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Lois Teye-Botchway April 10, 2023

Variability and Stability of TMS Treatment Targets for PTSD

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Biology

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Abstract

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Transcranial Magnetic Stimulation (TMS) is a non-invasive neuromodulation treatment shown to be moderately effective for Post-Traumatic Stress Disorder (PTSD). However, it is hypothesized that treatment efficacy can be improved by individualizing TMS targets using neuroimaging. Here, we aim to determine the variability of the DLPFC target between individuals, the variability between positive and negative target definitions, and the stability of the target across states after fear neurocircuitry task activation. In this ongoing sham-controlled TMS clinical trial, pre-TMS resting-state functional connectivity (RSFC) was used to define the area within the rDLPFC most strongly connected with the right amygdala for each participant. A second RS scan was collected in the same visit after the amygdala was activated by fMRI tasks. Neuroimaging data for seventeen patients were analyzed, which indicated significant variability in target location between participants ($F(1,16)=3005$, $p<0.001$, $\eta p2=0.99$). Results showed significant differences in the positive and negative target definitions ($F(1, 16) = 6.2$, $p = 0.02$, $\eta p2=0.28$). However, the change in target location between RS scans 1 and 2 was not significant ($F(1, 16) = 0.8$, $p = 0.38$, $\eta p2=0.05$), showing that the target remained stable after activation. The results suggest the importance of individualized targeting and the importance of further evaluation of negative- versus positive correlated DLPFC targets in the future. The lack of change in target location after amygdala activation suggests the rDLPFC target is stable within each individual. This has implications for combining TMS with trauma-focused therapy, in which the amygdala and fear neurocircuitry is activated. However, the small sample size limits the generalizability of the results, and further research with a larger participant pool is needed to confirm these findings.

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Acknowledgements

I would like to extend my deepest appreciation to Dr. Sanne van Rooij for her support and guidance throughout my project and for the past two years that I have been in her lab. Her dedication and encouragement have helped me to grow immeasurably and achieve success beyond my wildest dreams. I would also like to thank the other members of my committee, Dr. Anita Deveneni and Dr. Negar Fani, for their helpful feedback and support. Thank you to all the staff and interns at the Grady Trauma Project, who played an invaluable part in my project. An important thanks also to God, without whom I would not be here today. Finally, to all of my friends and support systems, thank you for being with me throughout my time at Emory, I truly do not know where I would be without you all.

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Introduction

What is PTSD?

Since the early days of modern neuroscience and psychology, scientists have been fascinated by what happens to someone after they witness or experience a severely traumatic event. Famous psychologists such as Sigmund Freud and Carl Rogers studied how traumatic events such as war, accidents, loss of loved ones, and sexual assault affected patients throughout their lifetime [1]. Although many significant developments have been made over the past century, trauma disorders have continued to be debilitating and difficult-to-treat forces in the lives of many around the world.

Post-traumatic stress disorder (PTSD) is a mental disorder that involves psychiatric symptoms after exposure to a traumatic event. Both exposure to traumatic events and PTSD diagnosis are prevalent in society today. Many U.S. and Canadian research studies have found that 70% of adults have experienced a traumatic event at least once. In comparison, lifetime PTSD estimates range between 6% and 9%, with higher levels in at-risk and underserved populations [2–4].

The American Psychiatric Association classifies four core symptomatic characteristics of PTSD in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), a standard that mental health specialists use to classify mental disorders. An individual is determined to have PTSD if they meet all the diagnostic criteria for one month after exposure to a traumatic event: re-experiencing symptoms of the event such as nightmares and flashbacks, avoidance of reminders of the traumatic event, adverse changes in mood, and signs of hyperarousal such as irritability and concentration problems [2].

Despite the increased recognition and research efforts in recent years, there is still much to learn about PTSD. In contrast to other mental health disorders, such as depression and anxiety, which have been studied extensively with more defined treatment options, PTSD is a relatively new diagnosis with continually evolving diagnostic criteria. It was not until 1980 that the APA officially

recognized PTSD as a mental health disorder, which brought it into the public eye nationally and drove an explosion in research. Even so, much of the early PTSD research focused on the experiences of war veterans and domestic and sexual abuse victims [5]. Further studies have shown that PTSD can arise from a wide range of traumatic events and in diverse populations, such as refugees, first responders, and individuals who experience chronic stressors. These breakthroughs in better understanding the pathology of PTSD have naturally led to increased attention to developing more effective ways of treating this disorder.

What are the treatments for PTSD? Are they effective?

There have been several breakthrough treatments for PTSD, which have been advanced by recent developments in the field. These studies concentrating on exploring the scope and impact of traumatic stress and prevention strategies for PTSD have improved understanding of its impact and led to more successful public health interventions [6].

Trauma-focused therapies such as Cognitive Processing Therapy (CPT), Prolonged Exposure Therapy (PE), Eye Movement, Desensitization, and Restructuring (EMDR), and others with an emphasized focus on trauma are the current standard for treatment. Medications such as selective serotonin reuptake inhibitors (SSRI) or selective norepinephrine reuptake inhibitors (SNRI) have also been shown to assist in treating symptoms [1].

Despite the successes of modern treatment options, there is still a significant subsection of patients whose symptoms do not improve after using trauma-focused therapy and medications. Studies have discovered that in patients who received PTSD treatment through primary care, their symptoms were chronic, with a 38% likelihood of recovery and a 30% likelihood of recurrence[7]. Past research has also shown that the non-response rates for selective serotonin reuptake inhibitors are somewhat high, at about 20–40%[8]. Additionally, about 30-50% of PTSD patients do not

respond to current treatments[9], while the non-response rate to cognitive–behavioral therapy (CBT) in PTSD can be as high as 50%[10].

There are several issues to consider when it comes to trauma-focused therapies. For instance, patients with limited education or literacy skills may be excluded from these treatments because they frequently involve complex tasks such as writing-based worksheets and homework. Moreover, access to behavioral health care may be limited, and patients may face stigma against such treatment, both of which can discourage individuals from seeking care or engaging in trauma-exposure treatment. Yet, trauma-focused therapy is still the most widespread treatment choice for PTSD by large government agencies and private practices[11]. So, for these treatment-resistant patients, what more can be done?

What is the neurobiology of PTSD and PTSD treatment non-response?

Understanding the neurobiology of PTSD and treatment non-response is crucial for improving the chances of successful treatment outcomes, as identifying the underlying neural causes can assist in developing treatments that target these specific mechanisms.

Impaired fear inhibition involving a lack of discrimination of danger and safety cues and deficiencies in the extinction of fear cues have been shown to be important biological markers of PTSD. One of the most studied hallmarks of PTSD neurobiology is exaggerated amygdala activity during fearful stimulation, which indicates dysregulation of inhibitory neurocircuits in the brain[12,13]. The amygdala is a subcortical brain structure involved in emotion processing, retrieval of emotional memories, and associative fear learning.

PTSD patients often exhibit decreased activation of the dorsolateral prefrontal cortex [14–16], a brain structure involved in complex cognitive and behavioral functions such as decision-making, working memory, and attentional control. Functional magnetic resonance imaging studies have

consistently found a negative correlation between decreased activity in the dorsolateral prefrontal cortex (DLPFC) and hyperactive amygdala levels in individuals with PTSD [17,18].

The neurobiology of PTSD treatment response is complex and involves changes in brain function in several key regions, including the amygdala and prefrontal cortex. Modulation of fear processing and fear extinction is one of the main principles underlying the efficacy of PTSD treatments. Fear processing refers to the cognitive processes involved in detecting, assessing, and reacting to threatening stimuli. In contrast, fear extinction is the process of recognizing that a stimulus that was once threatening is no longer a danger. The natural process of extinction of learned fear could be diminished by the underlying neuropathology linked to poor response to trauma-focused therapy (e.g., over-engagement of threat and salience network, decreased context processing, and emotion regulation).

The most consistent neural predictor of treatment nonresponse in PTSD is hyperreactivity of the amygdala, which is critical to the threat and salience, and fear learning circuits[19–21]. Additionally, right amygdala responsivity is positively associated with symptom severity in PTSD and other psychiatric disorders[12,22–24]. The maintenance of PTSD is linked to reduced amygdala inhibitory regulation and impaired fear inhibition. Additionally, lessened inhibitory control by the prefrontal cortex over the amygdala has been linked to the overstated fear reactions and impaired fear inhibition observed in PTSD [15]. This lack of inhibition results in an extreme fear reaction to stimuli that are not dangerous.

