THE HEART–BRAIN DIALOGUE

PATHWAYS TO TMS TREATMENT OPTIMIZATION

LAUREN ZWIENENBERG

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Lauren Zwienenberg

The work described in this doctoral dissertation was carried out at Synaeda Psycho Medisch Centrum and Stichting Brainclinics Foundation, in collaboration with Maastricht University

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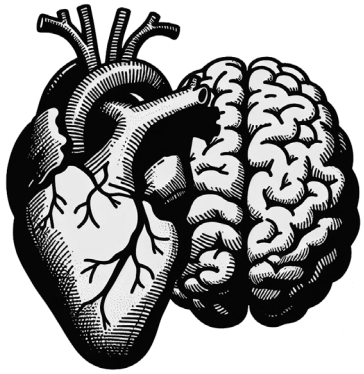

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INTRODUCTION

Elien.

Elien was a late child with siblings almost 10 years older than her. Her father was an alcoholic. He was absent throughout her childhood and her mother probably overcompensated for the lack of attention of her dad: they did everything together. When Elien was in secondary school, her parents divorced. She experienced a period of sadness, hopelessness for the future in which she lost pleasure and energy to do things, hence she mostly stayed at home and eventually did not finish school. Though, she did not seek help. She started working in a fashion store, which she enjoyed immensely. A few years later, her mother fell in love with another man, who became a much-loved father figure for Elien.

At 25, Elien moved out and she quickly met her soulmate. They got married and became parents of a lovely girl after an almost perfect pregnancy and delivery. Elien loved being a mother. Three years later, she was pregnant again, this time with a boy. The delivery was quick but intense. The period after the birth of her son, Elien felt gloomy, had no energy and experienced some mild psychotic symptoms. She tried to end her life by taking a large amount of medication. Her husband drove her to the emergency room and afterwards she stayed for almost two weeks at the intensive home treatment facility to stabilize. She was then treated with cognitive behavioural therapy and antidepressant medication, to which she responded well.

To rebuild her life, she started working again and she enjoyed family-life, but after a little while she started questioning life in general. Questions like 'is this it?' popped up regularly, accompanied, by amongst others, previously experienced sadness, hopelessness, nausea and decreased appetite. During that time, her stepfather died of cancer after knowing he was sick for only a few weeks. Due to how bad she felt already, she did not experience this period of mourning fully. The restart of cognitive behavioural therapy and medication resulted in a relief of symptoms within a few months. She continued her medication for a few extra months but was eventually able to live without.

Another stressful period followed: her son was not doing well at school and after a lot of testing was sent to a specialized school, the well-needed summer holiday turned out to be tense instead of relaxing, a friendship ended, and on top of that, her mother was diagnosed with breast cancer. Sitting at the dining table, Elien recognized her feelings from before: she knew exactly how it previously felt, however, this time it was much worse. She knew she needed help. She was prescribed medication again, but they did not relieve any symptoms. As a result, Elien took all her medication at once: she could not feel her pain if she was not alive. After admission to the emergency room, she was sent home, because she disagreed to everything they offered. Her family hid her medication, but two weeks later, she found them and tried to end her life again. This time, she was sent to an intensive care unit for two weeks. Eventually, after being stabilized, she was referred to Synaeda, a Dutch outpatient adult psychiatry clinic, and she was diagnosed with recurrent major depressive disorder.

DEPRESSION

Major depressive disorder (MDD), more commonly known as depression, is a mental health state in which patients, on more days than not, experience five or more of the following symptoms: a depressed mood, loss of interest, weight changes, sleep disturbances, psychomotor agitation/retardation, fatigue, feelings of worthlessness and excessive guilt, decreased concentration and thoughts of death and suicide, as presented in the Diagnostic and Statistical Manual of Mental Disorders (5th ed., DSM-5; American Psychiatric Association (APA), 2013). Approximately 11% of people worldwide experiences at least one depressive episode in their life (Herrman et al., 2022), with a prevalence around 4.4% (World Health Organization, 2017) and it is estimated that the likelihood of a suicide attempt is at least five times higher for individuals with depression (Nock et al., 2010). As presented in the case of Elien, depression is a very debilitating and sometimes life-threatening disease. In combination with the high prevalence, this makes it extremely important to have validated, effective treatments, both for quality of life of patients and to reduce stress on and costs of the mental health care system.

Over the years, various effective and valid antidepressant treatments were developed (Karrouri, 2021), aiming for the patient to remit and return to baseline level functioning. Treatment decision guidelines like the one established by the APA (2019) aid in determining which treatment is suitable at a given 'stage' of a depressive episode. This so-called stepped care model (Arns et al., 2022) aims to ensure that patients receive treatments matching their needs: starting with accessible, low-intensity, low-cost treatments with fewest side-effects. In case of non-response, a shift is made to more advanced treatments, often with more side-effects (van Straten et al., 2010). Psychotherapy and pharmacotherapy are proven effective and generally well tolerated, therefore they are considered the golden standard for first-line treatments. Depression-focused psychotherapy consists of many different methods like cognitive behavioural therapy (CBT), and in general aims for the patient to understand their symptoms and to regulate negative thoughts and behaviour that contribute to their depressive symptoms (Nyer et al., 2023). CBT is commonly linked to changes in brain regions like the prefrontal cortex (Yuan et al., 2022), amygdala and insula (Gorka et al., 2019; Zhou et al., 2021) and the subgenual anterior cingulate cortex (sgACC; Feurer, 2022). First-choice antidepressant medication (AD) are selective serotonin-reuptake inhibitors (SSRIs) like escitalopram, citalopram and sertraline, followed by serotonin-norepinephrine reuptake inhibitors (SNRIs) like venlafaxine. SSRIs block - as the name suggests - the reabsorption of serotonin in the synaptic cleft of the presynaptic nerve terminal of the neurons, increasing serotonin levels in the brain (Edinoff et al., 2021). SNRIs act on the serotonergic systems and, with increasing dosages, on the noradrenergic neurotransmission too (Sansone et al., 2014).

However, despite the general clinical efficacy of these available standard therapies, around 30% of depressed patients do not respond after two adequately dosed antidepressant treatments. These patients are considered treatment-resistant (TRD; Voineskos et al., 2020), or - probably better suiting - difficult-to-treat (DTD; Wilhelm, 2019). Available treatments for DTD are for example more extensive psychotherapy approaches, pharmacological augmentations like lithium and switches to other AD classes like tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs). Over the last decades, a field of research

emerged investigating neuromodulation treatments for depression in which vagal nerve stimulation (VNS), deep brain stimulation (DBS) and transcranial direct/alternating current stimulation (tDCS/tACS) are extensively studied as treatment options for DTD, with promising results (Figee et al., 2022; Kamel et al., 2022; Jog et al., 2023). Other neuromodulation treatments that are not only studied but are proven effective and used in clinical practice for DTD include electroconvulsive therapy (ECT) and transcranial magnetic stimulation (TMS). ECT is the most established and effective treatment for DTD with remission rates from 51-83% for non-psychotic DTD patients (Petrides et al., 2001; Wagenmakers et al., 2022). However, ECT is an invasive treatment requiring general anaesthesia. The treatment induces a short seizure by electrical currents that are sent through the brain (Trifu et al., 2021). ECT may cause several mild to severe side-effects, like headaches, nausea, cardiovascular events and (short- or longer-term) retrograde amnesia (Andrade et al., 2016). TMS on the other hand, is a relatively accessible, non-invasive brain stimulation (NIBS) treatment option with fewer and less severe side effects. Furthermore, TMS for depression has regulatory approval. Therefore, TMS is the best suited treatment option for DTD for outpatient clinical care, at the moment.

TRANSCRANIAL MAGNETIC STIMULATION FOR DEPRESSION

TMS is used to stimulate specific cortical brain regions also affecting functionally connected brain regions. TMS is administered by placing an electromagnetic coil on the scalp above the target brain area. A rapidly changing magnetic field is induced by the electric currents that pass through the coil. This changing magnetic field, or magnetic pulse, passes through the skin and skull and induces an electric field at the targeted brain region causing electrical currents to flow along the E-field gradient (Barker et al., 1985). This can result in depolarization of the nerve cells (neurons) and subsequent firing of action potentials. TMS can be applied repetitively (rTMS) with a certain stimulation frequency, measured in Hertz (Hz), opting for longer-lasting effects (Fox et al., 2012). Following rTMS, neurons are either excited or inhibited in the targeted brain area (Radhu et al., 2012; Klomjai et al., 2015), thereby modulating neural activity (Siebner et al., 2022). There are, however, clear inter-individual and intra-individual differences regarding

inhibition or excitation, suggesting brain-state-dependent influences (Baur et al., 2020). On the synapse level, TMS pulses can change the transmission efficacy, inducing neuroplasticity changes similar to long-term potentiation (LTP) or long-term depression (LTD). Common minor side-effects of TMS stimulation are muscle twitches and headaches at the stimulation site. The most serious risk of rTMS is an induced seizure, although the risk is as low as, or even lower than, using ADs if the safety regulations are followed (Rossi et al., 2021).

In 1985, Barker and colleagues first described TMS as a measure to study the human motor cortex through brain stimulation. Soon after, in 1987, Bickford and colleagues studied TMS effects on the peripheral nerve and were the first to report that "several of our normal subjects have noted mood elevation". At this time, TMS research focused on exploring neuromuscular functioning and its use in diagnostics. From the 1990's, the scope of TMS was expanded to cognitive neuroscience – studying language, perception and memory - (Pascual-Leone et al., 1994), often using TMS to disrupt brain functioning and assess the effects (Jahanshahi & Rothwell, 2000). With the development of rTMS and consequently longer lasting brain activity changes, TMS started to being tested for therapeutic purposes, initially focusing on depression. George et al. (1995) performed the first open label study and Pascual-Leone et al. (1996) the first randomized controlled trial (RCT) studying TMS for depression, with promising results. A large international multi-site study (O'Reardon et al., 2007) – independently replicated by George et al. (2010) – showed that active TMS has greater antidepressant effects compared to sham. This provided crucial evidence for the Food and Drug Administration (FDA) to approve TMS in 2008. The approval considered the Neuronetics Neurostar System for the treatment of depression in patients who did not respond to previous antidepressant treatment.

The actual working mechanisms of TMS for depression remain unknown. However, there is increasing evidence that the functional connectivity of the dorsolateral prefrontal cortex (DLPFC) to the sgACC plays a crucial role in antidepressant response (Padberg & George, 2009; Fox et al., 2012; Fox et al., 2014; Cash et al., 2019; Siddiqi et al., 2021; Elbau et al., 2023). It is hypothesized that rTMS not only activates

the DLPFC, but also transsynaptically activates subcortical regions, like the sgACC, subsequently modulating complete brain networks downstream. This causes normalization of communication within a specific network as well as communication with other brain networks (Bestmann et al., 2004; Beynel et al., 2020)

TMS PARAMETERS

Pulse parameters

Over the years, many more commercial and non-commercial devices were developed, with approximately one new device every two years (Gutiérrez-Muto et al., 2023), along with a wide variety of TMS coils. In 2013, the Brainsway Deep TMS device acquired FDA clearance for DTD followed by a variety of other devices (Cohen et al., 2022). Multiple of these TMS devices have FDA clearance on the basis of the hardware being 'substantially equivalent' to the predicate device. There are, however, clear differences between the observed pulses (Rossi et al., 2021). The Neuronetics Neurostar has a relatively short pulse width (185μs), whereas other widely-used devices like Magstim and MagVenture have pulse lengths around 300μs (Gutiérrez-Muto et al., 2023). Pulse shape (sine vs. square) and phase (biphasic vs. monophasic) are other pulse parameters that are continuously tested with the development of TMS devices that are able to generate a wide variety of pulse options. One extensively studied TMS device that is able to generate square wave pulses with different parameters (including pulse length and phase) is the controllable pulse parameter TMS (cTMS) device (Peterchev et al., 2008; 2011; 2013; 2014). A second, novel, device is the xTMS device, which is developed by the Engineering Department of Oxford University, and is based on the programmable TMS device (Sorkhabi et al., 2020). Using the xTMS, a great variety of pulse parameter settings can be programmed: different pulse lengths, phases, shapes, etc. (Sorkhabi et al., 2022; Ali et al., 2023; Wendt et al., 2023). As both devices are able to produce different parameters within one device, it accounts for inter-TMS-device differences, like coil and power influences, therefore qualifying for measuring differential effects of varying pulse parameter settings. The effects of different pulse parameters have mainly been tested on the primary motor cortex, using the motor evoked potential (MEP). The MEP is an electrical muscle response, resulting from stim-

ulation of the motor cortex – the brain region responsible for voluntary movement (Barker et al., 1985). MEPs can be assessed through physiological measurements and visualization of movement. The physiological measurement is performed using electromyography (EMG) with the placement of skin-electrodes over the target muscle: the abductor pollicis brevis. Visualization of movement is – as the name suggests – visually assessing movement in the hand, generally focusing on the thumb, as a measure of motor cortex activation (Pridmore et al., 1998). Information about the effects of various pulse parameters, as obtained by measured MEPs over the motor cortex, is currently extrapolated to other 'silent' brain regions, due to the lack of an easy-to-assess feedback mechanism in these brain regions.

Stimulation targets

Brain regions like the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), ventromedial prefrontal cortex (vmPFC) and dorsomedial prefrontal cortex (dmPFC) are increasingly studied as novel target locations for TMS for depression (Downar & Daskalakis, 2013; Downar et al., 2014; Kreuzer et al., 2015; Dunlop et al., 2020; Prentice et al., 2023). The primary target, however, has consistently been the DLPFC in both research and clinical practice. Various methods have been developed to define the DLPFC at scalp level.

The earliest TMS studies used the 5CM method, measuring the site 5cm anterior to the motor threshold hotspot (described in the paragraph below; George et al., 1995; Pascual-Leone et al., 1996). However, about one-third of 5CM targets lay outside of the DLPFC area (George et al., 2010), as the method does not consider inter-individual head size differences. Extensions to the 5CM method are the 5.5 and 6cm methods. These provide a slightly different placement aiming to improve the accuracy of DLPFC targeting, especially in individuals with larger head sizes. However, these still use predetermined measures and do not take individual head size differences into account. The 10-20 electroencephalogram (EEG) system-approach was developed by Herbert Jasper in 1958 as a standardized method for placing EEG electrodes on the scalp for brain activity recordings. 10-20 site corresponding to the DLPFC is F3 (left hemisphere) and F4 (right hemisphere) and can be determined by using an EEG cap or through numerous manual measurements

(Herwig et al., 2003). As this method uses relative distances, based on individual head measurements, it thus considers individual scalp (and therefore suggested brain region) differences better compared to the 5CM method. The Beam-F3 (Beam) method is a simplified approach of the 10-20 F3/F4 method, increasing efficiency of measuring the scalp measures to identify F3/F4 (Beam et al., 2009). A more reliable method that accounts for inter-individual differences in brain topography is magnetic resonance imaging (MRI) guided navigation. Neuronavigation requires an anatomical MRI scan of the brain for mapping the DLPFC to guide the TMS coil in the treatment session to the DLPFC target location on the scalp (Fitzgerald et al., 2009). Neuronavigation thus results in better session reliability, as the exact same site is stimulated over multiple sessions. However, it is more expensive and often not accessible for clinics. Increasingly investigated methods are functional MRI (fMRI) and E-field modeling. fMRI allows for network connectivity analyses and can be used to identify the scalp-position that is connected to deeper lying brain regions – often the sgACC - associated to better treatment outcome. E-field modeling individualizes even further using MRI to predict the distribution of the electric field generated by the TMS coil, opting for optimized coil positioning over an individual's DLPFC (Dannhauer et al., 2024).

In clinical practice, the Beam and 5CM method are most commonly used, as they are quick, easy and in-expensive to use. Although these two sites differ on average 2cm from each other (Fitzgerald et al., 2021), treatment results in similar response and remission rates (Fitzgerald et al., 2021; Trapp et al., 2023). This suggests that there are inter-individual differences regarding the stimulation site to which patients respond. Site-selection in clinical practice however does not consider these inter-individual differences (Fox et al., 2013).

