LICI, and lower excitation measured by RMT at baseline correlated with reduced suicidal thinking post-treatment. This indicates that inhibition and excitation levels may be involved in the mechanisms underlying suicidal ideations. Overall, these findings provide valuable insights into the potential predictors of treatment response in MDD patients undergoing TBS, which can be crucial for tailoring treatment approaches.
Nevertheless, it is essential to note that further research is required to establish a strong and reliable correlation in identifying biomarkers that can predict the response to TBS treatment. These biomarkers would help clinicians personalize TBS treatment, considering the interindividual differences in the pathophysiology of MDD. Potential predictors of treatment outcomes will contribute to more effective and tailored therapeutic approaches for patients with MDD.
## Appendix 1. Timeline of clinical assessments – Randomized Treatment

<table>
<thead>
<tr>
<th>Clinical Assessment or Procedure</th>
<th>Baseline</th>
<th>During treatment</th>
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<tbody>
<tr>
<td></td>
<td>Screening visit</td>
<td>First session</td>
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<tr>
<td>MINI</td>
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<tr>
<td>ATRQ</td>
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<tr>
<td>ATHF</td>
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<tr>
<td>CONMED</td>
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<tr>
<td>HRSD-17</td>
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<td>X</td>
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<tr>
<td>MADRS</td>
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<td>SRRS</td>
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<tr>
<td>YMRS</td>
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<tr>
<td>C-SSRS</td>
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<td>X^1</td>
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<td>MMSE</td>
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**Administered by trained RA/graduate student**

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<tr>
<td>QIDS-SR16</td>
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<td>ASRM</td>
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<td>BAI</td>
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<td>BSS</td>
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<td>Q-LES-Q-SF</td>
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<td>WEMWBS</td>
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<td>PSQI</td>
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<td>PCL-5^3</td>
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<tr>
<td>TMS side effects</td>
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<td>PTFQ</td>
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<td>MRI screening</td>
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<td>IPA-SF</td>
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**Self-report on iPad**

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<td>Urine</td>
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<td>TMS-EEG</td>
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**Procedures**

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<td>MINI = Mini International Neuropsychiatric Interview</td>
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<tr>
<td>ATHF = Antidepressant Treatment History Form</td>
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<tr>
<td>ATRQ = Antidepressant Treatment Resistance Questionnaire</td>
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<tr>
<td>CONMED = Concomitant Medication Log</td>
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<tr>
<td>MADRS = Montgomery–Asberg Depression Rating Scale</td>
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<tr>
<td>HRSD-17 = Hamilton Rating Scale for Depression 17-item</td>
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<tr>
<td>SRRS = Salpêtrière Retardation Rating Scale</td>
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<tr>
<td>YMRS = Young Mania Rating Scale</td>
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</table>

ASRM = Altman Self-Rating Mania Scale
BAI = Beck Anxiety Inventory
BSS = Beck Scale for Suicidal Ideation
Q-LES-Q-SF = Quality of Life Enjoyment and Satisfaction Questionnaire – Short Form
WEMWBS = Short Warwick Edinburgh Mental Well-Being Scale
PSQI = Pittsburgh Sleep Quality Index
LSEQ = Leeds Sleep Evaluation Questionnaire
PCL-5 = Posttraumatic Stress Disorder Checklist for DSM-5

[X]: As needed
| C-SSRS = Columbia-Suicide Severity Rating Scale | TMS side effects = TMS side effects questionnaire |
| MMSE = Mini Mental State Evaluation | PTFQ = Post-Treatment Feedback Questionnaire |
| TASS = TMS Adult Safety Screen | FPTFQ = Final Post-Treatment Feedback Questionnaire |
| QIDS-SR16 = 16-item Quick Inventory of Depressive Symptoms – self report | TBQ = Treatment Blinding Questionnaire |
| IPA-SF = International Physical Activity – Short Form | MRI = Magnetic Resonance Imaging |
| | TSSS = The Stanford Sleepiness Scale |