In a model of the neurobiology of PTSD treatment nonresponse, fear engagement via neuromodulation appears as a potential intervention to maximize prefrontal control over stress response systems found in the right amygdala[25]. The goals of such treatment include normalizing amygdala activity and enhancing the prefrontal cortex's inhibitory control over the amygdala. Evidence has also revealed that reducing or eliminating amygdala function could improve chronic

PTSD symptoms and treatment response. Still, surgery has many limitations, and ablation is not widely applicable to the general patient population[26].

What is Transcranial Magnetic Stimulation?

Transcranial Magnetic Stimulation (TMS) is a non-invasive neurostimulation technique that uses a magnetic field to stimulate nerve cells in the brain. TMS does not require anesthesia or the induction of seizures and has a limited number of relatively minimal adverse effects[27]. TMS works by creating a strong magnetic field in a coil placed over a patient's scalp in the vicinity of a targeted brain area field[28], as seen in Figure 2. This magnetic field generated by the coil depolarizes neurons and produces changes in neuronal activity. Repetitive transcranial magnetic stimulation (rTMS) is a treatment that involves the recurrent delivery of magnetic pulses to the brain in rapid 'bursts' (measured in Hz).

Multiple studies have shown how effective rTMS can be for treatment-resistant depression. After years of studies, the FDA finally approved the use of TMS for depression in 2008 [29]. Based on its efficacy for depression, some research has thus been conducted to address the possibility of using TMS as an alternative treatment for PTSD patients. TMS has specifically been considered as a treatment for PTSD in notable meta-analyses [30,31]. These investigations demonstrated that TMS had substantial effect sizes and outperformed control conditions. Additionally, there is growing evidence supporting the effectiveness of TMS for treating PTSD, either as a standalone therapy or in combination with other therapies[32] , but it has yet to be approved by the FDA. Limited sample sizes and significant levels of protocol variability, however, have made it difficult to draw firm conclusions on the most effective TMS treatment parameters for PTSD [25]

How is the target for TMS defined?

The standard clinical technique for rTMS for mood disorders such as Major depressive disorder (MDD) is associated with inadequate efficacy. In the past 20 years, since the advent of

research about TMS, limitations related to its use may be due to the imprecise nature of antiquated scalp-based targeting,

The original method for determining the coil position, as used by George et al. (1995)[33], is to find the site over the motor cortex that evokes a maximal finger twitch and then move the coil to a point 5 cm anterior, with the 5 cm based on an estimation from the Talairach Atlas, a 3-dimensional coordinate system of the human brain that is independent of individual differences in size and shape[34]. Despite its limitations, this method of determining the target for TMS treatments is still used in hospitals worldwide, such as Emory Brain Health Center.

What is individualized targeting, and why is it important?

When analyzing the state of the field currently, scalp-based targeting methods are less optimal than other neuronavigational methods, as they ignore variability due to head size, brain structure, and function. Thus, investigating the efficacy of individualizing TMS treatment targets can help the greater scientific community and has clear clinical implications.

In recent years, more advanced individualized TMS targeting methods that incorporate fMRI-guided neuronavigation individualized to each person has been developed. This year, a novel neuronavigational targeting protocol was approved by the FDA for Major Depressive Disorder[35]. This new FDA-approved method delivers much more stimulation in significantly less time than is typical for TMS treatment and has been shown to be very effective, as 70% of 29 participants reached remission after five days.

As more research is conducted, individualized targeting is becoming more widely accepted and researched for use in TMS treatments for psychiatric disorders. Exploring the use of MRI-based individualized targeting contributes to understanding if it can be feasibly implemented more widely. Thus, individual targeting for TMS through fMRI-based coil positioning is a hypothesis-driven approach to treating PTSD with non-invasive brain stimulation [34]. While TMS has been delivered

non-specifically to the general area of the DLPC for depression, individualized targeting uses a theoretical basis that draws from the neurobiology of PTSD maintenance by targeting the hyperactive amygdala. Tailoring treatment around each individual's unique functional connectivity with the amygdala allows the theoretical neurobiology model of PTSD to more directly guide the treatment approach and highlights the potential effectiveness of implementing individualization in more treatment centers.

How are MRIs used for targeting?

By utilizing brain imaging methods such as fMRI, TMS targeting has been refined from scalp-based measuring methods to sophisticated neuronavigational systems. MRIs allow researchers to target individual cortical locations with potentially millimeter accuracy[36].

The combined use of MRI and neuronavigation enable further developments in the efficacy of targeting TMS coils, as effectiveness improves by moving from anatomical positioning to positioning based on functional imaging. When using MRIs to develop individual targets, sites of activation found in a single individual's fMRI scans can be overlaid on their structural MRI and targeted directly with TMS. These neuronavigational approaches have directly benefited from advancements in neuroscience research and represent an important frontier in applying TMS to treat mental disorders.

Studies by Sack et al. (2009)[37] and Kammer, Vorwerg, and Herrnberger (2007)[38] have demonstrated the benefits of an advanced TMS neuronavigation system that allows storing the exact position and orientation of the TMS coil relative to an individual 3-D anatomical MR scan [39]. MRIs play a primary role in structural and functional neuroimaging for PTSD by providing a wide range of necessary evidence at each brain structure level and reflecting impaired functional connectivity, disequilibrium among functional brain networks, and impairment of brain structures closely interacting with the networks in PTSD [40].

How is correlation data used to find targets?

Resting-state functional connectivity MRI (rs-fcMRI) has become a beneficial method for identifying prospective treatment targets and examining the neural foundations of PTSD. While the brain is at rest, rs-fcMRI analyzes the intrinsic functional connectivity between neural areas. An increasing amount of research[41,42] has revealed that both positive correlations (i.e., synchrony) and negative correlations (i.e., anticorrelation) between brain regions are altered in individuals with PTSD compared to healthy controls.

For instance, a recent study found that inhibitory TMS treatment applied over the left DLPFC reduced negative and positive connectivity between the amygdala and other brain regions in patients with PTSD, suggesting that both types of connectivity may be important targets for intervention[43]. However, whether positive or negative correlations should be targeted in therapeutic interventions such as TMS remains a subject of debate and likely depends on several factors, including the disorder treated, TMS frequency, and neural target.

Understanding the complex interplay between synchrony and anticorrelation in rs-fMRI is an essential topic of ongoing study, with significant implications for introducing TMS as a new treatment for PTSD and other psychiatric disorders. These findings suggest that approaches such as TMS that can regulate both positive and negative correlations may be particularly successful in restoring a more balanced pattern of functional connectivity in individuals with PTSD. This further supports the advantages of individualized therapeutic strategies because PTSD-related neural networks may vary among individuals.

What area is targeted for PTSD? Why is it effective?

The dorsolateral prefrontal cortex (DLPFC) has been shown to have maximal efficacy as a target in TMS treatments. This anatomical location, located in Brodmann areas 9 and 46, is used because this region showed decreased activation levels in patients with depression[28,33,44,45].

In the late 1980s and early 1990s, neuroimaging studies first identified DLPFC hypoactivity in major depression. In the years since neuroimaging and stimulation, studies have provided some of the most vital support for DLPFC involvement in depression and PTSD. The DLPFC was found to indirectly regulate the amygdala, which makes it a valuable area of investigation since the amygdala is implicated in the pathophysiology of PTSD[46], as previously described. With rTMS, the DLPFC has been the stimulation target in most successful randomized controlled trials [47–49].

Recent research, including one by Oathes et al. in 2021[50], utilized fMRI recordings with TMS to map a causal communication between the right prefrontal cortex and the amygdala. Oathes et al. used rs-fMRI to evaluate the effects of TMS on subcortical amygdala network modulation. The authors found that the right amygdala's hyperactivity was shown to be significantly reduced by TMS. They discovered a higher functional connection between the DLPFC and the amygdala, which provides evidence of a mechanism underpinning the therapeutic effects of TMS on PTSD and other associated illnesses.

Jackson and colleagues (2021)[51] also showed that individual differences in baseline DLPFC-amygdala connectivity might impact the effectiveness of TMS treatment, highlighting the need for personalized treatment approaches. They also found that individuals with PTSD showed decreased activation in the DLPFC during a fear extinction task compared to healthy controls. These findings suggest that impaired functioning of the DLPFC, with its functional connectivity to the amygdala, may contribute to the maintenance of PTSD symptoms.