Stimulation intensity

DLPFC-TMS stimulation intensity is currently based on the motor threshold (MT). The MT is the lowest stimulation intensity that results in an MEP, indicating activation of the brain (Pridmore et al., 1998) which is evidence that you passed through the skull of the individual and reached the brain. EMG based MTs are generally lower than visualized MTs, however the EMG requires using more advanced technol-

ogy and training of technicians to reliably use the method, therefore visualization of MEPs is oftentimes used in clinical practices (Westin et al., 2014). Over time, the stimulation intensity of treatments gradually increased from 80%MT (George et al., 1995) to a maximum of 120%MT (O'Reardon et al., 2007). Generally, current TMS protocols for depression stimulate at an intensity of 110-120%MT, but increasing evidence exists that lower stimulation intensities are similarly effective on the group level. A study comparing treatment outcome with stimulation intensities of 80% and 120%MT showed no significant differences in treatment outcome between the groups (Chen et al., 2021). This suggests that either stimulating at 80%MT is sufficient for TMS treatment effects or there are inter-individual differences regarding the optimal stimulation intensity. Additionally, a recent double-blind RCT – the Stanford Neuromodulation Therapy (SNT) trial – showed promising results stimulating at 90%MT, although this was adjusted for the scalp-to-target-site-distance using MRI and employed individualized targeting based on fMRI (Cole et al., 2021). Personalization of stimulation intensity may improve treatment outcome and reduce side-effects even further.

Stimulation protocols

Within the scope of safety regulations (Rossi et al., 2009, 2021), TMS stimulation protocols differ regarding the frequency and number of pulses and the time between the pulse trains – the inter-train-interval (ITI). The base for currently commonly used TMS protocols revolves around the long thought hemispheric imbalance in depression. Throughout history, the right hemisphere was thought to be responsible for processing of negative emotions and the left hemisphere for positive emotions (Ley & Bryden, 1979; Reuter-Lorenz & Davidson, 1981; Mandal et al., 1991). In depression, the right hemisphere was therefore thought to be overactive in relation to the left hemisphere, as was measured in brain wave activity using the frontal alpha asymmetry (FAA) marker. A left-sided FAA (more inhibitory brain oscillations on the left hemisphere compared to the right) was initially found in depressed people (Henriques and Davidson, 1991; Pizzagalli et al., 2002) as compared to a right-sided FAA in healthy controls (Schaffer et al., 1983; Fingelkurts et al., 2006). Low frequency TMS (<5Hz) was found to reduce motor neuron plasticity, whereas high frequency (>5Hz) increas-

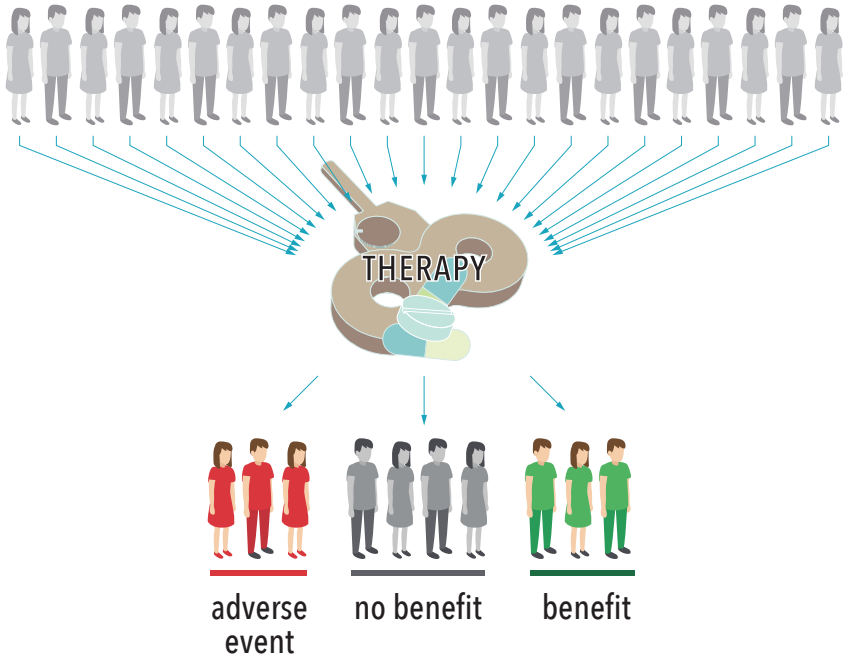
es the plasticity (Ridding & Rothwell, 2007). Therefore, low frequency TMS should inhibit the right hemisphere, causing a reduction in negative valence whereas the left – positive emotions – should be activated using high frequency protocols. Although this hemispheric imbalance theory has been disproven (Fitzgerald et al., 2006; Van der Vinne et al., 2017; Gibson et al., 2022), low frequency R-DLPFC (often 1Hz) and high frequency L-DLPFC are still commonly used treatment protocols.

Another frequently used TMS stimulation protocol is intermittent theta burst stimulation (iTBS). During iTBS, bursts of 3 pulses at 50Hz – so-called triplets - are applied for 2 seconds at a frequency of 5Hz, with an ITI of 8 seconds for a total of 600 pulses (Huang et al., 2005). An iTBS session can thus be performed in 3 minutes. The total amount of TMS pulses needed for optimal treatment response has been a topic of interest since the start of TMS treatment. First TMS studies used as little as 4000 pulses - 800 pulses in one session for 5 consecutive days - (George et al., 1995) which increased exponentially over time to 90000 pulses – 10 sessions with 18000 pulses per day, for 5 consecutive days – in the SNT trial (Cole et al., 2021). To date, no consensus has been found on the exact number of pulses – or sessions – needed for optimal TMS response (Fitzgerald et al., 2020).

An example of an adjustment within the scope of the safety parameters is the TMS Dash protocol (Carpenter et al., 2021). The Dash protocol is an adjustment of the commonly used 10Hz stimulation protocol. The original 10Hz protocol consists of stimulation trains of 4 seconds with an ITI of 26 seconds and a total of 3000 pulses, resulting in a total treatment duration of 37.5 minutes. The Dash protocol consists of an ITI of 11 seconds, shortening the duration of a full treatment to less than 20 minutes with similar clinical outcomes (Carpenter et al., 2021).

TREATMENT SELECTION TAILORED TO THE INDIVIDUAL

On the group level, all antidepressant treatment have comparable remission rates: from 30-50% (Figure 1.1). This is found even though they have different working mechanisms and, as can be read in the paragraph above about TMS, there are various treatment parameters to choose from. Generally, one third of patients responds, whereas two



one-size-fits-all psychiatry - *diagnostic approach, no biomarkers*

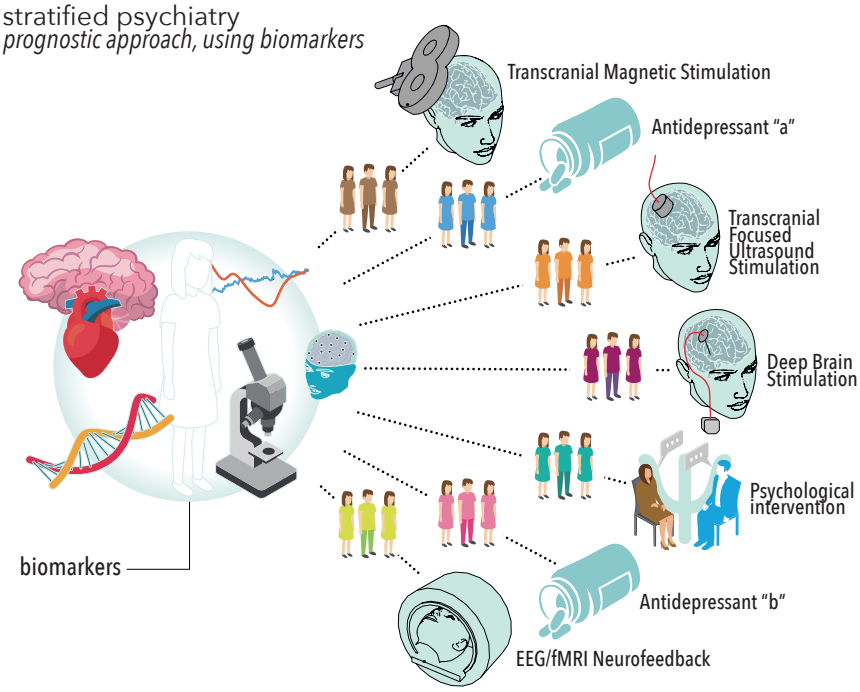
Figure 1.1. *A visualization of the current 'one-size-fits-all' psychiatry. Every individual follows the same route of treatment selection, not considering the heterogeneity of people with depression. Treatment outcome for all proven effective treatments is similar: one third of patients responds, whereas two thirds do not respond and/or stop treatment because of the experienced side effects from these treatments*

thirds do not respond and/or stop treatment because of the experienced side effects from these treatments (Saveanu et al., 2015; Arns et al., 2022). Combining the differential reaction to the same treatment with similar overall remission rates, this suggests a heterogeneity within the depressed patient population. Current classification methods could be the explanation for this heterogeneity. Psychiatric illnesses are classified by means of a broad range of symptoms, as described in the DSM-5, of which patients could experience all or a subset (García-Gutiérrez et al., 2020). In current one-size-fits-all psychiatry, this heterogeneity is not considered. Trial-and-error within the stepped care approach is the go-to method for treatment selection. A clinician, often in collaboration with the patient, selects the most suitable treatment

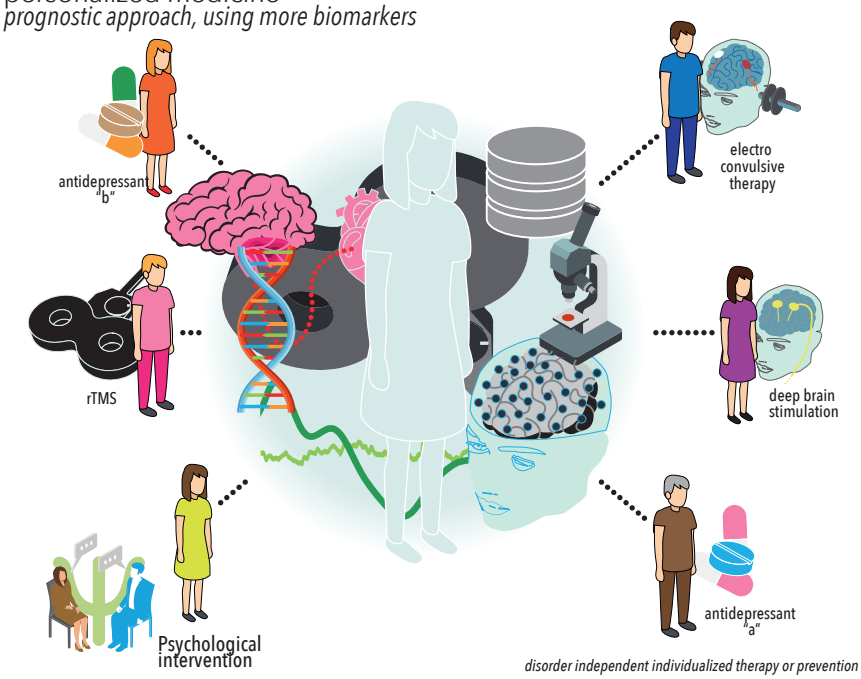
from the effective options available for the patient's current treatment stage. If the patient responds, treatment continues until remission or plateauing. In case of non-response or intolerable side-effects, an alternative treatment is chosen. This process repeats itself until the desired outcome is achieved. However, Rush et al. (2006) showed, in a large RCT; the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, that following every additional treatment step, remission rates decreased (from 36.8% after treatment one to 13% after treatment four) and relapse rates increased, especially after two treatment steps. In combination with the beforementioned heterogeneity of symptoms in depression, these findings advocate for a more nuanced treatment selection method tailored to individual patients. This would ensure adequate treatment earlier in the process, aiming to increase treatment effectiveness and avoid chronicity with all its consequences.

Personalized medicine, also referred to as precision psychiatry, aims to improve treatment efficacy by prescribing treatments based on an individual's biomarker profile (see following page, Figure 1.2; Bottom). Biomarkers are defined as "a functional variant or quantitative index of a biological process that predicts or reflects the evolution of or a predisposition to a disease or a response to a therapy" (FitzGerald, 2016). Precision psychiatry shifts from the symptom-based classification system as described before, towards biomarker-based disease types. The effect of a specific, on-label, treatment might be predicted effectively, but for the biomarker-negative group, there is no information on the best alternative treatment option (see for an example Drysdale et al., 2017). Furthermore, reliable biomarkers for psychiatric disorders are still at the early stage of discovery and translating research findings into clinically validated tools is a slow process. Additionally, even though various types of biomarkers have been shown to predict treatment response (partly), no biomarker - not even serotonin levels as part of the currently still influential but considerably debated serotonin hypothesis (Moncrieff et al., 2023) - was able to provide a conclusive optional treatment for most individuals yet (Kessler, 2018; Winter et al., 2024). While the potential of precision psychiatry is significant, these challenges must be addressed before clinical implementation is feasible.

stratified psychiatry
prognostic approach, using biomarkers



personalized medicine
prognostic approach, using more biomarkers



disorder independent individualized therapy or prevention

Figure 1.2. (Facing page) [Top] Stratified psychiatry: patient subgroups that are more likely to respond to an effective treatment that is approved for their disorder, are identified based on their biomarker profile. Stratification options are limited to evidence-based treatments only, herewith preventing any possibility of doing harm. [Bottom] Personalized medicine or precision psychiatry, aims to improve treatment efficacy by prescribing treatments based on an individual's biomarker profile. Precision psychiatry shifts from the symptom-based classification system to prescribe treatments, towards biomarker-based prescriptions.

Stratified psychiatry is a more pragmatic biomarker-based method for individualizing treatment selection (facing page, Figure 1.2; Top). Patient subgroups that are more likely to respond to an effective treatment that is approved for their disorder, are identified based on their biomarker profile (Arns et al., 2022; 2023), also considering predicted non-response to alternative treatments. Stratification options are limited to evidence-based treatments only, herewith preventing the possibility of doing harm by potential off-label prescriptions. The assessment of the needed biomarker(s) can be tailored to the limited treatment options, instead of going through a timely and costly assessment battery that should consider a much broader range of information.

ELECTROENCEPHALOGRAM

For a biomarker to be suited for implementation in clinical practice, it should be easy and quick to assess at low-cost. One technique that addresses the above is EEG. EEG is a non-invasive neuroimaging technique that records neuronal activity through electrodes placed on the scalp and was first described by Hans Berger (1929). This method allows for analyzing brain wave patterns at specific sites on the head, often following the 10-20 EEG system. Brain waves are commonly classified according to their oscillation frequency: delta (<4Hz), theta (4-7Hz), alpha (7-13Hz), beta (13-30Hz) and gamma (>30Hz) waves. In addition to studying brain wave oscillation frequencies, EEG allows for measuring brain responses to specific (cognitive, sensory or motor) events, using event-related potentials (ERPs). ERPs are time-locked EEG changes related to the processing of these events and give insight into the underlying cognitive processes. The description of ERPs is based on their amplitude and latency, for example, the most commonly studied ERP is the P300, as discovered by Sutton et al. in 1965, which is a positive deflection 265-400ms after auditory stimuli.