1 Administered only if BSS shows active suicidal thoughts
2 Administered only for clients > 65 years old
3 Administered only if comorbid PTSD diagnosis
4 Physician consultation will be after the initial telephone screening, and before the actual screening visit. It can also be planned as needed during the course of the study
References


https://doi.org/10.1093/cercor/12.4.386


Depression and Anxiety, 37(2), 134–145. https://doi.org/10.1002/da.22968


Hasler, G., Van Der Veen, J. W., Tumonis, T., Meyers, N., Shen, J., & Drevets, W. C.


https://doi.org/10.1017/S0033291720004729


https://doi.org/10.1001/archpsyc.56.4.315


https://doi.org/10.1113/JPHYSIOL.1993.SP019912


https://doi.org/10.1016/S0924-9338(97)83296-8


https://doi.org/10.1016/j.jpsychires.2007.03.001


Luborzewski, A., Schubert, F., Seifert, F., Danker-Hopfe, H., Brakemeier, E. L.,
alterations in the dorsolateral prefrontal cortex after treatment with high-frequency
repetitive transcranial magnetic stimulation in patients with unipolar major
https://doi.org/10.1016/j.jpsychires.2006.02.003

https://doi.org/10.1038/mp.2010.120

Luykx, J. J., Laban, K. G., van den Heuvel, M. P., Boks, M. P. M., Mandl, R. C. W.,
Kahn, R. S., & Bakker, S. C. (2012). Region and state specific glutamate
downregulation in major depressive disorder: A meta-analysis of 1H-MRS findings.
*Neuroscience and Biobehavioral Reviews, 36*(1), 198–205.
https://doi.org/10.1016/j.neubiorev.2011.05.014

Interindividual variability of the modulatory effects of repetitive transcranial
magnetic stimulation on cortical excitability. *Experimental Brain Research, 133*(4),
425–430. https://doi.org/10.1007/s002210000432

Modulation of corticospinal excitability by repetitive transcranial magnetic


Merk, A., Schubert, F., Quante, A., Luborzewski, A., Brakemeier, E. L., Grimm, S.,


https://doi.org/10.1016/j.neuropharm.2011.08.040


Northoff, G., & Sibille, E. (2014b). Why are cortical GABA neurons relevant to internal focus in depression? A cross-level model linking cellular, biochemical and neural

https://doi.org/10.1038/MP.2014.68


/PMC/articles/PMC4808719/


https://doi.org/10.1097/00004691-199807000-00005


https://doi.org/10.1016/J.BRS.2008.06.002


https://doi.org/10.1016/j.brainres.2004.06.009


https://doi.org/10.1002/NBM.3045

https://doi.org/10.1016/J.SCHRES.2020.02.001

https://doi.org/10.1093/schbul/sbt078

https://doi.org/10.1016/J.PSC.2018.04.002

https://doi.org/10.1016/j.clinph.2020.10.003


Salvadore, G., Van Der Veen, J. W., Zhang, Y., Marenco, S., MacHado-Vieira, R.,
predictors of clinical improvement to ketamine in depression. *The International
Journal of Neuropsychopharmacology, 15*(8), 1063–1072.

Sanacora, G., Gueorguieva, R., Epperson, C. N., Wu, Y. Te, Appel, M., Rothman, D. L.,
Krystal, J. H., & Mason, G. F. (2004). Subtype-specific alterations of γ-
aminobutyric acid and glutamate in patients with major depression. *Archives of
General Psychiatry, 61*(7), 705–713. https://doi.org/10.1001/archpsyc.61.7.705

Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of
depression: An emerging frontier of neuropsychopharmacology for mood disorders.
*Neuropharmacology, 62*(1), 63–77.

glutamatergic system to develop novel, improved therapeutics for mood disorders.
*Nature Reviews Drug Discovery, 7*(5), 426–437. https://doi.org/10.1038/NRD2462

Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott,
D. M., Abbafati, C., Adolph, C., Amlag, J. O., Aravkin, A. Y., Bang-Jensen, B. L.,
Bertolacci, G. J., Bloom, S. S., Castellano, R., Castro, E., Chakrabarti, S.,


Tong, Z., Yamaki, T., Harada, K., & Houkin, K. (2004). In vivo quantification of the


Walter, M., Henning, A., Grimm, S., Schulte, R. F., Beck, J., Dydak, U., Schnepf, B.,


https://apps.who.int/iris/handle/10665/254610