Overall, these studies suggest that the DLPFC's connection to the amygdala plays a critical role in the pathophysiology of PTSD and may be a promising target for non-invasive brain stimulation interventions. Despite these encouraging findings, more research is needed to better understand the role of the DLPFC in PTSD and how non-invasive brain stimulation techniques can be optimized for treatment.

What is DLPFC target stability?

DLPFC target stability refers to the consistency of the location and accuracy of stimulation targeting the dorsolateral prefrontal cortex (DLPFC). The stability of the DLPFC target is vital to determine because it affects the efficacy and reproducibility of TMS treatment. Studies have shown that the location of the stimulation site can vary across individuals and that inaccurate targeting of the DLPFC can lead to inconsistent and unreliable treatment outcomes. Therefore, efforts have been made to improve the accuracy and consistency of DLPFC targeting using TMS using previously discussed neuroimaging techniques such as fMRI and neuronavigation systems.

Several studies have investigated the accuracy and reliability of DLPFC TMS targeting, and the concept of target stability has emerged as a critical factor in the efficacy of TMS interventions. For example, Fitzgerald and colleagues (2009)[52] found that individual differences in DLPFC target stability predicted response to TMS treatment for major depression, suggesting that improving the accuracy and consistency of DLPFC targeting using TMS can lead to better treatment outcomes in various psychiatric disorders.

Overall, DLPFC target stability is an essential factor in the efficacy of TMS interventions for neuropsychiatric disorders. Effective TMS navigation techniques have been developed using individualized brain imaging data, in addition to recent advances in real-time TMS monitoring and control systems to maintain DLPFC target stability during a TMS session. Despite these advancements, further research is needed to optimize and standardize TMS navigation techniques to achieve optimal target stability.

Research Questions, Study Design, and Hypotheses

Due to the lack of current research about the inter-individual variability and intra-individual stability of the DLPFC target for TMS treatment, this brings three main questions: First, is there individual variability in the DLPFC target? Second, is there variability between the DLPFC targets

defined by positive and negative correlations with the amygdala? Third, is there stability between the DLPFC targets across states from pre- to post-fear and neurocircuitry engagement?

In this ongoing sham-controlled TMS clinical trial, pre-TMS resting-state functional connectivity (RSFC) was used to define the area within the rDLPFC most strongly connected with the right amygdala for each participant. A second RS scan (RS 2) was collected in the same visit after the amygdala was activated by fMRI tasks.

Hypothesis #1: Variability of DLPFC Target Between Individuals

First, is there individual variability in the DLPFC target? This is an essential first question to establish if there are noticeable and significant differences in the DLPFC targets for each patient. The null hypothesis is that the XYZ coordinates of the participants are not statically significant from each other. Or, in other words, H_0: XYZ Coordinates$_{(\text{Participant ni})}$ = XYZ Coordinates $_{(\text{Participant ni…})}$. If the null hypothesis is correct, we would expect the coordinates to be the same for all participants**.** The alternative hypothesis is H_A: XYZ Coordinates$_{(ni)}$ ≠ XYZ Coordinates $_{(ni)}$.

We hypothesize that there will be statistically substantial individual variability in the TMS target defined by the DLPFC's functional connectivity with the amygdala. This will show that individualization could be important to consider for future clinical applications of TMS.

Hypothesis #2: Variability of DLPFC Targets Defined by Positive Correlation and Negative (Anti) Correlation with the Amygdala

Secondly, is there any variability between targets defined by positive and negative correlations with the amygdala? The null hypothesis is that the XYZ coordinates of the positive Resting State (RS) 1 and negative RS 1 targets are not statically significant from each other. Or, in other words, H_0: RS1 Positive XYZ Coordinates$_{(ni)}$ = RS 1 Negative XYZ Coordinates $_{(ni)}$. If the null hypothesis is correct, we would expect the coordinates for the RS 1 Positive target definitions and RS 1 Negative definitions to be the same for all participants**.** The alternative hypothesis is H_A: RS 1

Positive XYZ Coordinates$_{(ni)}$ ≠ RS 1 Negative XYZ Coordinates $_{(ni)}$. If the alternative hypothesis is correct, we expect these two target definitions will differ significantly.

We predict there will be statistically significant differences in the coordinates of the positive and negative target definitions. This will show that, due to the lack of overlap of these target definitions, it will be essential to their differences and efficacy when creating targets in the future of TMS treatments for PTSD.

Hypothesis #3: Stability of DLPFC Target Across States

Finally, does rDLPFC targeting change across states from pre- (RS 1) to post-fear neurocircuitry engagement (RS 2)? The null hypothesis is that the XYZ coordinates of the RS 1 and RS 2 targets are not statistically significant from each other. Or, in other words, H_0: RS 1 XYZ Coordinates$_{(ni)}$ = RS 2 XYZ Coordinates $_{(ni)}$. If the null hypothesis is correct, we should expect coordinates for the RS 1 and RS 2 coordinates to be the same for all participants. The alternative hypothesis is H_A: RS 1 XYZ Coordinates$_{(ni)}$ ≠ RS 2 XYZ Coordinates $_{(ni)}$. If the alternative hypothesis is correct, we expect the RS 1 and RS 2 target coordinates to differ significantly.

We hypothesize that there will not be a statistically significant change in the target location within time points from pre-to post-fear neurocircuitry engagement. This will indicate the stability of the rDLPFC target and its usefulness as a method to target the amygdala through the DLPFC indirectly.

Methods

Participants

This study works in collaboration with the Grady Trauma Project, a large ongoing study on stress and trauma in underserved minority populations in Atlanta. This study drew on the recruiting infrastructure of the GTP to recruit and obtain screening assessments of PTSD patients.

Participants were recruited from a pool of patients seeking care at primary care clinics at Grady Memorial Hospital. Additionally, individuals in the Metro Atlanta area were contacted through social media advertisements, flyers, emails, texts, and phone calls and interviewed for trauma history and current emotional state via questionnaires such as the PTSD Checklist for DSM-5 (PCL-5) and Beck's Depression Inventory (BDI), which measures the presence and severity of PTSD and depression symptoms, respectively. Several inclusion and exclusion criteria were needed to verify participants' safe involvement in the study (Table 1).

Because this study is focused on the characteristics of the DLPFC target and not the effect of TMS on participants' PTSD prognosis, clinical symptoms were not the primary outcome of interest. However, the demographic factors of the participants, including their scores on the PCL-5 and BDI, were included in Figures 10-11 to describe the sample, as they provide an important context for interpreting the study results.

MRI Scan Parameters

Functional magnetic resonance imaging data were gathered on a 3T Siemens Trio MRI Scanner using a 32-channel head coil. Following a high-resolution structural T1 scan, a baseline resting-state (RS) scan is performed. During this scan, the participants were instructed to clear their minds and focus on small, white fixation cross on the screens above their heads.

Following the first RS scan, three functional MRI tasks were used to engage the fear inhibition neurocircuitry: a 7-minute threatening faces task comparing the response to fearful vs. neutral stimuli, a 10-minute Stop Signal Anticipation Task evaluating response inhibition, which is found to engage the vmPFC, and a 7-minute single visit Fear Conditioning task to measure fear learning, by pairing an aversive sound with a yellow or blue lamp (not extinction), shown to engage vmPFC, amygdala, and hippocampus. An additional 10-minute resting-state scan followed these scans (Figure 1).

TMS Targeting

Following the scans, we identified a region in the right dorsolateral prefrontal cortex (rDLPFC) of each participant most strongly functionally connected with the right amygdala. The rDLPFC was defined as Brodmann area 8, 9, 10, and 46, and the rDLPFC target was defined using RSFC with the right amygdala as the seed region and the peak within a DLPFC mask. DLPFC target data were mapped onto MNI coordinates using the Brainsight TMS Navigation system.

The target identified from negative RSFC and the second resting-state scan were used to compare against the first resting-state scan target to, respectively, determine the impact of positive versus negative targeting (Hypothesis 2) and stability of the DLPFC target (Hypothesis 3) (see statistical analyses section). For two participants, excessive motion during the resting state scans necessitated a 0.3mm increase in the motion sensor threshold (total 0.8mm) compared to the 0.5mm threshold set for all other participants.