EEG biomarkers have become increasingly important. Several biomarkers have been investigated in relation to antidepressant treatment response prediction and diagnostics. Klooster & Voetterl et al. (2023) systematically reviewed the robustness of various EEG and fMRI brain stimulation biomarkers for depression. The anti-correlation of the stimulation target and the sgACC and (lesion) network mapping showed potential as fMRI biomarkers. For EEG, the change in frontal theta power after tasks that increase rostral ACC activity, and the individual alpha peak frequency (iAF) emerged as the most promising biomarkers. The iAF is an individual's most prominent frequency within the alpha frequency band. Inter-individual iAF differences are significant, but iAF appears stable within a person, therefore it is considered a trait-marker (Grandy et al., 2013). Higher iAF is related to better cognitive performance in general (Rathee et al., 2020), while lower iAF is linked to a reduced cerebral blood flow (Jann et al., 2010). However, a slow iAF predicted better response to pharmacotherapy with sertraline, whereas no effects were found for escitalopram and venlafaxine (Arns et al., 2017). Van der Vinne et al. (2021) implemented the slow iAF, amongst two other biomarkers, for prospective pharmacotherapy stratification, which appeared feasible and resulted in significantly higher reduction of depressive symptoms. The association between the iAF and TMS treatment outcome showed that individuals with an iAF close to 10Hz (9.6.-10.4Hz) had better treatment outcome for 10Hz L-DLPFC TMS compared to those with lower or higher iAF frequencies (Corlier et al., 2019). Roelofs et al. (2020) independently replicated these findings. No effect was found for the 1Hz R-DLPFC protocol. Voetterl et al. (2023) examined stratification to different TMS protocols using Brainmarker-I, an age and sex normalized derivative of the iAF classified as decile scores (Voetterl et al., 2022). An iAF close to 10Hz was translated to a synchronization (sync) marker, slow iAF resulted in low decile scores (0-5) and fast iAF in high decile scores (6-10). Retrospective stratification of the sync marker group to the 10Hz protocol resulted in an increase in normalized remission rate of 29%. The high decile group showed increased normalized remission rates to 1Hz stimulation of 14% and 16% in the original and out-of-sample validation set, respectively. The low decile group showed low remission rates both in the 1Hz and 10Hz protocol. These findings are, however, based on retrospective data, therefore their prospective clinical relevance is yet to be determined.

HEART RATE IN DEPRESSION

A second biomarker technique is the measurement of heart rate. Heart rate assessments are already widely used in clinical practice, albeit not commonly in psychiatry, with various easily accessible options to measure this - even at home. The heart and brain are interactively connected (Lacey & Lacey, 1978), communicating with each other in four ways. Besides the nervous system which plays a key role in controlling cardiovascular homeostasis through heart rate and heart rate variability (HRV), they communicate physiologically by the pulsating (cerebral) blood flow, biochemically through hormones and neurotransmitters, and electrically by an electric field the heart continually emits, affecting the whole body (McCraty, 2015). These two intrinsically important organs are communicating continuously, suggesting an immensely important relation. Depression has been found to be an independent risk factor for developing cardiovascular diseases (Ehrenthal et al., 2010) and depressed patients generally have dysregulations in their autonomic nervous system, commonly consisting of an overall higher heart rate and lower HRV (Thayer & Lane, 2000; Kemp et al., 2010), suggesting increased sympathetic activation over parasympathetic activation. Furthermore, cardiovascular health metrics and psychiatric conditions, especially depression, are closely related (Penninx, 2017; Tonello et al., 2019). Recent genetic research has provided evidence supporting this notion, revealing genetic causal relationships between heart and brain metrics (Zhao et al., 2023). These findings suggest that adverse heart-related traits may genetically influence the development of various brain diseases, including psychiatric disorders such as depression. Heart rate and HRV have been studied as predictive biomarkers for antidepressant treatments response and could potentially be useful determinants in treatment decision making (Kircanski et al., 2019).

One possible way to measure the influence of the bottom-up connection – afferent influence - is measuring the electrophysiological response in the brain to every heartbeat, the heartbeat evoked potential (HEP), measured with EEG. The HEP is an ERP phase locked to the R-peak in the electrocardiogram (ECG; Schandry & Montoya, 1996). The HEP is considered an objective marker of interoceptive awareness – the perception of bodily signals – and represents cogni-

tive processing of the heartbeat (Zaccaro et al., 2024). The amplitude is higher during interoceptive tasks compared to tasks requiring exteroceptive awareness – perception of external signals. Terhaar and colleagues (2012) found that depressed patients have a lower HEP amplitude relative to healthy controls. The physiological processes underlying the HEP remain under investigation, although research points towards influence from baroreceptor signalling from the aortic arch, carotid sinus and carotid arteries (Pollatos & Schandry, 2004; Park et al., 2019; Engelen et al., 2023).

The heart-brain connection – a functional rather than anatomical connection - of the nervous system is mediated by the vagus nerve (VN). The VN is part of the parasympathetic nervous system (PNS), the part of the autonomic nervous system that is activated during rest and connects the brain directly to the heart. Iseger et al. (2017; 2020) reported on various overlapping nodes within the frontal-vagal pathway with nodes in the depression network. Specifically, the DLPFC, the sgACC and the VN. As mentioned, the DLPFC is the target location for TMS for depression; the sgACC and VN are target locations for DBS and VNS for depression, respectively. During DLPFC-TMS, transsynaptically the cortical signal transfers into subcortical regions and eventually activates the VN. VN activation results in similar effects as through autonomic activation of the PNS including lower heart rate, higher HRV, lower blood pressure (Iseger et al., 2020). Various types of ADs – SSRIs like sertraline and escitalopram, and venlafaxine and mirtazapine (Terhardt et al., 2013; Olbrich et al., 2016) - also influence heart rate. DLPFC-TMS thus results in heart rate decelerations if the stimulation site is the scalp-level entrance into the above described transsynaptic network. This suggests that TMS could be used as a top-down – efferent -, network-based target-engagement method to individually localize the DLPFC-sgACC connected scalp site, and with that, the hypothesized best entrance into the depression network. Iseger and colleagues (2017) examined this with Neuro-Cardiac Guided TMS (NCG-TMS): assessing heart rate changes during TMS stimulation over a grid of different frontal and central 10-20 EEG system locations. Results showed that heart rate decelerations were site-specific with clear inter-individual differences. These findings were independently replicated (Kaur et al.,

2020) and replicated in a larger study (Iseger et al., 2021), both assessing healthy controls.

Consistent and replicable support was found for the underlying hypotheses of NCG-TMS as a target engagement method (Iseger et al., 2020). However, addressing heart rate deceleration by itself appeared not as robust as a biomarker for clinical practice, since outcomes appeared somewhat variable. Heart rate is not only influenced by TMS stimulation, but also by respiration (so called respiratory sinus arrythmia; RSA) and other (non-)intentional behaviour as well. To account for this, a novel implementation was developed, called Heart-Brain Coupling TMS (HBC-TMS; Dijkstra et al., 2023). This method relies on the induced heart rate oscillation frequency during rTMS stimulation. Heart rate decelerations are thus expected during stimulation and heart rate normalization during the ITI, each consecutive stimulation round. A 10Hz protocol with 5 seconds of stimulation, followed by an ITI of 11 seconds would therefore result in an oscillation frequency of 0.0625Hz (4 stimulation trains of 16 seconds in 64 seconds). HBC-TMS was successfully validated with iTBS, resulting in heart rate oscillations of 0.1Hz (1 stimulation train every 10 seconds). The location with the highest HBC was suggested to be the site with strongest DLPFC-sgACC connectivity, therefore hypothesized to be the optimal stimulation site. In a recent fMRI study, Dijkstra and colleagues (2024) provided evidence comparing various neutral, positively and negatively connected frontal (and exploratory also parietal) sites with the amount of HBC. Their findings showed an accuracy of 86% for predicting which of the stimulated frontal sites had the highest sgACC anti-correlation, based on HBC. Although this needs to be replicated, their results suggest HBC assesses DLPFC-sgACC connectivity. Furthermore, HBC-TMS was shown to be site-specific, even when comparing between two proven effective stimulation sites (Beam and 5CM), with inter-individual differences, as was described before for NCG-TMS (Dijkstra et al., 2023). This is suggestive of HBC-TMS being an easy to measure biomarker with the potential for stratification between proven effective stimulation sites.

AIMS AND OUTLINE OF THIS THESIS

The overarching goal of this thesis is to develop methods to improve antidepressant treatment outcomes, focusing on TMS for depression. For this, we assessed the potential of the HEP as a differential biomarker for stratification in **Chapter 2**. We compared baseline HEP of remitters and non-remitters to treatments with the ADs sertraline, escitalopram, venlafaxine, and TMS. In **Chapter 3**, we aimed to validate previous NCG-TMS findings assessing heart rate decelerations during TMS stimulation in depressed patients, since this was only investigated in healthy controls before. The subsequent chapters assessed the novel implementation method: HBC-TMS. **Chapter 4** reports on the validation of HBC-TMS findings of site-specificity and inter-individual differences in a larger dataset. Additionally, and more importantly, we developed a method to further optimize HBC-TMS. This allowed for between-subject comparisons, and for the determination of a frontal excitability threshold. Lastly, we examined the reliability of the optimal HBC location and frontal threshold over time. With HBC-TMS as a prefrontal activation readout measure, studies investigating the effect of TMS pulses on the motor cortex could be validated for the DLPFC. In **Chapter 5**, we examined HBC-TMS as a readout measure for DLPFC activation to compare different pulse parameters: pulse length and pulse shape, using the cTMS and xTMS device. Lastly, we describe in **Chapter 6** how prospective HBC and Brainmarker-I based treatment stratification is implemented in clinical practice, assessing real-world clinical data from two Dutch outpatient clinics. We examined the feasibility and effectiveness of Brainmarker-I and HBC based prospective stratification and investigated the relation between HBC and the iAF and a wider EEG power spectrum.

2

HEARTBEAT EVOKED POTENTIAL IN MAJOR DEPRESSIVE DISORDER: A BIOMARKER FOR DIFFERENTIAL TREATMENT PREDICTION BETWEEN VENLAFAXINE AND RTMS?

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ABSTRACT

Currently, major depressive disorder (MDD) treatment plans are based on trial-and-error and remission rates remain low. A strategy to replace trial-and-error and increase remission rates could be treatment stratification. We explored the heartbeat-evoked-potential (HEP) as a biomarker for treatment stratification to either antidepressant medication or rTMS treatment.

Two datasets were analysed: 1) the international Study to Predict Optimized Treatment in Depression (iSPOT-D; n=1008 MDD patients, randomized to escitalopram, sertraline or venlafaxine and n=336 healthy controls) and 2) a multi-site, open-label rTMS study (n=196). The primary outcome measure was remission. Cardiac field artefacts were removed from the baseline EEG using Independent Component Analysis (ICA). The HEP-peak was detected in a bandwidth of 20ms around 8ms and 270ms (N8, N270) after the R-peak of the ECG signal. Differences between remitters and non-remitters were statistically assessed by repeated-measures ANOVAs for electrodes Fp1, Cz and Oz.

In the venlafaxine subgroup, remitters showed a lower HEP around

the N8 peak than non-remitters on electrode site Cz (p=.004; d=.497). The rTMS group showed a non-significant difference in the opposite direction (d=-0.051). Retrospective stratification to one of the treatments based on the HEP resulted in enhanced treatment outcome prediction for venlafaxine (+22.98%) and rTMS (+10.66%).

These data suggest that the HEP could be used as a stratification biomarker between venlafaxine and rTMS, however future out-of-sample replication is warranted.

INTRODUCTION

Although there are various antidepressant treatments for major depressive disorder (MDD), remission rates remain low (Rush et al., 2006). One reason could be the current way of treatment selection which is based on trial-and-error, with first-choice treatments consisting of psychotherapy and antidepressant medication (AD). A possible strategy to increase remission rates could therefore be treatment stratification between equally effective treatments based on biomarkers (Arns et al., 2022). In this regard specifically heart rate (HR) and heart rate-variability (HRV) have been suggested as useful candidates (Kircanski et al., 2019).

Against this background, an overall higher heart rate (HR) and lower heart rate-variability (HRV) have been reported in patients with MDD, indicating dysregulation of their autonomic nervous system (Kemp et al., 2010). It is therefore not surprising that MDD has been found to be an independent risk factor for developing cardiovascular diseases (Ehrenthal et al., 2010). Interestingly, it was shown that both HR and HRV can be normalized during neuromodulation treatment. However, this effect does not seem to be long lasting and was not found in treatment with antidepressant medication (Kemp et al., 2010; Iseger et al., 2021). In addition, treatment with venlafaxine can lead to a higher HR and lower HRV (Terhardt et al., 2013).

Afferent influence from the heart on the brain might play an important role in cognitive processing and emotions, as it was found in ear-

lier reports of gut and stomach influences over brain networks such as the default mode network (DMN), however knowledge is still limited (Porciello et al., 2018; Cao et al., 2020). The heart and brain have an interactive connection (Lacey & Lacey, 1978). Stimulation with rTMS to the dorsolateral prefrontal cortex activates the downstream frontal-vagal pathway (Iseger et al., 2020), which results in lower HR and higher HRV (Iseger et al., 2017; Iseger et al., 2021; Zwienenberg et al., 2021). Besides the frontal-vagal pathway, there are also bottom-up influences from the heart to the brain. The heart and brain are connected in four ways (McCraty, 2015): 1) physically by the pulsating (cerebral) blood flow; 2) biochemically through hormones and neurotransmitters, 3) electrically by an electric field of the heart that continually affects the whole body, and 4) the nervous system that controls HR and HRV. Although the knowledge about the afferent influence from the heart on the brain is still limited, this might play an important role in cognitive processing and emotions.

One way to operationalise the heart's effect on the brain is to look at the neural electrophysiological response in the brain that is phase locked to the R-peak in the electrocardiogram (ECG), or the so-called heartbeat evoked potential (HEP; Schandry & Montoya, 1996). The HEP, known as an objective marker of interoceptive awareness, has a decreased amplitude in MDD patients relative to healthy controls (Terhaar et al., 2012) and can be increased by cardiac awareness training (Schandry & Weitkunat, 2009). Studies on the localization and intracranial recordings of the HEP generators show the contribution of the insular cortex, cingulate cortex, amygdala, and somatosensory cortex (Kern et al., 2013; Park et al., 2017; Salomon et al., 2018). Current knowledge proposed furthermore that the HEP provides a sensitive cortical index of cardiac processing reflecting changes in emotional and arousal states (Park & Blanke, 2019).

Given the low remission rates in MDD treatment, functional subgroups within MDD patients are suggested to respond differently to treatments. Based on the knowledge that 1) interoceptive awareness could be used as a somatic marker for depression, 2) the HEP is correlated to interoceptive awareness and 3) treatment with venlafaxine and rTMS have opposite outcomes on HR and HRV, we tested

whether the HEP could function as a biomarker for treatment stratification (Arns et al., 2022) in MDD and thereby help parsing inherent heterogeneity into more homogenous subgroups. Therefore, we aimed to investigate whether there are differences in resting state baseline HEP amplitude between remitters and non-remitters to antidepressant medication and rTMS treatment.

MATERIALS AND METHODS

DESIGN

Sample 1: iSPOT-D
This study is based on data acquired from the international Study to Predict Optimized Treatment in Depression (iSPOT-D), an international, randomized, prospective, practical, clinical open-label trial aimed at finding biomarkers for antidepressant treatment response, in which MDD participants were randomized to escitalopram, sertraline or venlafaxine-XR in a 1:1:1 ratio. A complete description of the study methods is published elsewhere (Williams et al., 2011; Arns et al., 2016).

Sample 2: rTMS
rTMS data was acquired from a multi-site, open-label study. rTMS treatment consisted of either low frequency 1Hz stimulation on the right DLPFC, high frequency 10Hz stimulation on the left DLPFC or stimulation at both sites. rTMS was complemented with cognitive behavioral therapy. A complete description of the study methods is published elsewhere (Donse et al., 2017).

PARTICIPANTS AND TREATMENT

Sample 1: iSPOT-D
1008 nonpsychotic adult MDD patients and 336 matched healthy controls participated in the study (for the consort diagram and demographic features of the whole sample, see Arns et al., 2016). MDD patients were treatment-naïve, or medication was washed-out (5 half-lives). The Mini-International Neuropsychiatric Interview (MINI-plus; Sheehan et al., 1998) according to the DSM-IV criteria, and the Hamilton Rating Scale for Depression (HRSD17), a score ≥16,

were used to confirm the primary diagnosis of nonpsychotic MDD at baseline visit. All participants took part in the EEG assessment. After eight weeks of treatment the participants came in for clinical assessment. An overview of patient demographics can be found in Table 1.

The total amount of usable baseline EEG measurements was 1296, with a final total of 769 MDD (552 protocol completers) and 247 HC: 124 were excluded due to no/bad peak detection, 144 due to reversed polarization of the R peaks and 12 did not meet requirements on the minimal number of segments. In 94% of the cases, there was one cardiac-field artefact (CFA) independent component (IC) excluded, in 4% there were no ICs excluded, due to unclear CFA and in 2% of the cases two ICs were excluded.

Sample 2: rTMS
The total sample consisted of 196 patients, 98 female, 98 male, aged 18-78 (43.2 ± 12.9). Patients underwent at least 10 rTMS sessions, with an average of 20.9 sessions (SD=7.5). The Beck Depression Inventory, second edition Dutch version (BDI-II-NL; BDI; van der Does, 2002), was assessed at baseline, every fifth session and at the last session. A baseline BDI ≥ 14 confirmed the diagnosis of MDD. An overview of patient demographics can be found in Table 2.1.

Of the initial dataset, 163 remained with complete data (one excluded due to missing data; 17 excluded due to missing the ECG channel; seven due to inverse peak polarization; seven because of bad peak detection; and one due to insufficient segments). In 99% of the cases, there was one CFA component excluded, in 1% there was no component excluded, because it was unclear which component represented the CFA

PRE-TREATMENT ASSESSMENTS: EYES CLOSED RESTING STATE

EEG recordings were performed using a standardized methodology and platform (Brain Resource Ltd., Australia). Details of this procedure (Williams et al., 2011; Arns et al., 2016) and details on the reliability and across-site consistency of the EEG assessment (Williams et al., 2005; Paul et al., 2009) have been published earlier. In short, participants were seated in a sound and light attenuated room that was controlled at an ambient temperature of 22°C. 26 channels were

used for the EEG acquisition: Fp1, Fp2, F7, F3, Fz, F4, F8, FC3, FCz, FC4, T3, C3, Cz, C4, T4, CP3, CPz, Cp4, T5, P3, Pz, P4, T6, O1, Oz and O2 (Quikcap; NuAmps; extended 10-20 electrode international system). For the ECG measurements one electrode was placed on the inner left wrist. Both EEG and ECG data were simultaneously collected for two minutes with eyes closed (EC). Participants were asked to remain relaxed during the assessment. Data were offline referenced to averaged mastoids with a ground at AFz. Horizontal eye movements were recorded with electrodes placed 1.5 cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3mm above the middle of the left eyebrow and 1.5cm below the middle of the left lower eyelid. Electrode impedance was maintained at <10kOhms for all electrodes. Sampling rate of all channels was 500Hz. A low pass filter with an attenuation of 40dB per decade above 100Hz was employed prior to digitization.

PREPROCESSING EEG AND ECG DATA

Data were preprocessed using Brain Vision Analyzer (BVA) 2.2.0.7383 (Brain Products GmbH). The EEG channels were filtered with infinite impulse response (IIR) filter with a high pass of 0.05Hz and a low pass of 40Hz. The ECG channel was filtered with a high pass of 0.05Hz and low pass of 25Hz.

ECG markers R- and T-peak were detected using automatic peak detection but were manually verified for correctness. ECG data with negative R-peaks were excluded from the analyses. In case of incorrect peak detection, the settings were changed from automatic to positive peak detection. In case peak detection was still incorrect after changing the settings, the data was excluded from analyses.

Segmentation was based on the latency of the R-peak with a total length of 700ms: 200ms before and 500ms after the R-peak. Overlapping segments were allowed.
After segmentation, a DC Detrend for segments correction was applied to the data with interval lengths based on time; 200ms before the start of the segment to 200ms after the end of the segment.

Artifact rejection (AR) was carried out using automatic segment selection on all EEG channels (maximum amplitude was 150μV; van Dinteren et al., 2017). Cases that consisted of <90 segments before artifact rejection were excluded from the analysis, since this suggests the peak detection was incorrect and/or the patient had an abnormal heartbeat. After artifact rejection cases with <45 segments were excluded to maintain an adequate signal-to-noise ratio.

For the removal of volume conducted CFA from the heart-related brain potential, independent component analyses (ICA) have been shown to be effective in removing artefactual components from the HEP (Terhaar et al., 2012). Thus, a restricted infomax ICA with normal PCA sphering was automatically carried out on the whole data of all EEG channels and the ECG channel. ICs were then compared to the ECG. ICs suggesting reflecting a CFA were semi-automatically identified and satisfied two criteria: 1) the plotted average had to account for the R- and T-peak time course, 2) the topographical maps of the ICA inverse weighs must show a dipole with the maximum around Oz and the minimum around Fp1/Fp2. On average 1-2 CFA-ICs per subject were identified by one rater. These were then excluded from back-projection to the EEG-channels.

Subsequently, the average of all segments was calculated per subject. Next, group averages for both remitters and non-remitters of both samples were calculated. A new channel was created containing the group average signal of all EEG channels of the iSPOT-D sample. Based on this new channel, the timing and amplitude of the R- (N8) and T-peak (N270) of the ECG signal were determined for usage in the following analyses.

Mean values of 20ms, 10ms before and after the peak, were computed and used to extract the area under the curve (AUC; μV^2) in BVA and exported to SPSS (IBM SPSS Statistics for iOS, Version 27.0 Armonk, NY) per individual and channel. Within these bandwidths the amplitude of the peak was checked for all channels. The electrodes with the highest AUC, lowest AUC and a third topographically located in the middle were selected from the iSPOT-D dataset for the analyses in both samples, allowing for stratification. This resulted in the use of electrodes

Fp1, Cz and Oz in the analyses. See Table 2.1 in the supplement for the average peaks and Table 2.2 in the supplement for the timing and respective AUC values of these electrode sites.

STATISTICAL DESIGN

The primary outcome measure was remission and resting state EC EEG was used since EC is considered a more 'interoceptive brain state' (Marx et al., 2003). Remission was defined as a score of ≤ 7 on the HRSD17 (Sample 1) or a BDI <12 (Sample 2) after eight weeks or at the last session. HEP AUC values were inspected and were log transformed to assure a normal distribution. Differences in age, sex, education, and baseline severity of Sample 1 were already reported by Arns et al. (2016). For Sample 2, differences between remitters and non-remitters were tested using t-tests. Significant variables were considered covariates in the analyses.

All statistical analyses in SPSS for treatment prediction were conducted on protocol completers. Differences between remitters and non-remitters were tested with Repeated-measures ANOVAs (RM ANOVAs) with the factor being Remission type (remitters, non-remitters), the within-subjects variable was electrode Site (Fp1, Cz and Oz), the between-subjects variable was Sex (male, female). Baseline Severity and Age were added as covariates. The iSPOT-D analyses had an extra between-subjects variable: Treatment Arm (escitalopram, sertraline, or venlafaxine-XR).

Significant (interaction) effects regarding remission were further investigated using RM ANOVAs with the data split by the significant variables. A conventional alpha of $p \leq .05$ is used. The effects of Site were investigated by creating topography plots of the effect sizes (ES) over all EEG sites. ES are reported in Cohen's d. Post-hoc partial correlations were calculated.

To assess the clinical relevance of the HEP, the normalized positive predictive value (N-PPV) of the HEP is examined, based on the Youden's *J* cut-off.

RESULTS

Sample 1: iSPOT-D

The demographics of the participants with usable EEG data and who completed protocol can be found in Table 2.1.

Table 2.1. Demograpics of protocol completers

	Sample 1: iSPOT-D					Sample 2
	MDD	HC	escitalopram	sertraline	venlafaxine-XR	rTMS
N	552	247	180	193	179	163
Females	301	141	92	106	103	87
Mean age in years, (SD)	38.9 (12.9)	36.5 (12.8)	39.0 (13.0)	38.6 (12.5)	39.1 (13.3)	43.1 (13.1)
Pre HRSD17, (SD)	21.63 (3.92)	1.21 (1.57)	21.67 (3.92)	21.72 (4.05)	21.49 (3.80)	31.52 (10.26)
Pre BDI, (SD)	-	-	-	-	-	31.26 (10.0)
HRSD17 week 8, (SD)	9.66 (6.33)	1.13 (1.44)	9.26 (6.68)	9.77 (5.92)	9.96 (6.42)	-
BDI week 8, (SD)	-	-	-	-	-	14.39 (12.47)
% Remission	44.7	-	48.9	42.5	43.0	53.4

REMITTERS VS. NON-REMITTERS

The RM ANOVA on the N8 peak yielded a significant between subjects Remission X Treatment effect ($F(2,1036)=3.129$, $p=.045$). With the data split by treatment, the RM ANOVA showed a between-subjects effect of Remission ($F(1,171)=3.940$, $p=.049$) for the venlafaxine group. In this group, a univariate general linear model (GLM) ANOVA with the three sites showed a significant effect of Cz ($F(1,173)=8.369$, $p=.004$, $d=.497$), with venlafaxine remitters having a lower HEP than non-remitters (Figure 2.3a on page 45). A significant partial correlation between the percentage improvement on the HRSD17 and the AUC of Cz for the venlafaxine group, covaried for age and baseline severity, was found ($r^2=2.2\%$, $p=.05$).

The analyses on the N270 peak yielded no significant effects.

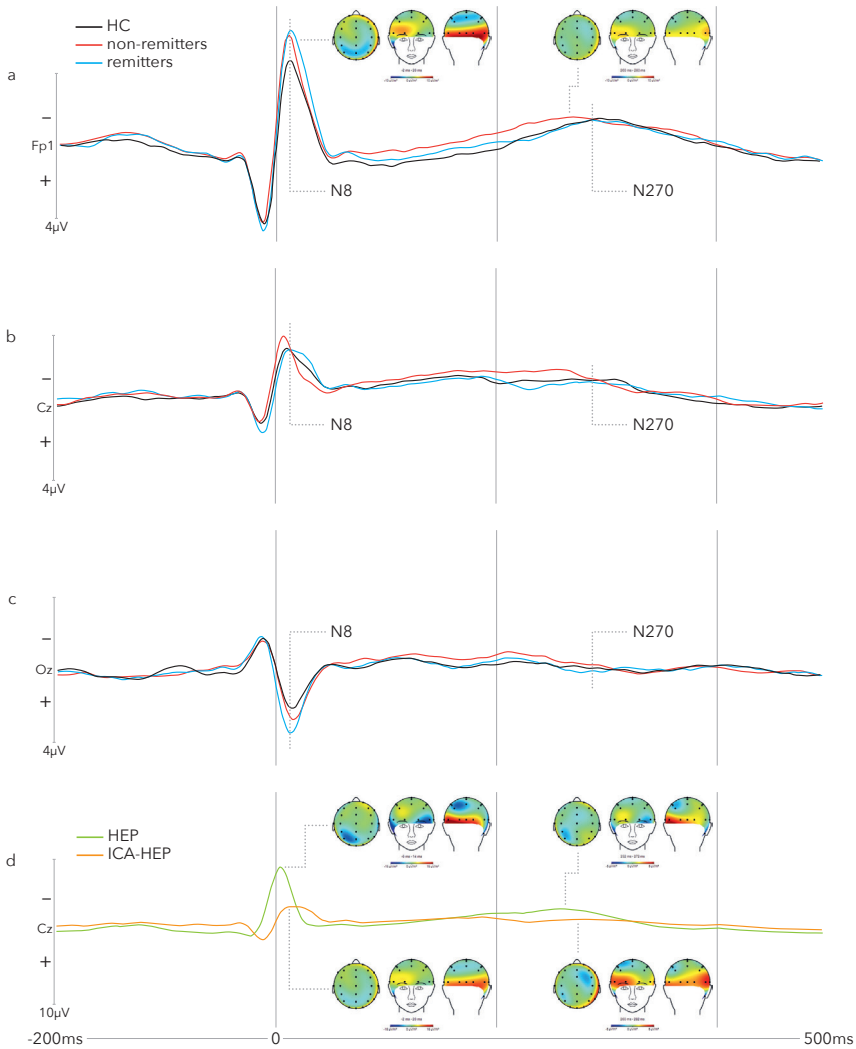


Figure 2.1. *Average HEP of iSPOT-D study for Fp1 (a), Cz (b) and Oz (c). Visualized are the differences for HC (black) all non-remitters (red) and all remitters (blue) after ICA correction (ICA-HEP). Topographical plots show the top, front, and back view of the EEG amplitudes for all electrode sites for both peaks. d) Shows the original EEG signal at Cz before (green) and after (orange) ICA-correction. The topographical plots show the EEG amplitudes for both the non-corrected HEP (top) and the ICA-HEP (bottom). Please note that negative amplitudes are plotted upwards and positive amplitudes downwards*

Sample 2: rTMS

No differences between remitters and non-remitters were found for age ($t(161)=-.169$, n.s.), and sex ($t(161)=-.449$, n.s.). Baseline severity was different between remitters and non-remitters ($t(161)=5.72$, $p<.001$), with non-remitters having higher baseline severity.

The RM ANOVAs for both N8 and N270, as well as the partial correlation between the AUC values of both peaks with the BDI change, correlating for baseline severity, showed no significant effects. Interestingly, the effect size of Cz appeared small between remitters and non-remitters but was found in the opposite direction as for venlafaxine ($d=-0.051$), meaning rTMS remitters had a higher HEP than non-remitters (Figure 2.3a on page 45).

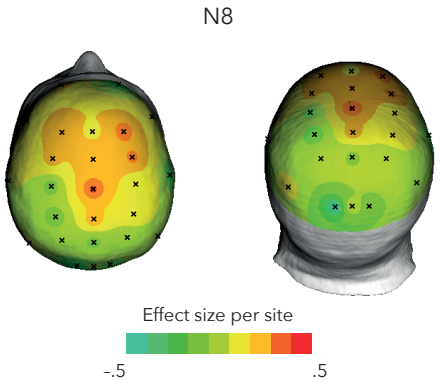


Figure 2.2. *Topography plot showing effect sizes (Cohen's d) of the differences between remitters and non-remitters of the N8 HEP for the venlafaxine group. A significant effect was found at Cz.*

SENSITIVITY ANALYSIS

The ECG AUC value was analyzed per group to verify whether differences in ECG between remitters and non-remitters caused the significant effects in HEP. A one-way ANOVA of the ECG signals was run for the venlafaxine group. This ANOVA showed no significant effects ($p=.107$, $d=.243$), which suggests that the HEP differences related to remission originate from brain activity and are not directly caused by the electrical field of the heart. In addition, adding the ECG AUC as a covariate to the above analyses did not change the results, suggesting the significant differences were not driven by the ECG channel.

STRATIFICATION

As can be seen in Figure 2.3a, although non-significant, the rTMS sample yields differences in HEP between remitters and non-remitters that are in opposite direction of the venlafaxine results, which opens the possibility to stratify between the two treatments. Although escitalopram (d=0.067) and sertraline (d=-0.011) also showed opposite effects, the effects sizes were non-significant and both smaller than the venlafaxine and rTMS sample and were thus disregarded in the stratification analysis.
To test the predictive value of HEP as a biomarker for stratification between treatments we calculated the normalized positive predictive value (N-PPV). The N-PPV shows the added value of using the HEP as a stratification biomarker over the PPV without using the HEP for treatment prediction.

The Youden's J of Cz for the subgroup venlafaxine was highest at an AUC value of 1.17 (J=0.236), this was set as cut-off for the predictions. When an individual's HEP on Cz was higher than the cut-off and the person was in the rTMS sample, remission was predicted. Whereas a person from the venlafaxine sample was assigned remitter status with a HEP value below that cut-off. These outcomes were retrospectively compared to the real remission rates, which resulted in the calculation of the N-PPV, of which the results can be found in Figure 2.3b, showing increased remission rates for venlafaxine of 22.98% and 10.66% for the rTMS group.