TMS Protocol

The region identified from the first resting state scan was targeted with twice daily TMS sessions (active or sham) over ten consecutive weekdays using neuronavigation (See Figure 2). Each of the 20 sessions consists of 1800 stimulations @1Hz over the rDLPFC, 120% motor threshold. Two sessions per day, 30 minutes each, are delivered with a 10-minute break to reduce fatigue. Participants were informed that they would receive either active or sham TMS treatments. After the TMS treatment sessions, the participant and researcher completed a data form asking if they thought the treatment the participant received was active or a sham.

To ensure the reliability and reproducibility of the results, the entire study was implemented with a randomized, sham-controlled, and double-blinded design. An independent investigator oversaw the process of randomizing the treatment groups, while all investigators and patients were blind to the treatment condition of patients to reduce bias. Once the study concluded, all

participants were offered the chance to receive open-label treatment, in which both the patient and the researcher were aware of the type of treatment being administered.

Data Analysis

The dependent variable is each participant's XYZ MNI coordinates that located their personalized DLPFC target of the area with the highest positive correlation to the amygdala after RS 1. The MNI system standardizes brain location representation using XYZ coordinates relative to the anterior commissure at the center of the MNI brain template. X, Y, and Z correspond to left-right, anterior-posterior, and superior-inferior positions, respectively, and are reported in millimeters from the origin, the intersection between the anterior and posterior commissures.

For hypothesis 2, a second target was defined as the area with the highest negative correlation to the amygdala after RS 1. The independent variable is the positively and negatively correlated DLPFC targets.

For hypothesis 3, a third target was defined as the area with the highest positive correlation to the amygdala after RS 2. The second independent variable is the two resting states from pre- vs. post-fear neurocircuitry engagement (rest 1 and rest 2).

We measured each participant's personalized DLPFC target of the area with the highest positive correlation to the amygdala after Rest 1, then compared that to their respective DLPFC target of the area with the highest negative correlation to the amygdala after Rest 1.

All statistical analyses were conducted using IBM SPSS 28.0. For statistical interpretation, the statistical significance was set to $p \leq 0.05$. To test our three hypotheses, we used two different repeated measures ANOVA models.

To test Hypothesis #1 and Hypothesis #3, we used a TargetCoordinates (3; X, Y, Z) * Time (2; RS 1 vs. RS 2) within-subject repeated measures ANOVA. For Hypothesis #1, we analyzed the

between-subject effects to measure individual variability in targeting. For Hypothesis #3, we compared the X, Y, and Z target coordinates between RS1 and RS2.

To Test Hypothesis #2, we use a TargetCoordinates (3, X, Y, Z) * TargetDefinition (2; RS 1 Positive vs. RS1 Negative) within-subject repeated measures ANOVA. In this 3*2 Repeated Measures ANOVA, we compared the X, Y, and Z target coordinates between positive and negative target definitions.

Results

Participants

The study sample consisted of 17 participants (n=13 women; n=5 Black) with (sub-threshold) PTSD after experiencing at least one traumatic event in their lifetime. Details on demographic variables are described in Table 2.

Hypothesis 1 – Variability of DLPFC Target Between Subjects

Statistical analyses using a 3 (X, Y, Z Coordinates) 2 (RS 1, RS 2) repeated measures ANOVA showed a significant between-subjects effect, $F(1,16)=3005$, $p<0.001$, $\eta p^2=0.99$ (Table 3), suggesting substantial differences in target locations between participants across states.

Hypothesis 2 – Variability of Positive and Negative Target Definitions

A 3 (X, Y, Z Coordinates) x 2 (RS 1 Positive vs. RS1 Negative Target Definition) repeated measures ANOVA showed a significant effect of positive versus negative target definition, $F(1,16)= 6.2$, $p=0.02$, $\eta p^2=0.28$ (Table 4), such that targets defined using positive RSFC with the right amygdala resulted in different targets than defined using negative RFSC with the right amygdala. The interaction between XYZ coordinates and target definition was also significant, $F(2,32) = 7.1$, $p =0.03$, $\eta p^2=0.31$. A subsequent paired-samples t-test indicated that the effect of target definition for positive versus negative RSFC was only significant for the Y coordinates ($t(16) = 3.7$, $p < 0.001$, $d = 0.89$).

Hypothesis 3 – Stability of DLPFC Target Across States

Finally, the 3 (X, Y, Z coordinates) x 2 (Rest 1, Rest 2) repeated measures ANOVA demonstrated that the differences in participants' target location between RS 1 and RS 2 were not significant $F(1,16)=0.8$, $p=0.38$, $\eta p^2=0.05$ (Table 4).

Discussion

In this neuroimaging study, we show that DLPFC targets defined using positive RSFC with the right amygdala varied significantly between participants. Results show that DLPFC targets defined using positive RSFC with the right amygdala resulted in significantly different targets than those defined using negative RFSC with the right amygdala. Additionally, this study has demonstrated that the DLPFC target was stable over states. These findings suggest that the rDLPFC exhibits inter-individual variability, inter-target variability, and intra-individual stability.

Overall, the results of this study have important implications for the field of neuroscience, as they highlight the importance of understanding the variability and stability of the DLPFC across individuals. Our study's significance lies in its potential implications for personalized TMS and PTSD treatments. This work is crucial because it provides valuable evidence in the debate about the need for individualized targeting, showing that there are noticeable differences in the DLPFC targets, and the target is stable.

The DLPFC Target Varies Between Participants

Our first finding from this study shows that the DLPFC target varies significantly across our sample population, which supports our original hypothesis. The substantial differences between participants indicate considerable variability and diversity of participants' targets. In other words, each participant had unique targets in their respective DLPFC regions that varied considerably within the sample size. This also implies that the variability between participants was more significant than the variability caused by change across states.

When examining a 3D scatter plot with each participant's respective Rest 1 and Rest 2 targets, one can also observe the diversity in participants' individualized targets based on the variability in the location of the data points. Some participants had more consistent and tightly clustered data points, indicating greater consistency in their targeted coordinates from Resting State 1 to Resting State 2. In contrast, other participants had more widely dispersed data points, indicating greater variability in the location of their targeted coordinates. This diversity in targets and target changes after engagement may reflect individual differences in brain function or structure and could be related to factors such as age, gender, genetics, or environmental influences.

This finding is consistent with previous literature that suggests individual differences in prefrontal cortex function may be related to differences in emotion regulation and decision-making processes[12,18,19]. This result contributes to the growing body of literature on personalized neuroimaging-based treatments by highlighting the importance of individual differences in DLPFC-amygdala functional connectivity.

The study conducted by Jackson et al.[51] shed light on the importance of considering individual differences in baseline DLPFC-amygdala connectivity in TMS treatment. The study results showed that variability in functional connectivity between these two brain regions might have more consequences than previously known, underscoring the need for personalized treatment approaches. The results of their study are significant, as they suggest that targeting the DLPFC using TMS may have varying efficacy depending on the individual's baseline connectivity between the DLPFC and amygdala.

Our study supports and extends the findings of Jackson and colleagues by demonstrating significant individual variability in DLPFC targets, which suggests that variability in functional connectivity to the amygdala may play a critical role in the pathophysiology of PTSD. However, we have not related findings to PTSD severity. This underscores the need for further research to

understand the underlying mechanisms driving individual differences in DLPFC functional connectivity and to develop more personalized neuronavigational-based treatment approaches that account for such differences.

Positive and Negative Target Definitions Varied Significantly

This study showed significant differences in positively and negatively correlated coordinates across our sample population. After creating an estimated mean of variance graphs (figure 16), it appeared that the differences between the positive and negative target definitions were most likely driven by the Y coordinate. Our results contribute to the growing body of literature[34,44] on personalized neuroimaging-based treatments by highlighting the potential importance of individual differences in positive and negative functional connectivity patterns.

Although other studies have not investigated the differences between positive and negative DLPFC-amygdala target definitions, prior studies have shown that negative correlations involving the amygdala are linked to reduced clinical PTSD symptoms. At the same time, past research has also investigated functional connectivity between the prefrontal cortex and other brain regions[41–43]. In contrast, our results suggest that there may be individual differences in the stimulation or inhibition of the functional connectivity patterns within this region. Whether therapeutic interventions like TMS should target these positive or negative correlations is still debated. However, our findings suggest that targeting one type of correlation over the other may result in different targets, which warrants further and more extensive research.

The DLPFC Target is Stable

Our study found that the DLPFC target did not change after fear and neurocircuitry engagement, indicating the stability of this region as a potential target for TMS. This finding aligns with previous literature that suggests the DLPFC is a critical brain region involved in emotion regulation and decision-making processes, and its stability as a target for TMS has been indicated in

previous studies[28,30,44]. Our results provide further evidence to support the use of TMS as a potential treatment for mental health disorders by highlighting the stability of the DLPFC target.