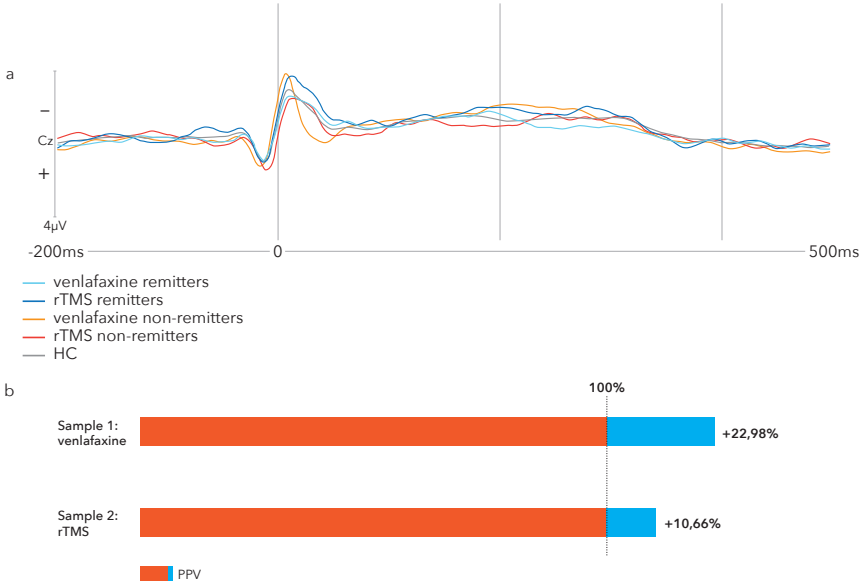


Figure 2.3. a) Average HEP signals on Cz of HC and the (non-)remitters from the venlafaxine and rTMS sample. Please note that negative amplitudes are plotted upwards and positive downwards. b) Normalized PPV when stratifying based on the HEP.

DISCUSSION

We explored the differences in baseline HEP between remitters and non-remitters of MDD patients to either AD or rTMS treatment and looked whether the HEP could be used as a biomarker to predict remission outcomes in depression, and for stratifying between treatment with venlafaxine and rTMS. Significant differences between remitters and non-remitters were found for the N8 peak at Cz in the venlafaxine group, where remitters showed a smaller HEP amplitude, whereas no differences were found for the SSRI's sertraline and escitalopram. For rTMS, numerically the opposite was found, although non-significant, with a larger N8 peak at Cz for remitters, with a small effect size. By calculating the normalized PPV, the data showed added value for both treatments when stratifying based on the HEP between treatment with rTMS and venlafaxine. Although using the HEP as a stratification biomarker already results in an added value of 23% for the venlafaxine group and 10% for the rTMS group, when you

compare this added value to the decreased value if these participants were stratified to the opposite treatment (-7.33% for the venlafaxine and -31.79% for the rTMS group) the added value of using the HEP as a stratification biomarker appears even larger.

The differences reported for venlafaxine were found at Cz, which directly overlies the somatosensory cortex. The somatosensory cortex is directly connected to the insula, the processor of interoceptive signals (Salomon et al., 2018). Adding to that, the somatosensory cortex is mainly innervated by the noradrenergic system (Rodenkirch et al., 2019), which agrees with the specificity of this effect being confined to the SNRI venlafaxine.

Terhaar et al. (2012) reported MDD patients having a lower HEP than HC. Although they focused on a different HEP latency window, we found HC having a lower HEP than MDD patients over the whole segment, including their latency window. This could be explained by the different methods used in both studies. In their study, participants had to count their heartbeat during the HEP measurement, an interoceptive task. The HEP has been proven to be sensitive to changes in directed attention (Montoya et al., 1993) which has been confirmed by Al et al. (2021) showing that the HEP decreases during somatosensory tasks compared to resting state. In addition, Terhaar et al. (2012) used the data of 32 participants some of which already used an AD. Mussgay et al. (1999) stated that medication use has no effect on heartbeat perception, but this could have had an influence on the HEP. Concluding, the lower HEP is the outcome of a lower interoceptive awareness in MDD, likely driven by a reduced noradrenergic tone. This implies that HEP is purely a perception concept in MDD and that the reason for a lower interoceptive awareness should be searched for somewhere else than in physical influences.

Limitations of our study included our choice of HEP timing being data driven, not theory driven, based on the observed group differences. Prior studies examined different HEP latencies from 20-592ms after the R peak (Terhaar et al., 2012; Wei et al., 2016; Petzschner et al., 2019) Post-hoc sensitivity analyses within the window of 170-270ms after R peak yielded no different results. Possibly, using a

group-ICA as a method of determining the latency of the HEP component can add to the knowledge about the topography of interest of the HEP in MDD.

Using ICA as a means of cardiac field artifact correction is a generally powerful method, but no study demonstrated a complete removal of the CFA by means of ICA. Terhaar et al. (2012) excluded two CFA components, one concerning the R-peak of the ECG, the second concerning on the T-peak of the ECG. In our preprocessing, we looked for two CFA components, but in most cases only one CFA component could be found. This could mean not all CFA is removed. Besides not removing all CFA, the ICA can cause removal of actual EEG signals. We focused on removing most CFA, but we intended to not remove EEG signal. Petzschner et al. (2019) assumed that with equal cardiac activity in both groups, it is safe to assume that the CFA is constant in those groups and will therefore not affect the differences between remitters and non-remitters. To assure this equal cardiac activity, we both visually and statistically compared the ECG signal amplitude across conditions and no significant differences were found. Also, when covarying for ECG, the results did not differ.

CONCLUSION

We found that the HEP is a potential differential biomarker in MDD treatment prediction between treating with venlafaxine and rTMS. However, this was the first study to investigate predictive applications, so validation and replication needs to be done thoroughly, looking at the timing and localization of the HEP and at different outcome measures as a means of determining remission rates as to looking at removing the cardiac field artifact.

SUPPLEMENTAL MATERIALS

Analyses comparing MDD with HC were performed on all usable EEG data.

Differences in HEP between MDD and HC were tested with Repeated-measures ANOVAs (RM ANOVAs) with within-subjects variable electrode Site (Fp1, Cz and Oz), and a between-subjects variable was Sex (female, male). Age was furthermore considered as a covariate with Group (MDD, HC) as factor.

MDD vs. HC
Differences between MDD and HC on the N8 peak were found using a RM ANOVA showing a significant between-subjects effect between MDD and HC ($F(1,1007)=5.016$, $p=.025$). Univariate ANOVAs per site with covariate Age showed significant effects for all three sites, albeit with small effect sizes: Fp1 ($F(1,1014)=8.235$, $p=.004$, $d=-0.089$), Cz ($F(1,1014)=4.142$, $p=.042$, $d=-0.128$), and Oz ($F(1,1014)=5.948$, $p=.015$, $d=-0.169$).

Table S2.1. *Timing (ms from 0) and AUC values of the N8 and N270 peak.*

		ms	AUC (μV^2)	ms	AUC (μV^2)
iSPOT-D	ICA-HEP	8	-0.981	270	-0.267

Table S2.2. *Overview of the most positive and most negative site, and a site topographically in between, for the N8 and N270 peak and their respective AUC values.*

	N8		N270	
	Site	AUC (μV^2)	Site	AUC (μV^2)
Most positive	Oz	1.52	T3	0.0455
Most negative	Fp1	-2.81	FCz	-0.293
In between	Cz	-1.34	C3	-0.527

3

NEURO-CARDIAC GUIDED RTMS AS A STRATIFYING METHOD BETWEEN THE ‘5CM’ AND ‘BEAM-F3’ STIMULATION CLUSTERS

This chapter is published as:

Zwienenberg, L., Iseger, T. A., Dijkstra, E., Rouwhorst, R., Dijk, H. van, Sack, A. T., & Arns, M. (2021). Neuro-cardiac guided rTMS as a stratifying method between the '5cm' and 'BeamF3' stimulation clusters. *Brain Stimulation, 14*(5), 1070–1072. doi: 10.1016/j.brs.2021.07.005

DEAR EDITOR,

Fitzgerald (2021) reviewed the question *'Targeting repetitive transcranial magnetic stimulation in depression: do we really know what we are stimulating and how best to do it?'*. Two clusters of stimulation targets in use for rTMS treatment in major depressive disorder (MDD) emerge, one surrounding the more posterior '5-cm' rule and another surrounding the more anterior 'Beam-F3' target. Given the fact that large effectiveness studies have demonstrated comparable response (47-58%) and remission (29-37%) rates for the 'Beam-F3' cluster (Blumberger et al., 2018) and '5-cm' cluster (Carpenter et al., 2012) respectively, we hypothesize if these comparable rates could be explained by inter-individual differences. This is in line with Fitzgerald's notion that *'...some patients are just likely to be better off treated at a posterior site and others more anteriorly, due to the connectivity of the networks or individual elements of prefrontal anatomy...'*. Thus, effectively addressing such inter-individual differences and 'stratify' patients to either the 'Beam-F3' or '5-cm' cluster could result in increased response and remission rates, whilst at the same time not 'doing any harm' given that both heuristics are already widely implemented and used in clinical practice. In analogy, one could consider this an 'SSRI-vs-SNRI', where we know that group level response and remission rates are the

same when patients are randomized, but when stratifying people to one or the other using a biomarker such as EEG alpha asymmetry, this could significantly enhance clinical response on the individual level (Arns et al., 2016; van der Vinne et al., 2021).

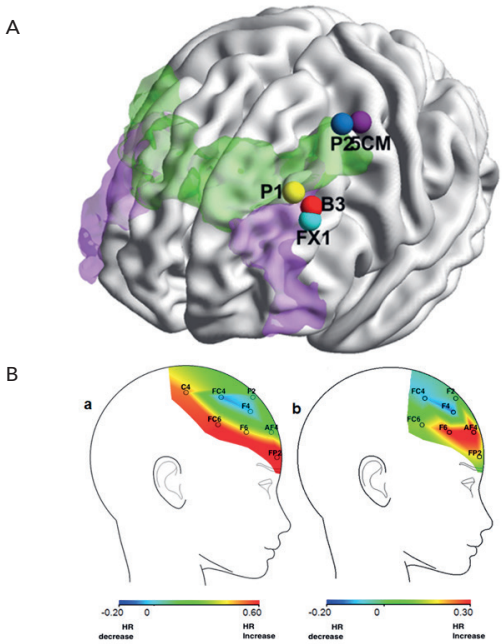
One technique that may be considered is neuro-cardiac guided rTMS (NCG-TMS). This technique aims to activate the frontal-vagal pathway, resulting in downstream effects observed as heart rate (HR) decelerations (Iseger et al., 2017; 2020). This frontal-vagal network shares overlapping functional nodes with the deregulated brain network associated with MDD, including the DLPFC, sgACC and vagal nerve (Iseger et al., 2020). Since Fitzgerald's review, Iseger et al. (2021) further replicated and validated the NCG-TMS method by stimulating multiple 10-10 EEG sites with 10Hz. The specific HR deceleration at stimulation sites F3/F4 and FC3/FC4, respectively corresponding to the 'Beam-F3' and '5cm' clusters, as well as the acceleration of HR at all other stimulation sites (Figure 3.1Ba) was replicated.

However, until now, the Iseger et al. (2021) study as well as prior studies only included healthy participants. Therefore, before considering the NCG-TMS method as a site-stratification method for MDD treatment, it requires replication in an MDD population.
Thirty-three MDD patients (average age 52.0, 15 male) who received rTMS for treatment of MDD, underwent an NCG-TMS assessment either at session 10 of treatment, or after unsuccessfully completing treatment. All patients provided written informed consent.

NCG-TMS procedures were identical to those used in Iseger et al. (2021), except that site C4 was not included since no HR deceleration was expected based on previous studies. In short, 10Hz trains (5 sec. duration, 100% MT, 30 sec. ITI) were applied to 7 different 10-10 locations on the right hemisphere (F4, FC4, F2, F6, FC6, FP2, AF4) with either a Deymed DuoMAG XT-100 (Deymed Diagnostic, Czech Republic) or Magstim Super Rapid^2 (The Magstim Company Ltd., UK), with a 70mm figure-8 coil. Stimulation was right sided, which is the first stimulation choice at the neuroCare Nijmegen clinic. Furthermore, Iseger et al. (2020) demonstrated in an individual participant meta-analysis a lack of laterality differences for NCG-TMS, therefore laterality is

not expected to differ. Every location was randomly stimulated 3 times across all sites. A custom EEG cap without electrodes (ANT-Neuro) was used in order to locate the 10-10 system locations. During stimulation, the patient was asked to sit relaxed, breath normally and to avoid talking, since this could influence HR.

No side effects or adverse events were reported. In line with previous findings in the healthy control group (Figure 3.1Ba; from Iseger et al., 2021), we found HR decelerations for F4 and FC4, as well as HR accelerations in all other locations (Figure 3.1Bb). Figure 3.1C shows the overview of optimal stimulation sites for all subjects.



C

Site	F4	FC4	F2	F6	FP2	AF4	FC6
Optimal site for % of subjects	27.3%	21.2%	18.2%	9.1%	9.1%	9.1%	6.1%

Figure 3.1. *A) shows the potential stimulation sites on the cortex in MNI space. P1='dysphoric' site, P2='anxiosomatic' site, FX1=optimal site of sgACC, B3=BeamF3 location, 5CM=5cm location (Reprinted with permission from: Fitzgerald, 2021). B) shows group level topographical plots of RR interval changes. The scale represents the inversed z-scores. Blue indicates HR deceleration, orange/red indicates HR acceleration, as a result of 10Hz TMS. The healthy control group (Ba), and the MDD sample (Bb) both show HR deceleration at F4/FC4 and acceleration at the other sites. C) Shows the overview of optimal stimulation sites for all subjects based on NCG-TMS*

Taken together, the NCG-TMS method confirmed, in a data-driven way, in two independent samples (MDD as well as controls), the 'Beam-F3' and '5-cm' clusters Fitzgerald described in his review. Nearly half (48%) of all subjects demonstrated the 'Beam-F3' or '5-cm' cluster to be the individually best stimulation site (i.e. the site with largest HR deceleration), confirming inter-individual variability between optimal NCG-TMS sites. Although less than 50% seems few, the NCG-TMS was probed at 100% of MT. In practice, TMS is often applied at 120% of MT, thereby compensating for spatial inaccuracies, thus likely increasing the likelihood of appropriate targeting. Future research should investigate targeting other than '5-cm' and 'Beam-F3' cluster-sites for efficacy and side-effects more systematically.

Given the similar effectiveness of both target clusters in MDD on the group level (Carpenter et al., 2012; Blumberger et al., 2018) and given the fact that both methods are already widely used, it is suggested that NCG-TMS could be used as a means of treatment-stratification. The question then is whether such an a priori NCG-TMS based determination of the individual target would also translate into higher clinical efficacy by reducing inter-individual variability? As indicated by Fitzgerald, answering that question takes substantial sample sizes due to comparing two active treatment groups. To address this question, we are currently planning a large naturalistic prospective trial in three clinics to investigate the effect of the NCG-TMS based stratification approach on response and remission rates in MDD. In addition, we are currently investigating how this method may also be used to determine individual frontal thresholding opposed to relying on the motor-threshold.

4

TRANSCRANIAL MAGNETIC STIMULATION INDUCED HEART-BRAIN COUPLING AS A TARGET ENGAGEMENT TECHNIQUE: DEFINING A FRONTAL EXCITABILITY THRESHOLD

Based on:

Transcranial Magnetic Stimulation induced Heart-Brain Coupling as a Target Engagement Technique: Defining a Frontal Excitability Threshold.
Zwienenberg, L., Dijkstra, E., van Dijk., H., Rouwhorst, R., Middleton, V., Downar, J., Sack, A.T. & Arns, M. (*In preparation*).

ABSTRACT

Transcranial magnetic stimulation (TMS) induced Heart-Brain-Coupling (HBC) has been proposed as a prefrontal-specific target-engagement biomarker. Here we replicate and validate HBC-TMS as a technique for target localization and establishing prefrontal excitability thresholds.

HBC-TMS was assessed in 36 healthy participants across 2 experiments and contrasted to resting state HBC in 420 participants. TMS induced HBC-power and HBC-logpower were assessed at four prefrontal sites. A novel method was developed to ascertain the frontal threshold (FT) which was compared to the conventional motor threshold (MT) and assessed for its test-retest reliability. The test-retest reliability of the site demonstrating the highest HBC power were assessed in 10 healthy participants across 5 HBC-TMS experiments.

HBC-TMS showed site-specific effects, with Beam-F3/4 sites being twice more likely to show HBC relative to 5CM sites across individuals. The FT correlates to MT, but at significantly lower intensity (7.75%MSO, d>.85) and is reliable over time (r>.80). Lastly, participants that demonstrate HBC have more reproducible best-HBC sites across time.