These results further show that fear and neurocircuitry engagement did not have differential effects on DLPFC targets. The findings indicate that the XYZ coordinates remain stable between rest 1 and rest 2 scans and that any differences in target locations were not significantly influenced by the change across states.

Yet, the significant findings of variability between the positive and negative target definitions lend credibility to our findings of DLPFC target stability. Our results show that despite our small sample size, the data is likely powered enough to show a substantial effect. The significant effect sizes suggest that an increased sample size would lend greatly to the validity of these results. It also means that the statistical test being used is susceptible enough and can detect minor effects in the data, which suggests that the sample size is large enough to detect any significant differences in the population with high probability.

While previous studies have investigated the use of TMS in targeting the DLPFC[28], this study specifically focused on the stability of this target after fear and neurocircuitry engagement. Further research is needed to replicate and extend our findings and to explore the potential of personalized TMS interventions based on individual differences in DLPFC target stability.

Limitations

Several limitations need to be considered when interpreting these results. One challenge of interpreting the data is determining whether the lack of significant differences in the RS 1 vs. RS 2 target differences is due to the absence of an effect by the fear engagement tasks or if the study was underpowered due to the relatively small sample size used in the study. With a small sample size, it is possible that the results are not generalizable to the larger population, and the statistical power of the analysis may be limited. Additionally, the limited sample size may constrain the ability to detect

small but significant effects. Increased sample size would increase the study's statistical power and reduce the risk of Type II errors. Therefore, caution should be exercised when applying these findings to other populations or using them to make broader generalizations.

An effort to increase generalizability included the careful monitoring of movement during the MRI scans. Participants were instructed to remain still during the scan, and their head was stabilized using a cushioned headrest. Any movement during the scan was recorded and could be accounted for in the analysis. Additionally, the study's double-blinded nature is essential in minimizing the risk of bias and confounding factors. However, it also presents challenges when interpreting clinical results, as it makes it difficult to draw any conclusions about the clinical significance of the findings. Thus, the clinical outcomes were only mentioned as demographic factors and were not analyzed or extrapolated further. Further research with larger sample sizes, control groups, and more diverse populations is needed to increase the generalizability and validity of the findings.

Future Directions

To build on the results that individuals' DLPFC targets vary, future studies could investigate whether this variability in DLPFC targeting is related to treatment response or non-response. An experiment could divide participants into groups based on their response to treatment (i.e., responders and non-responders), and the stability of their respective DLPFC stimulation could be subsequently compared. Investigating if treatment non-response is related to participants' increased DLPFC target instability could be beneficial for better individualizing TMS treatments. These analyses will be performed upon unblinding of the parent clinical trial after completion.

Furthermore, studies could investigate the role of individual differences in DLPFC anatomy and function in TMS treatment response variability. It could be interesting to examine whether differences in DLPFC morphology, such as cortical thickness and gray matter volume, could be

associated with differences in treatment response. Investigating these individual variabilities may help to identify subgroups of patients who could benefit most from TMS treatment. Ultimately, a better understanding of the factors that influence treatment response could lead to more effective TMS approaches that improve patient outcomes and quality of life.

As a follow-up to the finding that negative and positive target definitions significantly differ, an interesting approach could use randomized controlled trials comparing the effectiveness of TMS treatment targeting positively correlated DLPFC-amygdala regions versus negatively correlated regions. Exploring the clinical implications of using positive and negative DLPFC target definitions for TMS could provide insight into using brain stimulation for other psychiatric disorders. For example, Fitzgerald and colleagues (2009)[52] found that individual differences in DLPFC target stability predicted response to TMS treatment for depression, suggesting that improving the accuracy and consistency of DLPFC targeting using TMS could be important for treatment outcomes and efficacy.

Lastly, future work could focus on determining the feasibility of implementing neuroimaging-based TMS treatments in clinical applications. Since our work has shown that the DLPFC target is variable and stable, it will be critical to determine if this type of treatment has a clinical benefit. The use of fMRI neuronavigation is novel due to the increased complexity, cost, and time, which are barriers for smaller TMS treatment centers. So, it will be significantly beneficial for future studies to help understand if there is a clinical benefit to making this form of treatment more standardized.

Conclusion

Overall, these findings show that the stability and variability of the rDLPFC target's connectivity with the amygdala is a promising starting point for TMS for PTSD. Although this study has not addressed the feasibility of implementing individualized targeting, we have shown that using

the rDLPFC to target the amygdala is stable across states and shows variability between people and between positive and negative target definitions. The findings from this study benefit the larger scientific community by informing the next stage of developing personalized TMS protocols for individuals with PTSD, potentially improving treatment outcomes. Knowing that the DLPFC target is stable and variable is a pivotal foundation to determine whether this method is reliable and is related to increased treatment efficacy. Further research will help understand these findings' potential clinical applications fully.

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Tables and Figures

Figure 1.

Functional MRI Tasks to Engage Fear Inhibition Neurocircuitry. The diagram illustrates the three functional MRI tasks used to engage the fear inhibition neurocircuitry: the 7-minute Fearful faces task, the 10-minute Stop Signal Task Inhibition, and the 7-minute single visit Fear Conditioning task. These tasks were designed to measure the response to fearful vs. neutral stimuli, response inhibition, and fear learning, respectively, and engaged various brain regions, including the vmPFC, hippocampus, and amygdala. An additional 10-minute resting state scan followed the scans.

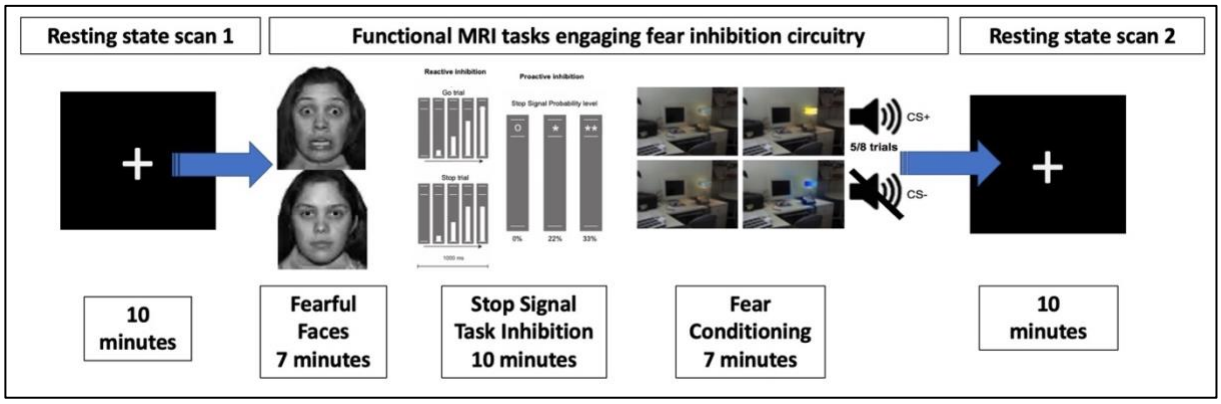


Figure 2.

Example of TMS coil placement on a volunteer's head using individualized MRI scans. The figure shows a 3D reconstruction of the volunteer's head with the center of the TMS coil positioned over the left dorsolateral prefrontal cortex (DLPFC) region of interest. The coil is placed at a 45-degree angle to the scalp, and the handle points towards the back of the head. The coil is held in place by an adjustable arm mounted on a headrest, allowing for precise and stable placement during TMS sessions. MRI scans ensure accurate and consistent coil placement across TMS sessions and between participants.

Figure 3.

Neuronavigation of the rDLPFC target. The image displays a patient's sMRI in native space, with a right Resting State Functional Connectivity (RSFC) map overlay in warmer colors, indicating greater RSFC with the right Amygdala. The green coil shows the location of the TMS coil in real-time, which is precisely targeted to the rDLPFC using millimeter precision (<2mm from the target), as indicated by the red dot on the top right. The axial T1 image with RSFC map overlay and green crosshair indicates the precise location of the TMS coil relative to the target.

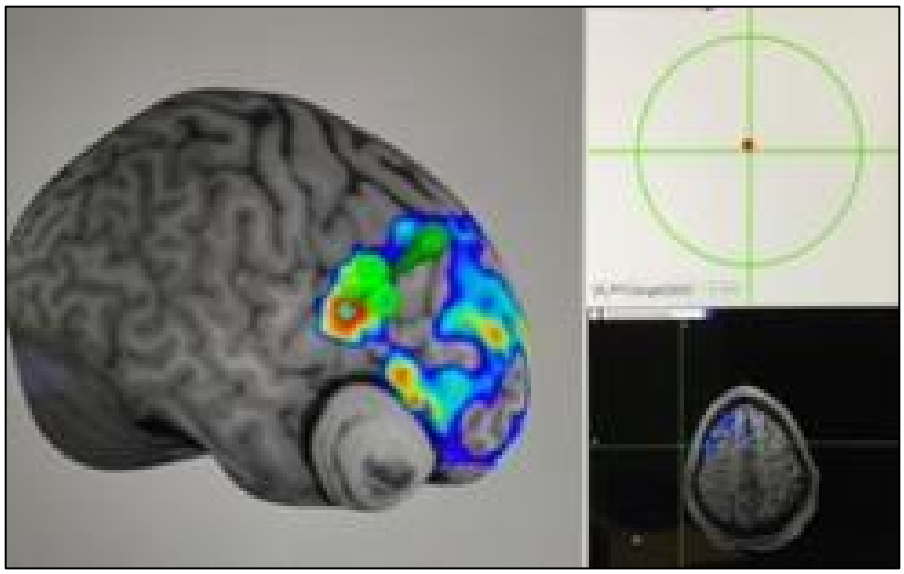


Table 1.

Inclusion Criteria Table for Study Participants. This table presents the criteria used to determine the eligibility of participants for the study.

Inclusion Criteria	Exclusion Criteria
Men and women 18-65 years old (all ethnicities and races).	Having active suicidal intent or plan as defined by: ▪ a positive answer to questions 4 and/or 5 on the Columbia-Suicide Severity Rating Scale (C-SSRS) or ▪ more than one suicide attempt in lifetime; or ▪ suicide attempt in the past twelve months; or ▪ the clinician's opinion, is likely to attempt suicide within the next six months.
Diagnosed with sub-threshold PTSD according to the DSM-5 criteria, if they meet 3 out of 4 symptom clusters on the PCL-5 (Must include hyperarousal symptoms)	Taking psychotropic medications, including antidepressants, antipsychotics, benzodiazepines, and anticonvulsants.
At least one symptom to meet for criterion C (avoidance symptoms)	Diagnosed with the following conditions: ▪ a neurological disorder, including a history of seizures, ▪ cerebrovascular disease, ▪ primary or secondary tumors in CNS, ▪ stroke, ▪ cerebral aneurysm or movement disorder, or ▪ any lifetime history of loss of consciousness for more than 5 minutes due to head injury ▪ psychotic disorder or bipolar affective disorder
At least two symptoms to meet for criterion D (negative changes in cognitions and mood)	History of cranial surgery, metallic particles in the eye or head (exclusive of mouth), implanted cardiac pacemaker or any intra-cardiac lines, implanted neurostimulators, intra-cranial implants (e.g., aneurysm clips, shunts, stimulators, cochlear implants, or electrodes) or implanted medical pumps.
At least one symptom to meet for criterion E (hyperarousal)	Current substance abuse or dependence as indicated by a score of 6 or higher on the Drug Abuse Screening Test (DAST)
Capable and willing to provide informed consent.	Current alcohol abuse or dependence as indicated by a score of 8 or higher on the Alcohol Use Disorder Identification Test (AUDIT)
Able to adhere to the treatment schedule ▪ 9 am to 3 pm sessions Monday through Friday for 2 weeks transportation supplied if necessary	For women, being pregnant or sexually active and not using birth control.
Having a BMI under 40	Currently participating in another clinical study or enrolled in another clinical study within 30 days prior to this study or started (new) treatment for PTSD within 3 months before this study. Or Previous treatment with TMS

Table 2.

Demographics of Study Sample. The table displays the completion status, sex, race, ethnicity, and type of criterion A traumatic event of the study sample. N represents the number of participants, and percentage represents the proportion of participants in each category." Figure 1: Descriptive statistics of age in the study sample (n=17). This table displays the descriptive statistics of age in the study sample, including mean, median, mode, range, and standard deviation. The mean age was 40.35 years (SD=12.93), with a median age of 43 years and a mode of 27 years. The age range of the sample was 21 to 63 years.

		Frequency (N)	Percent %
Completion Status	Completed without Open Label Treatment	8	38.1%
	Completed with Open Label Treatment	6	28.6%
	Dropped Out	5	23.8%
	Half Completed	2	9.5%
Sex	Female	13	76.5%
	Male	4	23.5%
Race	White	9	52.9%
	Black	5	29.4%
	Asian	1	5.9%
	Hisp/Lat	1	5.9%
	Mixed	1	5.9%
Ethnicity	Not Hisp	15	88.2%
	Hisp/Lat	2	11.8%
Type of Traumatic Event	Sexual Assault	7	41.2%
	Physical Assault	3	17.6%
	Sudden Death	2	11.8%
	Assault with a Weapon	1	5.9%
	Combat/Exposure to a War-Zone	1	5.9%
	Life-Threatening Illness/Injury	1	5.9%
	Transportation Accident	2	11.8%
Age	18-29 years old	4	23.5%
	30-39 years old	4	23.5%
	40-49 years old	5	29.4%
	50+ years old	4	23.5%

Figure 4.

Change in PCL-5 Scores over Time. To measure the effects of the TMS treatment on PTSD symptoms, the PTSD Checklist for DSM-5 (PCL-5) was collected pre-TMS in week 1, after TMS in week 4, and 3 months later. The graph displays the change in PCL-5 scores over the study, with some scores decreasing, some increasing, and some remaining stagnant. Individual participants are labeled with a Subject Identifier Number (SID) to maintain confidentiality. A paired-samples t-test indicated that PCL-5 scores were significantly lower post-treatment (M = 26, SD = 14) than for pre-treatment (M = 42, SD = 12), $t(14) = 6.1$, $p < 0.001$, $d = 10.4$).

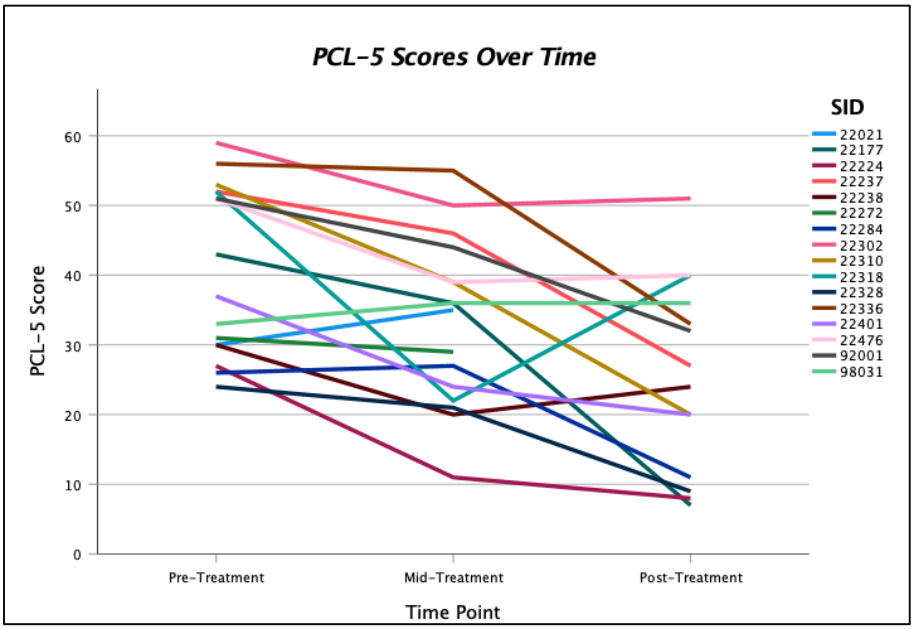


Figure 5.