HBC-TMS serves as a reliable and reproducible technique for indexing prefrontal target engagement and FT. FTs are consistently lower than MTs, which has implications for generalizing MT as a proxy for frontal TMS applications. Future studies may utilize HBC as a prefrontal-specific marker for optimization of TMS parameters (pulse shape, pulse width, coil orientation, etc.) rather than generalizing parameters obtained from motor cortex using MEPs. Using the HBC-TMS based FT instead of MT in clinical practice could be used for target personalization, target-engagement verification, and result in potentially higher remission rates.

INTRODUCTION

Transcranial magnetic stimulation (TMS) targeting the dorsolateral prefrontal cortex (DLPFC) is a safe and effective treatment for major depression (Carpenter et al., 2012; Blumberger et al., 2018). To further improve treatment outcomes for TMS, there is a growing interest to refine TMS parameters, such as the stimulation target and intensity. Conventionally, TMS parameter optimization relies upon markers derived from primary motor cortex, such as motor evoked potentials (MEPs) and motor thresholds (MTs). However, therapeutic TMS often targets other regions, such as dorsolateral prefrontal cortex (DLPFC), and ideally, target-engagement markers would rely upon effects from stimulation of this target directly, rather than simply inferring that parameters optimized via MEP effects will automatically generalize to DLPFC stimulation.

To address this issue, a target engagement method targeting the DLPFC was recently developed, called Heart-Brain-Coupling TMS (HBC-TMS; Dijkstra et al., 2023). This method is based on activation of a frontal-vagal network, which includes nodes within the DLPFC and subgenual anterior cingulate cortex (sgACC; Dijkstra et al., 2024) and results in downstream activation of the vagus nerve. This frontal-vagal network has been implicated both in depressive symptoms as well as the regulation of parasympathetic autonomic function (Makovac, Thayer & Ottaviani, 2017; Iseger et al., 2020). When this network is targeted with TMS, heart rate is modulated resulting from transsyn-

aptic parasympathetic activation (Iseger et al., 2017; Makovac, Thayer & Ottaviani, 2017; Zwienenberg et al., 2021). Specifically, heart rate decelerates during the TMS stimulation trains and normalizes during the inter-train-interval (ITI), leading to a specific modulation frequency in heart rate that follows the TMS duty-cycle. This has been demonstrated for two different TMS protocols: iTBS (2s stimulation and 8s ITI) and 10Hz TMS (5s stimulation and 11s ITI) resulting in 1/10=0.1Hz and 1/16=0.0625Hz HBC-frequencies, respectively (Dijkstra et al., 2023).

Stimulation intensity for prefrontal TMS in current clinical practice relies on the motor threshold (MT), derived from stimulation of the primary motor cortex. However, prior work has demonstrated that prefrontal excitability could differ substantially from the excitability of the motor strip (Iseger et al., 2021, Tik et al., 2023), leading to incorrect inferences regarding the optimal parameters of stimulation for the DLPFC. For example, overestimation of the excitability threshold for DLPFC-TMS could result in excessively intense stimulation, with the resultant side effects of scalp pain, uncomfortable muscle contractions, or in extreme cases, syncopal episodes during treatment (Rouwhorst et al., 2022). Ideally, a DLFPC-specific marker such as HBC could be used to generate a more accurate estimate of frontal excitability – the frontal threshold (FT).

The primary aim of this study was to replicate site-specificity of HBC in a larger sample, and to develop a methodology to reliably use HBC-TMS to determine a FT. HBC-TMS data were contrasted to non-TMS resting state HBC, to establish a fixed threshold that would reliably indicate engagement of the frontal vagal network and thus represent the FT. In addition, we assessed test-retest reliability of FT. Finally, we compared the within-subject relationship between the FT and the conventional MT.

METHODS

We performed three experiments. Experiment 1 was designed to a) replicate previous HBC findings, b) examine the influence of pain on HBC and c) investigate test-retest reliability of the FT. Experiment 2

was aimed at identifying the cut-off value for establishing a frontal excitability threshold, by contrasting HBC-values obtained from a non-TMS resting state to TMS-induced HBC. Experiment 3 was executed to examine spatial test-retest reliability, factoring in FT. Importantly, the validation of the HBC processing pipeline was performed concurrently on synthetic data (see Supplemental materials).

PARTICIPANTS

Experiment 1. 45 healthy participants (average age 36.67 (11.77); 21 female) were included after providing written informed consent. Exclusion criteria were 1) neurological/psychiatric disease, 2) age under 18 years, and 3) standard exclusion criteria for TMS. This study was approved by the ethical committee of Maastricht University (ERCPN-OZL_246_168_12_2021) and data was collected at six different Dutch outpatient TMS clinics.

Experiment 2. 420 participants (healthy subjects (n=25) and patients (n=395)) were included who underwent a resting state EEG assessment (including ECG) as part of routine care, to extract resting state HBC. In addition, a total of 98 unique participants (54 female, age 16-73) were included who underwent HBC-assessments in different studies: n=36 HC from Experiment 1 (of which 32 were recorded twice), n=52 MDD from Rouwhorst et al. (in preparation) and n=10 HC from Dijkstra et al. (2023). HBC-TMS data from MDD patients were assessed as part of routine care. Recordings took place at seven different sites, resulting in 525 ECG datasets.

Experiment 3. Ten healthy participants (average age 29.5 (3.66); nine female) were included after providing written informed consent. The same exclusion criteria were applied as for Experiment 1. Data was collected at Salience Health (Plano, Texas, USA) as part of regular care.

STIMULATION PROTOCOL AND TMS DEVICE

Stimulation parameters followed a specific 10Hz protocol (5s on, 11s off) with an intensity sweep of 15 stimulation trains from low to high intensities defined as 2% maximum stimulator output (MSO) steps, with one stimulation train per intensity, and with the 15th intensity matching

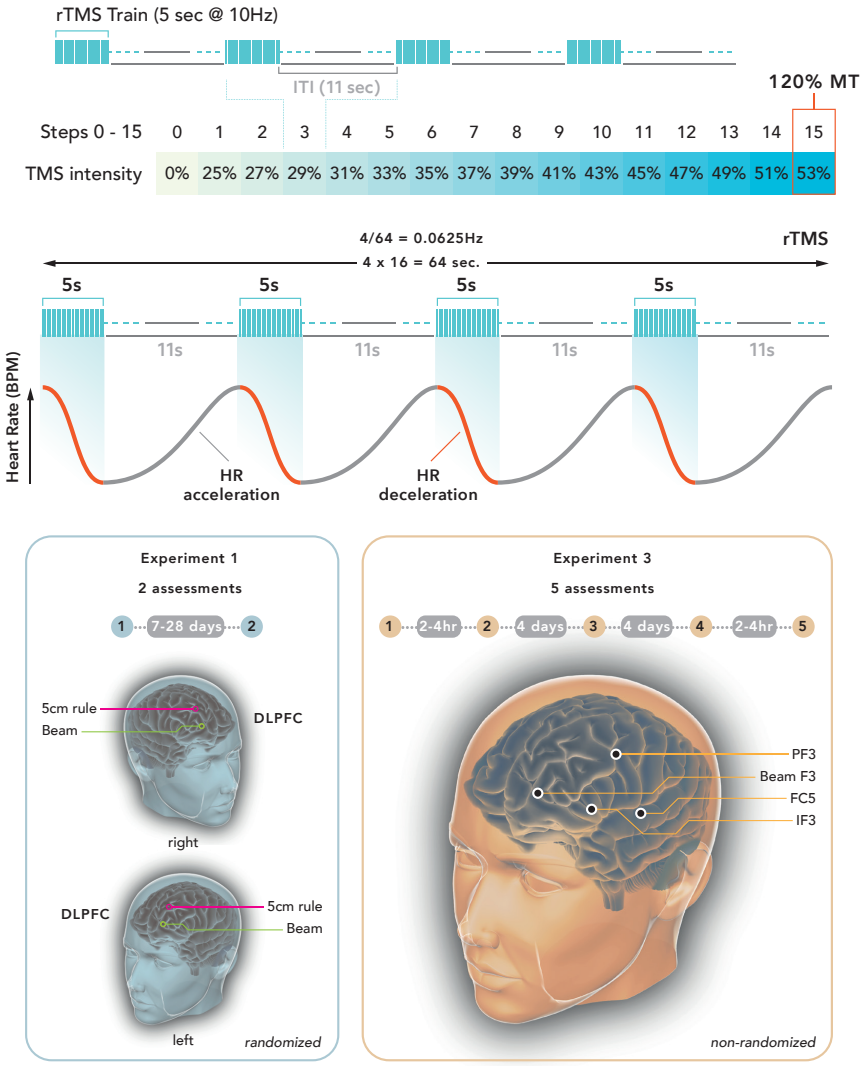


Figure 4.1. Visual overview of the HBC-TMS stimulation protocol (top), TMS induced heart rate oscillations (middle) and designs of experiment 1 and 3 (bottom).

to the individual's 120%MT. The starting intensity was thus defined as 28%MSO below 120%MT. The stimulation was preceded by 16 seconds of no stimulation, resulting in a total duration of 256 seconds of stimulation per site. Heart rate was measured using a Polar H10 band (Polar Electro 2022) connected through Bluetooth with the Heart-Brain Connect app (version 1.32, 2022, Brainclinics). See Figure 4.1 for a visualiza-

tion of the stimulation protocols, HBC-TMS induced HR oscillations and the designs of Experiment 1 and 3.

Experiment 1. Participants received two HBC-assessments, in which the Beam and 5CM location on both right and left prefrontal cortex were stimulated with TMS while measuring heart rate, separated by one to four weeks between assessments. TMS was applied with either a DuoMag XT-100 system (Deymed Diagnostic; DuoMag 70BFX LQC coil) or a MagPro R20 system (MagVenture; MCF-B70 coil). The order of testing the 4 locations was randomized across participants during the first assessment and remained the same within-subject for the second assessment. Stimulation rounds alternated between hemispheres to prevent cross-over effects, and the same targeting method (Beam/5CM) was always used consecutively (e.g., BeamF3 – BeamF4). After every stimulation round, participants were asked to rate painfulness of stimulation from 0-10 (10 as most painful) at that site on a numerical rating scale (NRS-11). Participants were instructed to sit still and to not talk during the assessment. To control for inter-researcher variability, both assessments in each participant were performed by the same researcher.

Experiment 2. In this separate set of participants, HBC data were collected during an EEG assessment that also included one lead to collect ECG data (C7 referenced to M2). The EEG was part of routine care and was collected during a 3-minute resting state (RS) with eyes open. Please see the supplemental materials for further details.

Experiment 3. In this third set of participants, each individual underwent five HBC-assessments spread over 8 days: trial A on day 1 in the morning, trial B 2-4 hours after trial A, trial C on day 4 in the morning, trial D on day 8 in the morning, and trial E 2-4 hours after trial D. During each assessment, participants were stimulated at four different sites on the left hemisphere in the same order: PF3, FC5, BeamF3, and IF3 (the PF3 and IF3 locations correspond to heuristics for locating two optimized DLPFC targets based on the lesion-network maps of depression developed by (Siddiqi et al, 2021; Mir-Moghtadaei et al., 2022). TMS was applied with a MagPro R20 system (MagVenture), equipped with a focal figure-of-eight coil. If a participant found a site to be too uncomfortable to continue the intensity sweep, the remaining TMS

trains were continued at the last tolerable intensity (*n*=3).

COIL POSITIONING AND MT

Experiment 1. The TMS coil was positioned over the Beam and 5CM site at an angle of 45° relative to the parasaggital plane. The 5CM site is defined as the site 5cm anterior to the scalp position of the motor hotspot in a parasagittal line. Beam sites were defined using the Beam-F3 algorithm and software (Beam et al., 2009). If the distance between two sites was <1cm, the two sites were taken as one site (*n*=1). MT was determined before both assessments and defined as the lowest stimulation intensity that, in 4 trials, induced at least 2 visible thumb twitches of the contralateral hand. MT was measured for both hemispheres and the average was used to determine the stimulation intensity sweep as described above.

Experiment 3. The coil outlines for each site were determined and marked on two caps on the participant's head: a MagVenture cotton cap and a MagStim mesh cap. Site PF3 and IF3 were mapped using scalp heuristics as above (Mir-Moghtadaei et al., 2022). FC5 was measured using a pre-measured mesh cap corresponding to a 10-10 EEG electrode cap. Beam mapping took place using BeamF3 software (Beam et al., 2009). The individual MT was determined during trial A, defined as the lowest stimulation intensity that, in 10 trials, induced at least 5 visible thumb twitches. In case the participants were not taking any medication that could affect the MT, this intensity was used for all five assessments. Otherwise, the MT was measured before each trial (*n*=1).

DATA ANALYSIS

ECG data were analyzed according to the methods described in Dijkstra et al., 2023. The time-frequency power of 0.0625Hz was computed using a 10 cycle Morlet wavelet that was min-max normalized and normalized between 0 and 1 over all measurements within the subject. Additionally, in the current analysis the computed HBC-power was log-transformed to obtain a parametric distribution that could be compared between measurements (within and between subjects, see supplemental materials). These analyses were used to create individual HBC reports, which were visually inspected for artefacts.

In case of artefacts and deviations, the raw ECG data was corrected in KubiosHRVScientific (version 4.1.0, 2023, Kubios Oy) by manually scoring the R-peaks (Experiment 1; *n*=5, Experiment 3: *n*=3). The optimal site in the HBC report was established based on a combination of the HBC-power values, HBC-logpower values and visual inspection, aiming for sites with HBC-logpower values above the cut-off value as below described, thus showing actual HBC. Stimulation intensities 3-13 were used for the analyses, based on the finding that more data is needed to perform adequate frequency analyses on these slow waves, as described in the supplemental materials. In the Supplemental materials we describe the validation of the HBC method and processing. For analyses within participants the HBC-power was used, whereas the HBC-logpower was used for comparison between participants. Repeated measures ANOVAs were Greenhouse Geisser corrected if the assumption of sphericity was violated.

Experiment 1. Of the 45 participants a total of 9 were excluded because they found stimulation too painful (*n*=4), because data was lost (*n*=1) or data had low signal quality (*n*=4), resulting in 36 participants who completed the first HBC-assessment. Another four participants did not attend the 2nd assessment thus 32 participants completed both assessments. For two participants in assessment 1 only three sites could be analyzed due to inadequate signal quality for the site. Four (assessment 1) and one dataset (assessment 2) were adjusted in Kubios. To replicate previous HBC findings (Dijkstra et al., 2023) we performed a repeated measures ANOVA with a within-subjects factor of intensity (3-13) comparing HBC-power for the site with the highest HBC-power. To compare HBC-power for all sites we added another within-subject factor of site (4 levels: Beam and 5CM left and right). A chi-square test was performed to rule out order effects and data were visually inspected to investigate dose-response effects. We used a Pearson correlation and Independent samples T-test to examine the relationship between the FT and the MT. Further, (intraclass) correlations were calculated to chart the test-retest reliability of the FT and MT. Finally, we performed curve fitting analysis on the percentage of difference between the FT and the actual stimulation intensity.

Experiment 2. Visual inspection of the data resulted in a further exclusion of 39 ECG traces due to artefacts, noise, or ectopic heartbeats.

The remaining 486 datasets were analyzed according to the methods described above.

Experiment 3. Of the ten participants in this set, one participant underwent only three assessments because of sickness, and one participant underwent five, but the data of the last assessment was lost, resulting in four complete assessments for this individual. To study spatial test-retest reliability, we calculated the percentage of overlap regarding the site showing the highest HBC-power on average and Fleiss' Kappa to calculate the average agreement over the complete measurements for the site showing highest HBC-power. Fleiss' kappa was performed on the complete datasets (n=8). Then, we visualized the HBC-logpower over the assessments for participants showing high (>50%) and low (<50%) overlap. Finally, we performed a Chi-square test on the average percentage of overlap for each location to see if some locations are more prone to have more overlap.