Change in BDI Scores over Time. To measure the effects of the TMS treatment on depressive symptoms, the Beck Depression Inventory (BDI) was collected pre-TMS in week 1, after TMS in week 4, and 3 months later. The graph displays the change in BDI scores over the study, with some scores decreasing, some increasing, and some remaining stagnant. Individual participants are labeled with a Subject Identifier Number (SID) to maintain confidentiality. A paired-samples t-test indicated that BDI scores were significantly lower post-treatment (M = 16, SD = 12) than for pre-treatment (M = 28, SD = 12), $t(14) = 4.1$, $p < 0.001$, $d = 11.5$)

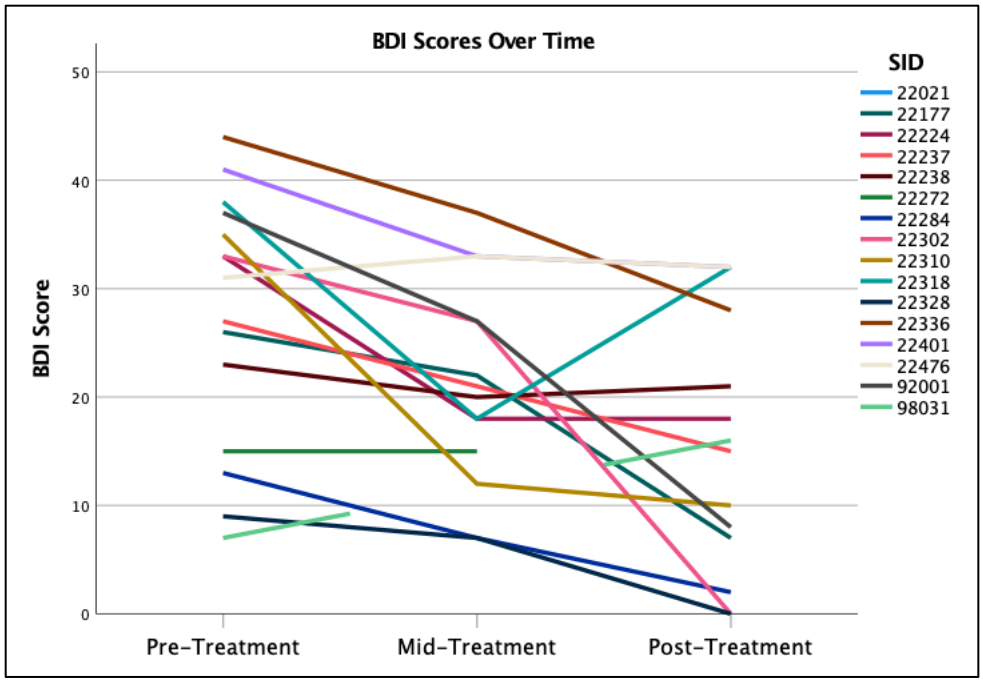


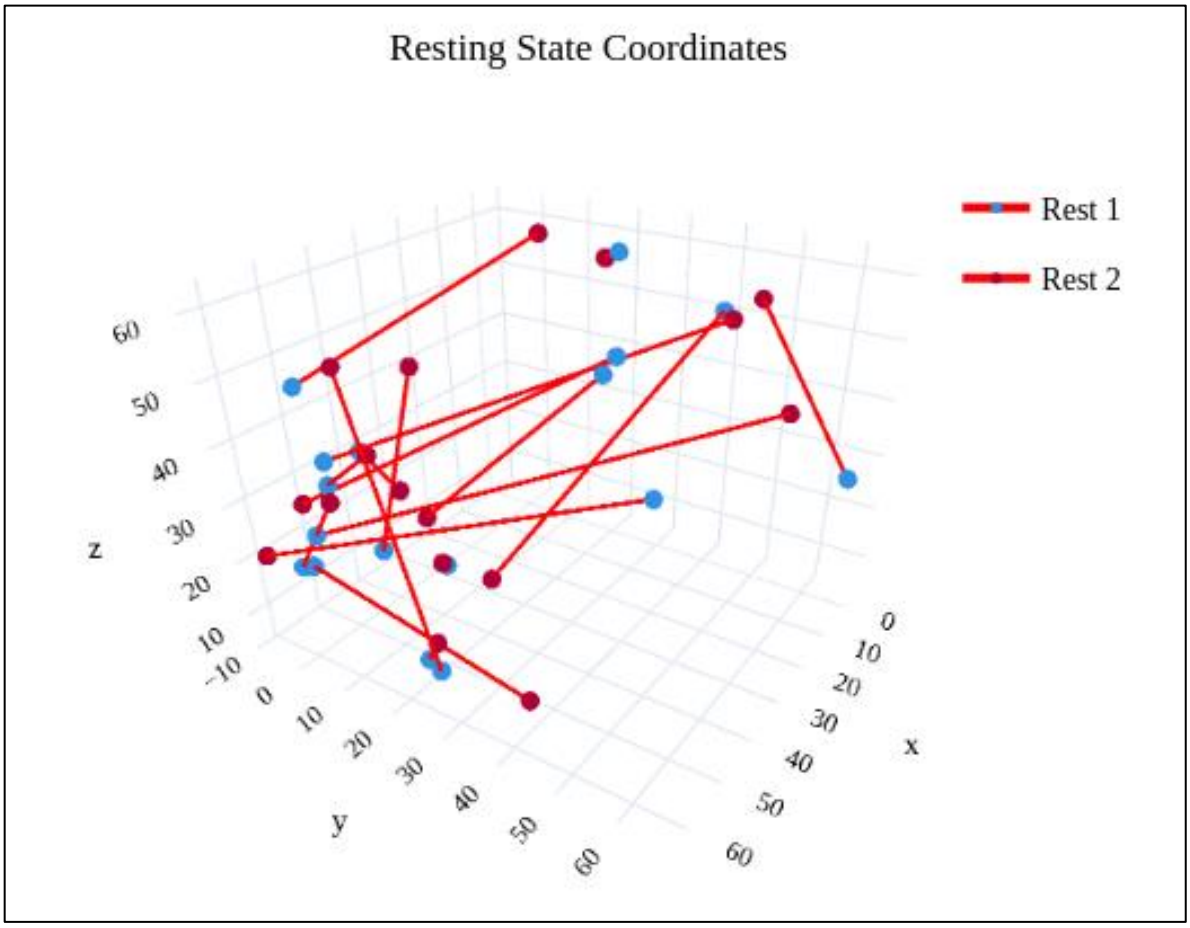
Table 3.

Results of ANOVA test for within and between-subject effects- The table shows the results of an ANOVA test examining the within and between-subject effects of individual variability and neurocircuitry engagement on the DLPFC target. The two Within-Subject Factors are XYZ Coordinates, with 3 levels (X, Y, and Z), and Rest 1 vs. 2, with 2 levels (Rest 1 and Rest 2). Significance was computed using alpha=0.05.

Source of Variation	Type III Sum of Squares	df	Mean Square	F	P-value	Partial Eta Squared	Observed Power
Between Subjects	100957.7	1	100957.7	3005.2	<0.001	.995	1.000
Error	537.5	16	33.6				
Across States (Rest 1 vs Rest 2)	36.5	1	36.5	0.811	0.381	0.05	0.14
Error	720.0	16	45.0				

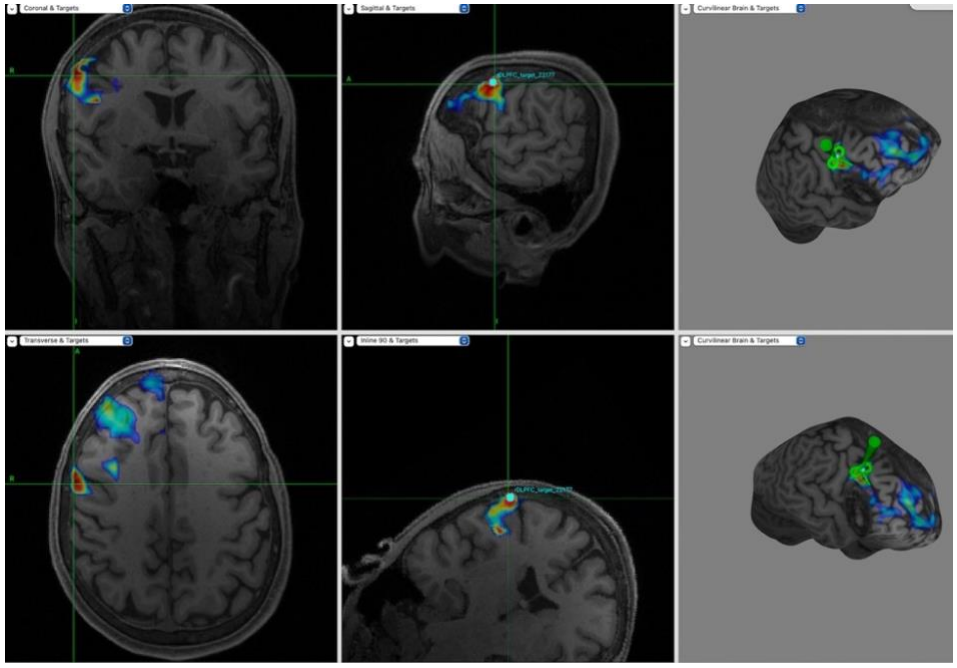
Figure 6.

3D scatter plot showing the XYZ coordinates of participants' targets. The x, y, and z-axes represent the XYZ coordinates, and the unit of measurement is in mm. Lines between points indicate target change from Rest 1 to Rest 2. Rest 1 target coordinates are represented by blue dots, while Rest 2 targets are represented by red dots. The lines between points indicate the change in target location between Rest 1 and Rest 2. The scatter plot shows the distribution of target locations across the two resting states, with some participants having very little change in target locations while others had more noticeable changes. The link to an interactive, draggable version of this graph can be found here: https://plotly.com/~loistb/2/.



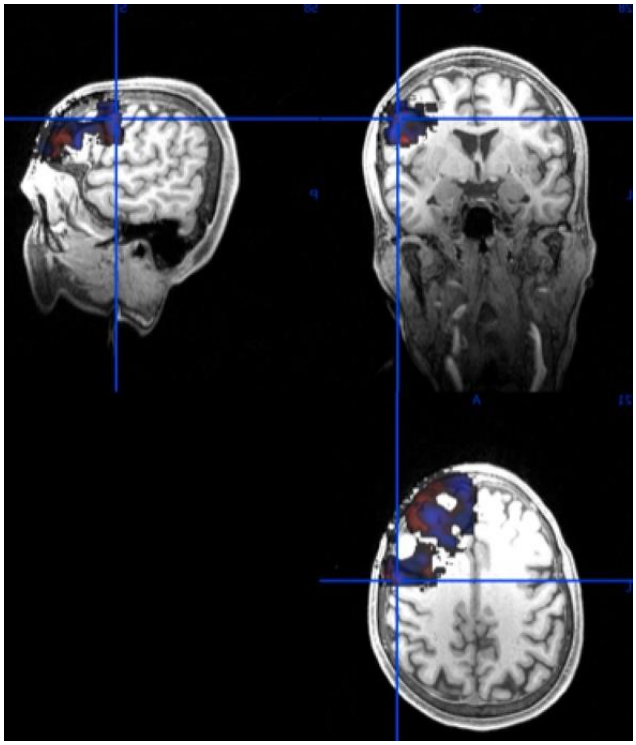
Figures 7-9.

Example of variability between RS 1 and RS 2 targets. Brain activation map showing areas of increased activity during the task. Figures generated using Yale BioImage Suite Medical Image Analysis Software [Version 1.2, 2020/08/05]. The targets displayed are for SID 22177.



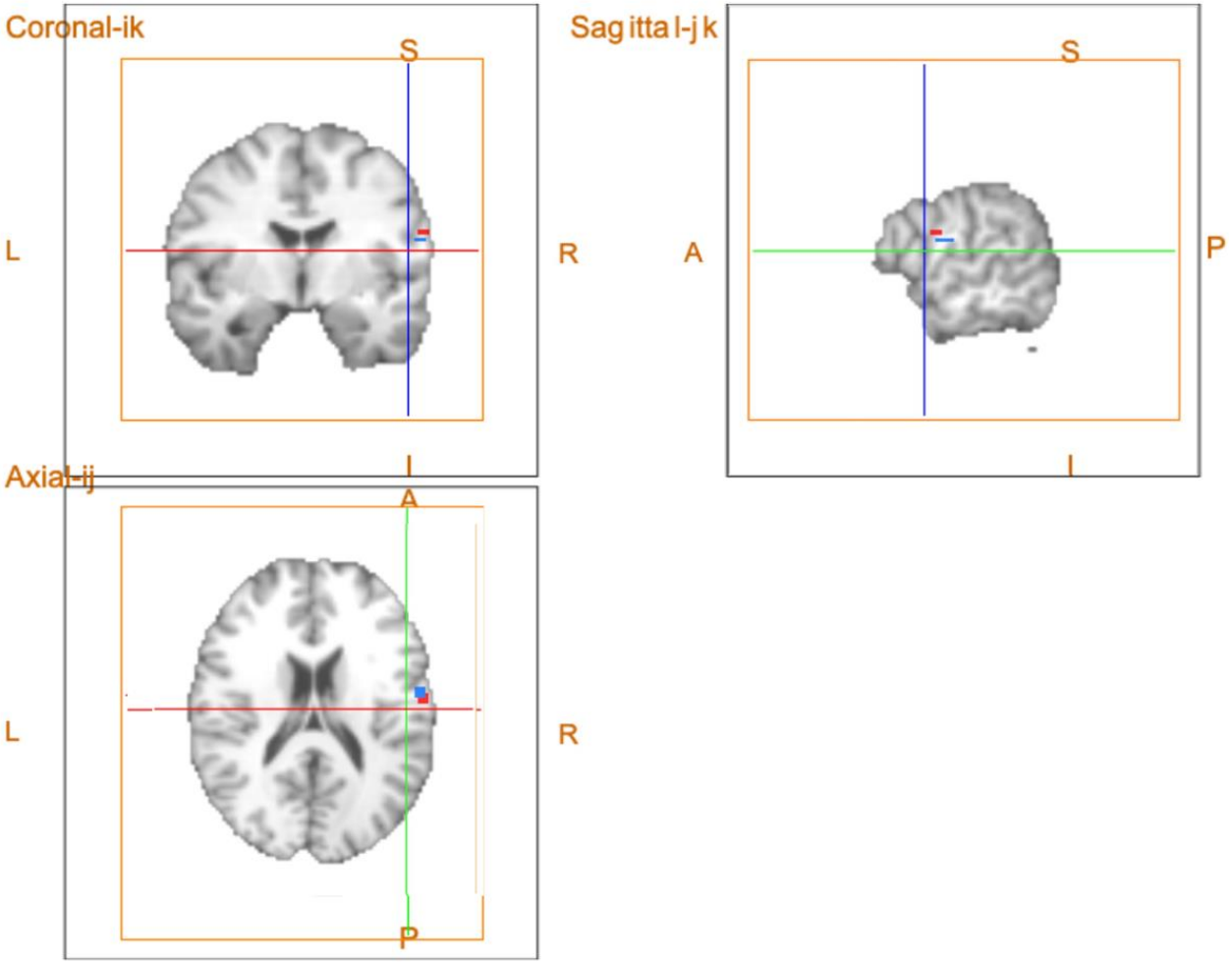
Rest 1 Target:

(60, 9, 35)



Rest 2 Target:

(62, 3, 23)



Change in the target location. Rest 1 Target is indicated by a red dot, while Rest 2 Target is indicated by a blue dot. Figure generated using MRIcro software.

Table 4.

Results of ANOVA test for within and between-subject effects- The table shows the results of an ANOVA test examining the within and between-subject effects of individual variability and positive and negative correlation on the DLPFC target. The two Within-Subject Factors are XYZ Coordinates, with 3 levels (X, Y, and Z), and Rest 1 vs. 2, with 2 levels (Rest 1 and Rest 2). Mauchly's Test of Sphericity was met (Mauchly's W=0.809, p=0.205, Approx. Chi-Square = 3.171). Significance was computed using alpha=0.05.

Source of Variation	Type III Sum of Squares	df	Mean Square	F	P-value	Partial Eta Squared	Observed Power
Between Subjects	165894.2	1	116894.2	1736.1	<0.001	.991	1.000
Error	1077.3	16	67.3				
Target Definition (Positive vs Negative)	328.3	1	328.3	6.2	0.024	0.280	0.649
Error	845.2	16	52.8				
Interaction of XYZ coordinates * Target definition	6332.5	2	3166.3	7.1	0.03	0.309	0.908
Error	14173.5	32	442.3				

Figure 10.

Estimated Marginal Means of Coordinates by Correlation Group. The graph shows how the means of XYZ coordinates vary across different levels of the independent variables, particularly in the Y direction. X-axis represents XYZ coordinates, and Y-axis represents the estimated marginal means for each level of the independent variables of Rest 1 Positive Target Definitions, Rest 2 Positive Target Definitions, and Rest 1 Negative Target Definitions.