RESULTS

Experiment 1. Replication. The repeated measures ANOVA of all sites (Figure 4.2, following page) showed a significant effect on HBC-power of intensity (F(1.966,62.911)=14.176, p<.001, h_p^2=.307), but no effect of site (F(3,96)=1.382, p=.253, h_p^2=.041) or site by intensity interaction (F(6.834,218.678)=.951, p=.467, h_p=.029). For the best site, the repeated measures ANOVA yielded a significant effect of intensity (F(2.420,23.116)=8.309, p<.001, h_p^2=.192). No correlation was found between the start location and the site with the highest HBC (r=.095). The Beam sites were more often identified as the individual site with highest HBC-power (n=24) compared to the 5CM sites (n=12; χ^2 =4, p=0.0455, Cramér's V=.333).

The influence of perceived pain on HBC is described in the Supplemental materials; in brief, across 2 independent analyses, we found
1) no differences between the average pain score of the location with highest HBC-power and the other location(s), and
2) no correlation between the normalized pain and HBC-power scores.

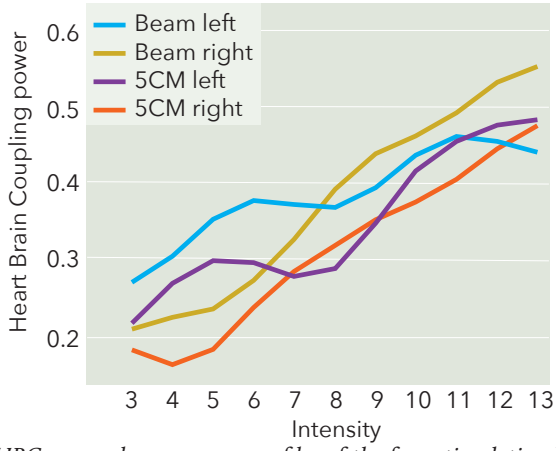


Figure 4.2. HBC-power dose-response profiles of the four stimulation locations of experiment 1, assessment 1.

Experiment 2: TMS-induced HBC was contrasted with ECG data recorded during a non-TMS resting-state (RS). The distributions of the HBC-logpower values were computed and compared for both conditions, depicted in Figure 4.3A, using a two-sided independent t-test. HBC-logpower was significantly higher than the RS HBC-logpower at 0.0625Hz (t=26.73, p<0.001, d=0.623). For completeness, since some of the participants took part in both the RS as well as the HBC-TMS assessment, a dependent t-test was performed between the RS and a random sample of the same size from the HBC-TMS assessments. This confirmed the difference between the two distributions (t=20.75, p<0.001, d=0.593) with similar effect size.

To determine an HBC based frontal excitability threshold, a one-tailed confidence level of 90% based on the RS distribution was employed. The determined HBC-logpower threshold was found to have a value of 9.5, which was visually verified by two researchers by inspecting border cases yielding that 9.5 indeed represents a reliable cut-off to indicate HBC-TMS (Figure 4.3B). A FT was established if two consecutive stimulation intensities showed an HBC-logpower of at least 9.5.

Experiment 1. FT Validation. For all participants, we were able to find an MT in both assessments. When comparing assessment 1 and 2, a strong correlation was found between the average MT (r=.981, p<.001),the left MT (r=.961) and right MT (r=.958).

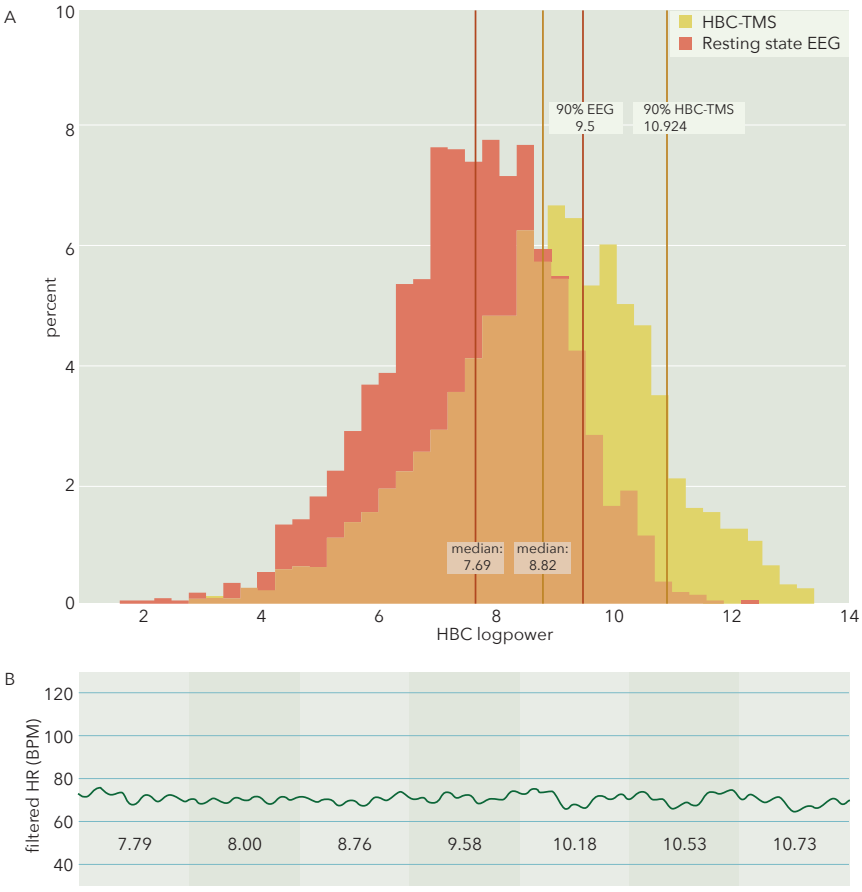


Figure 4.3. (A) Distributions of HBC-logpower at 0.0625Hz for resting state eyes open (in red) and HBC (in blue) segments of 16 seconds. (B) Example of an ECG signal (RR-interval as BPM) with the corresponding HBC-logpower values per stimulation intensity; at first showing no HBC (HBC-logpower below cutoff; 9.5) and with increasing intensity starting to show HBC, which is clearly visible in the ECG signal.

For 3 subjects no FT could be established. When comparing assessment 1 and 2, a strong correlation was found between the FTs of the best site ($t(26)=1.393$, $p=.175$, $d=.268$, $r=.805$), of the left ($t(22)=-1.632$, $p=.534$, $d=-.132$, $r=.729$) and right ($t(23)=1.173$, $p=.253$, $d=.239$, $r=.798$) hemisphere.

When comparing the MT and FT per hemisphere (Figure 4.4), we found a strong correlation and significant difference between the left MT and FT of assessment 1 ($t(29)=6.939$, $p<.001$, $d=1.267$, $r=.884$) and

assessment 2 (t(25)=5.449, p<.001, d=1.069, r=.800) and for the right hemisphere of assessment 1 (t(30)=4.479, p<.001, d=.805, r=.842) and assessment 2 (t(26)=5.896, p<.001, d=1.135, r=.868). The average difference between the MT and FT was 9.2%MSO on the left and 6.3%MSO on the right hemisphere and these differences were of large ES (d>.851).

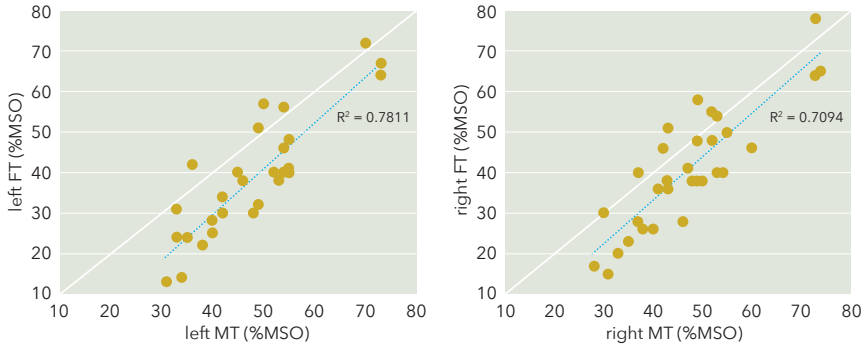


Figure 4.4. Correlation plot of MT with FT (both as %MSO) of the left and right hemisphere, with line of best fit (blue) and 45° reference line (white).

The differences between the FT and MT and 120%MT (as %MSO) can be found in Table S1, in the supplemental materials. Assuming most prefrontal TMS protocols utilize 120% MT, in Figure 4.5 we visualized what this implicates if such stimulation intensity would have been expressed as a function of FT. It implicates that at lower FTs the differences are larger with possible 'over-stimulation' of almost 250% FT in some cases, illustrating the importance of further development of a frontal excitability threshold. This relation followed a logarithmic function (logarithmic fit (F(1,28)=46.800, p<.001; R^2=.626). There was a significant difference for men and women (t(28)=-2.032, p=.001, d=-.748), with women having bigger differences (mean difference 28.36%MSO; Figure 4.5). We found a weak but significant correlation between percentage difference and age (r=-.382, p=.037), which was gone when controlling for the FT (r=.011, p=.956).

Experiment 3. Site test-retest reliability. Considering all data, there was a 68% overlap over the five assessments regarding best site, with a weak, but significant reliability (*Fleiss' Kappa:* (κ)=.182, p=.006; n=8). However, when visualizing the HBC-logpower for the participants

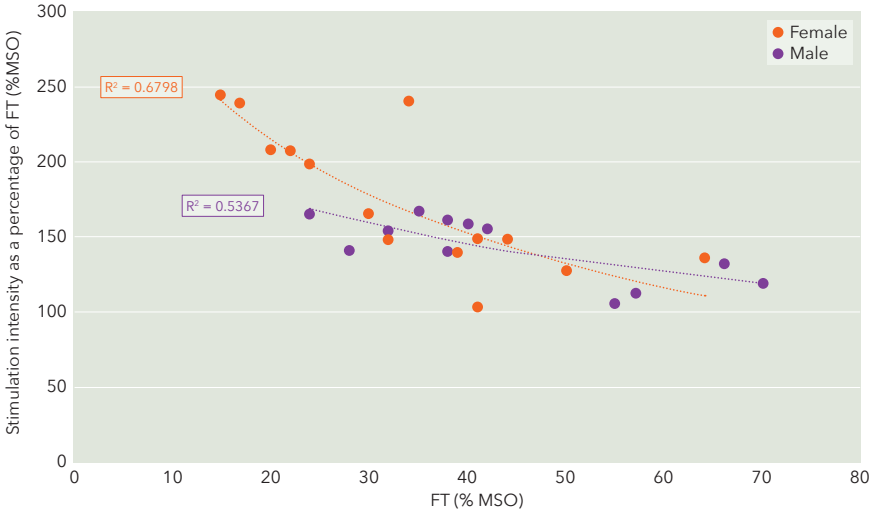


Figure 4.5. Correlation plot of percentage difference between the FT and the difference between FT and the conventional therapeutic stimulation intensity (120%MT) with a significant logarithmic fit explaining 62.6% of the variance over all data, 68% for females and 53.7% for males.

with high (>60%) and low (<60%) overlap (Figure 4.6), we see that those with an average HBC-logpower value of the first three assessment above the coupling cut-off value (N=4) showed a high consistency (89%) in best site, while those with low HBC-logpower values (N=6) did not (53%).

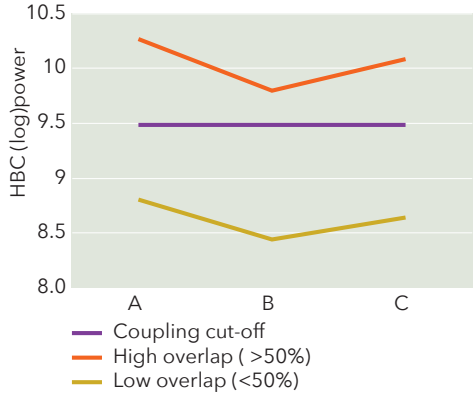


Figure 4.6. Overview of the average HBC-logpower, with a median split based on the percentage of overlap of their site showing most HBC-power over the HBC-assessments. Data shown is of complete datasets for all participants, which results in the first three trials: A (Day 1 AM), B (Day 1 PM), and C (Day 4 AM).

When looking at the reproducibility for each location individually, we found an average overlap of 26% for PF3, 22% for FC5, 29.5% for BF3 and 22.5% for lF3. This distribution was not significantly different from chance level (χ^2 =1.760, p=.624).

DISCUSSION

This study is a replication and extension of previous work on HBC-TMS (Dijkstra et al., 2023). We found that HBC was site-specific, with intraindividual differences in HBC between stimulation of evidence-based DLPFC-sites with Beam-F3/4 sites being twice more likely to be the individual site with strongest HBC. We further demonstrated that these effects were not induced by the painfulness of stimulation. In addition, we developed a method to establish an individual frontal excitability threshold that correlates strongly, but is significantly lower than MT. Finally, with multiple (3 to 5) repeated assessments we determined an overlap of best stimulation location of 68%, which was higher when only sites with HBC over the FT criterion logpower value of 9.5 were considered. These findings have several possible implications.

As we were able to replicate previous results on HBC-TMS and confirm that these effects were not driven by pain of stimulation, it suggests that HBC-TMS could be used as an effective and agile readout for DLPFC target engagement. The results seem to verify that HBC is caused by transsynaptic activation of the parasympathetic nervous system through stimulation of the prefrontal cortex. This is backed up by the study of Dijkstra et al. (2024) in which it was found that the location with the highest HBC-power is also the location with the highest anticorrelation to the sgACC.

Further, using HBC-TMS we determined that even though FT correlates to the MT, it is significantly lower, with an average of 7.75%MSO, with the differences representing a large ES, yet asymmetrically with lower MTs notably associated with even lower FTs. As such, in clinical practice, patients receiving stimulation at 120% of the MT may be receiving stimulation at a substantially higher mul-

tiple (up to 250%) of the threshold at which transsynaptic effects of stimulation are evident based on ECG readouts (see Figure 4.5). This might further explain why earlier studies that utilized subthreshold TMS (e.g. 80%MT or 90%MT) in MDD have also shown clinical benefit similar or superior to that with 120% MT (Cole et al., 2020; 2021; Chen et al., 2021). Using HBC-TMS to determine an individual FT thus would allow for lower treatment stimulation intensities, with the potential of decreasing side effects of TMS. Importantly however, not every participant showed HBC (n=3; 9%), possibly because the frontal-vagal hotspot for those individuals laid outside the grid of locations utilized in this study. This again points to interindividual variability of DLPFC-connectivity, and it might be worthwhile to perform HBC mapping of a wider range of sites overlying the DLPFC in future studies.

Lastly, the test-retest reliability of the best site in study 3 showed an overlap of 68% over all five measurements considering all subjects and all assessments but showing a weak, but significant internal consistency (κ=.182). However, it is important to keep in mind that the MT 'hotspot' can only be found if we elicit a motor response, therefore the HBC 'hotspot' can only be localized if we actually detect suprathreshold HBC. Bearing this point in mind, we found that the participants who showed more overlap regarding the location with the highest HBC-power also showed higher HBC-logpower values that were above the FT cut-off (Figure 4.6). This shows the potential of HBC-TMS site selection.

Nonetheless, it appears that the best HBC-TMS site hotspot is less spatially stable than the MT hotspot (Malcolm et al., 2006), which could potentially be explained by the anatomical differences between stimulated areas. The DLPFC has more interindividual variability, especially regarding sulcal pattern and microstructure (Bruno et al., 2022), whereas the primary motor cortex and specifically the 'hand knob', the representation of the hand in the motor cortex, appears to be very stable between individuals (Ahdab et al., 2014). Besides, it is possible that the optimal stimulation site lies somewhere in between the stimulated grid and therefore it is indirectly stimulated by all stimulation rounds, with different effects each time. In clinical

practice, the MT is often measured multiple times during treatment, for example every fifth assessment, resulting in different intensities and locations. A similar protocol could be used for HBC-assessments; thus, a larger and denser grid of sample points, alongside a more precise neuronavigational approach than simple scalp heuristics, could potentially yield a more stable HBC hotspot in future work. However, at this point, this takes up more time compared to MT testing. So future studies should focus on finding a more immediate HBC measure, comparable to the MT.

This series of studies come with some potential limitations. The sample size of study 3 was small and unbalanced regarding the sex of participants (90% female), thus the test-retest reliability should be investigated in a bigger and more representative sample in future studies. Also, in experiment 1 and 3, data was collected over a period of 5-28 days, while TMS treatment for MDD typically involves a longer period of time. It would therefore be valuable to investigate HBC-TMS at the beginning and end of an actual treatment course in the future. Adding to that, there might be some variation in targeting the proposed sites in experiment 1 and 3, as no neuronavigation was used. In experiment 1, this resulted in a small difference between stimulated sites between assessments (0.6 cm) and in experiment 3, a mesh cap was used, aiming for a stable difference between sites, although slight differences in the placement of the cap were still possible. However, despite these issues, the present findings offer good generalizability to standard clinical practice, particularly as many TMS clinics do not employ neuronavigation. We should also note that the influence of state-dependence on TMS outcome is a topic of increasing interest, and research elsewhere has noted differential effects of TMS during different brain states (Sack et al., 2023). In the current set of experiments, we did not take brain states into account, though they could possibly account for the differences that we found over time. Another limitation to the HBC-method in general is that it can be uncomfortable to undergo, demonstrated by four participants quitting the assessment because of pain. However, we note here that the FT is typically substantially lower than the conventional 120% of MT; thus, in future work, it may be possible to sample a lower range of intensities and thereby improve tolerability. Lastly,

the present results do not directly address the possibility of habituation (i.e. a potential lessening of the bradycardic response to stimulation with successive trains of stimulation even at a fixed intensity), which might result in an apparently lower HBC value at higher intensities. However, such habituation could potentially also apply to the therapeutic/neuroplastic effects of TMS itself, which might exert themselves primarily in the initial trains of stimulation. Thus, further work will be required in general to clarify the dynamics of efficacy for successive trains of stimulation, both in terms of their effect on HBC and in terms of their effect on depression and other therapeutic indications in clinical practice.

Despite these limitations, the current study has several important implications. First, we have replicated and validated previous work on HBC-TMS, showing site specificity between evidence-based stimulation locations. Adding to that, we have established and tested a method to determine the FT, which showed a high test-retest reliability. Thus, besides using HBC-TMS for individualization of stimulation location and intensity, it is a straightforward measure to employ in future parameter-optimization studies that have previously employed MEPs as an imperfect proxy for transsynaptic activation in the DLPFC, whose threshold may be different from that of primary motor cortex. HBC-TMS may therefore have essential applications not only in clinical practice, but also in basic research on the optimization of parameters (e.g., optimal pulse width, pulse shape, pulse pattern, and coil angle) for stimulation of the DLPFC that do not rely upon generalization from effects in the motor cortex as a proxy, but instead rely on effects mediated directly through stimulation of the intended region itself.

SUPPLEMENTAL MATERIALS

1. DETERMINATION OF THE FRONTAL EXCITABILITY THRESHOLD USING HBC-TMS

1.1 Participants and data collection

For parametrization of Heart-Brain Coupling (HBC) data to establish a frontal excitability threshold (FT) cut-off value, we utilized the largest dataset available, combining all data collected so far. The sites where the HBC-assessments took place are: Brainclinics (52 MDD patients; 5 HC), neurocare (19 HC; Amsterdam & Den Haag), Neurowave (12 HC) and Synaeda (13 HC) Leeuwarden and Drachten).

1.2 Analysis and validation

The power at 0.0625 Hz was computed as described in (Dijkstra et al., 2023). However, since the goal was to compare between subjects, the normalized power was not suitable. To be able to compare between subjects, the power was log-transformed to meet a normal distribution (HBC-logpower).

Given the low frequency of interest, data at the edges (first and last two intensities) are in some cases attenuated by the analysis methods. For dose-response analyses, data recorded during intensity 3 to 13 were considered, given the higher signal-to-noise ratio for these intensities. See Paragraph 2 of the supplemental materials for extended explanation.

1.3 Determining the frontal threshold (FT)

The HBC-TMS dataset resulted in a total of 4775 16-sec. segments recorded for intensities 3 to 13 recorded from the 101 participants. ECG data of patients was obtained using a custom designed EEG system (Brainmarker Platform, Brainclinics Foundation, Nijmegen), using a 40-channel Compumedics Grael amplifier. Data was recorded for three minutes of rest with eyes open (RS-EO). An electrode placed at C7 referenced to M2 was used to acquire the ECG. After visual inspection, RR intervals were semi-automatically annotated using an custom algorithm using several Python packages (©2001-2022 Python Software Foundation, numpy (Gramfort et al., 2013), scipy (Virtanen et al., 2020), hrvanalysis (https://github.com/Aura-healthcare/hrv-anal-

ysis). The resulting 243 clean recordings were analyzed using the same method used for the HBC method described in the main manuscript and subsequently segmented into 16 second segments similar to the HBC segments per intensity. This resulted in a total of 2508 segments and their corresponding average HBC-logpower values.

To evaluate the clinical applicability of this threshold, the number of measurements demonstrating HBC and the number of participants undergoing HBC-TMS were determined. The results, shown in Figure S4.1, indicate that using an HBC-logpower threshold of 9.5, implicates that for 79.8% of participants HBC will be found at any of the four stimulated locations; and 59.9% of all measurements showing HBC. For a majority of participants (79.8%) an optimal and individualized target location can be ascertained using the HBC assessment and stimulation can be applied at an intensity in accordance with current practices. For the remaining 20.2% of the participants undergoing the HBC-assessment it would not be possible to determine an individualized frontal threshold, possibly indicating that the standard Beam-F3/4 and 5 cm sites for those subjects are not adequate in targeting the frontal-vagal pathway, possibly requiring a different stimulation site.

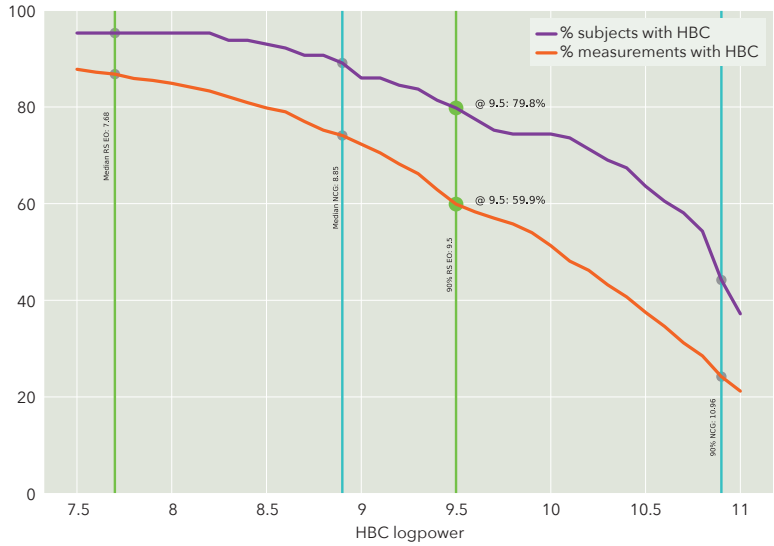


Figure S4.1. Rate of measurements that do show HBC when averaged over the whole measurement, depending on HBC-logpower.

2. VALIDATING THE HBC PROCESSING PIPELINE USING SYNTHETIC DATA

To further validate the HBC measure developed in this work, we simulated ECG data changing in response to TMS stimulation with HR decreases followed by a recuperation during the subsequent rest period (ITI), in line with Figure 4.1 from the main manuscript. The PQRST complex was modeled using a Daubechies wavelet. The inter beat interval (IBI) lengths were based on an average heart-rate of 60 BPM and were modeled to lengthen during the 'stimulation phase' and shorten during the 'resting phase'. As a response to the increasing TMS intensity the amount of increase and decrease of the IBI intervals was increased with each 16 second period. From this, the expected HR was computed (See Fig. S4.2). We hypothesized – also based on visual inspection of many individual HBC examples - the HR would decrease shortly and quite steeply after the start of the rTMS stimulation train and then slowly recover during the rest period (ITI). Using this model, a synthetic ECG trace was reconstructed, as depicted in Figure S4.2.

We then set out to manipulate several properties of the model and investigate how this influences the HBC measure. As can be seen in Figure S4.2A, when heart rate is modelled as always responding to the TMS stimulation in the same manner (lacking a dose-response effect), the HBC metric is somewhat affected in the first two and the last two intensities (see 'meanTFR@0.0625Hz' and 'meanTFR/intensity@0.0625Hz' panels). This is understandably related to the sudden start and end of the measurement where the ECG signal is cut off and the resulting HR fluctuations not fitting well to the very long wavelets needed for the analyses at these low frequencies. However, when a dose-response effect is modelled, the metric performs well (Fig. S4.2B). Further modeling showed that adding 'noise' reflecting breathing or very slow fluctuations, as well as mechanical noise in the ECG, can sometimes affect the HBC metric in the first and last two intensities in a similar manner as described for the first example (Fig. S4.2C). A higher signal-to-noise ratio was therefore expected for intensities 3-13.

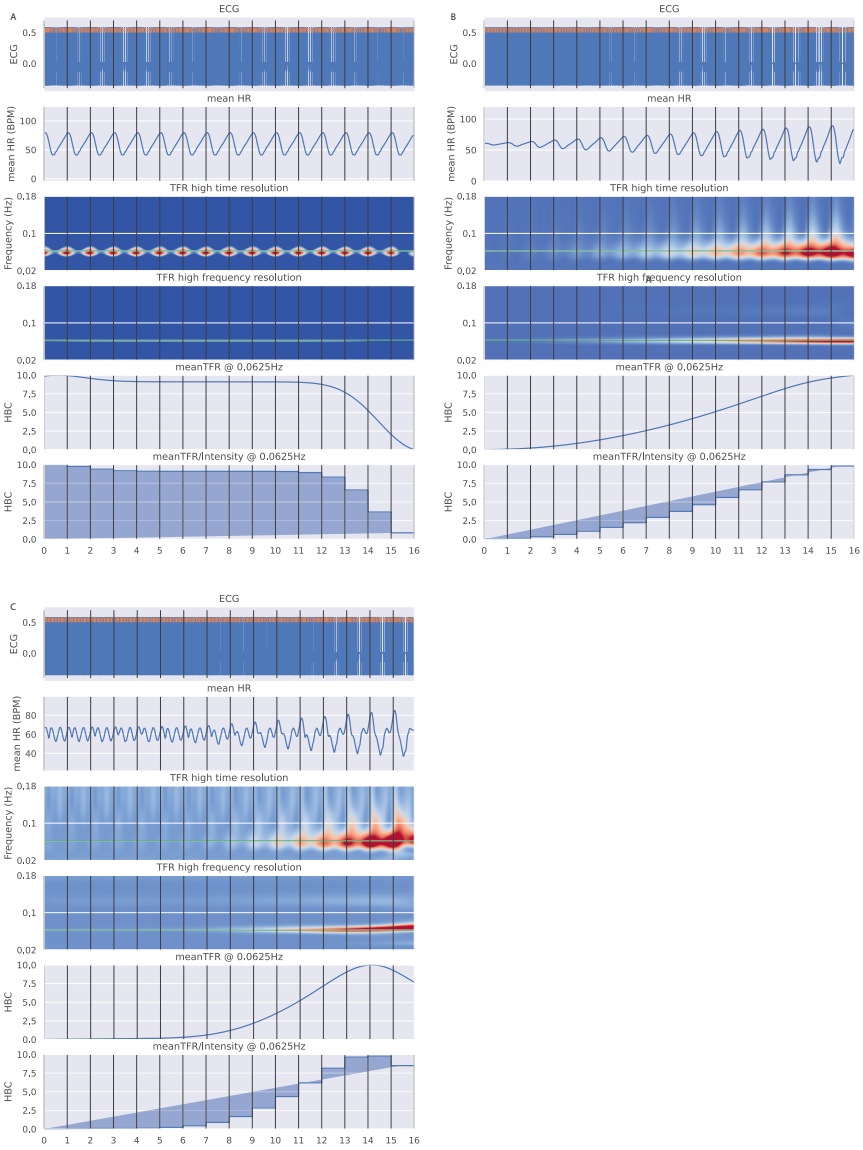


Figure S4.2. Visualization of the analysis used to compute the HBC metric A) when there is no dose-response, but HBC at all intensities, B) when HBC is developing as a dose-response effect and C) when adding breathing effects on the heart rate.

3. VALIDATION OF THE HBC MEASURE THROUGH CURVE FITTING

Based on the model presented in Figure 4.1 in the main manuscript we expected the heart-rate to start decreasing during stimulation and to start to rebound during the inter-train-interval, whereby the 1/16=0.0625 Hz band was determined to reflect HBC. In order to validate this, we averaged the time-series across subjects for 1) resting state; 2) low TMS-intensity (intensity 3) and 3) high TMS-intensity (intensity 13). Using curve-fitting, we subsequently fit a sine wave function of 0.0625 Hz to this data with the hypothesis that this fit would be better than a linear fit. Both functions were also fit to the resting state data where we expected a linear fit, due to the absence of rTMS induced HBC. Results are depicted in Fig. S4.3 where A) shows the response of the heart-rate to high TMS intensity (sine: y=sinπ(2*π*x*0.0625+0.53)*1.07-0.0001: R^2=0.92, linear: y=x* -0.025: R^2=0.09) and B) the HBC for RS-EO (sine: y=sin(2*π *x*0.0625+6.05)*-0.11-0.0003: R^2=0.54, linear: y=x*0.0056: R^2=0.23).

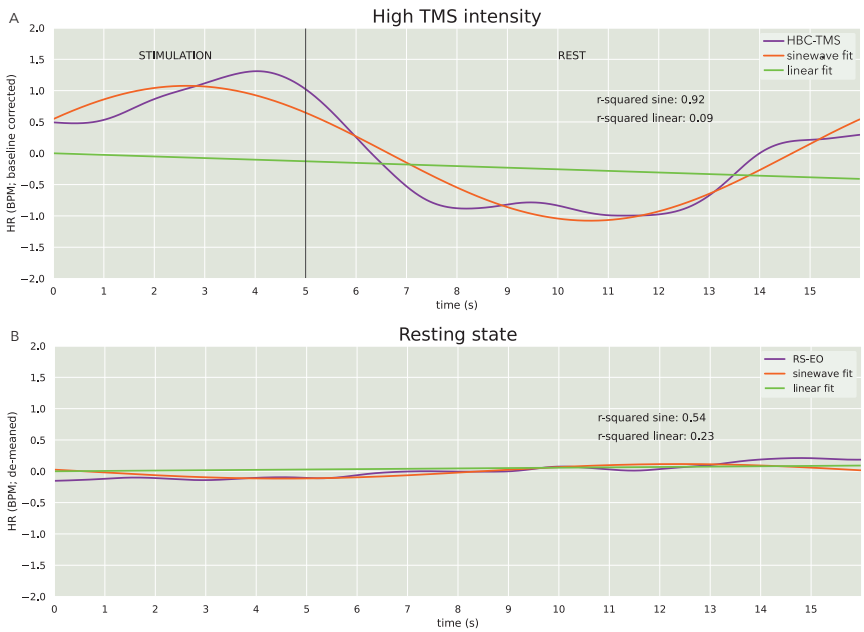


Figure S4.3. Curve-fitting for HR changes. A sine-wave fit to 0.0625Hz as well as a linear fit was performed for the HR changes A) in response to the rTMS stimulation protocol and B) resting state eyes open (RS-EO). During the high rTMS intensity stimulation the sine wave of 0.0625 Hz resulted in an R^2 of .92, and the linear fit an R^2 of .09. For RS-EO the sine fit yielded an R^2 of .54, while the linear fit resulted in an R^2 of .23.

4. DOES PERCEIVED PAIN MEDIATE TMS-INDUCED HBC?

The participants of Experiment 1 were asked to rate their perceived pain on a scale from 0 to 10 for every site where TMS was applied. In total 36 subjects with complete data were included in the current analysis, yielding a total of 140 measurements.

For each participant, the optimal HBC-location was determined as described in the main manuscript (e.g. highest normalized HBC-power). Then, the most painful location was identified as the location(s) with the highest pain score (if two locations had the same pain score, both locations were included in the analysis). As visualized in Figure S4.4 (left), the average pain score for the optimal HBC-location, relative to other locations did not differ (t=1.00; p=0.31). Then, we correlated normalized pain scores and normalized-HBC and found no significant correlation (Fig. S4.4 (right), r=0.13, p=0.12).

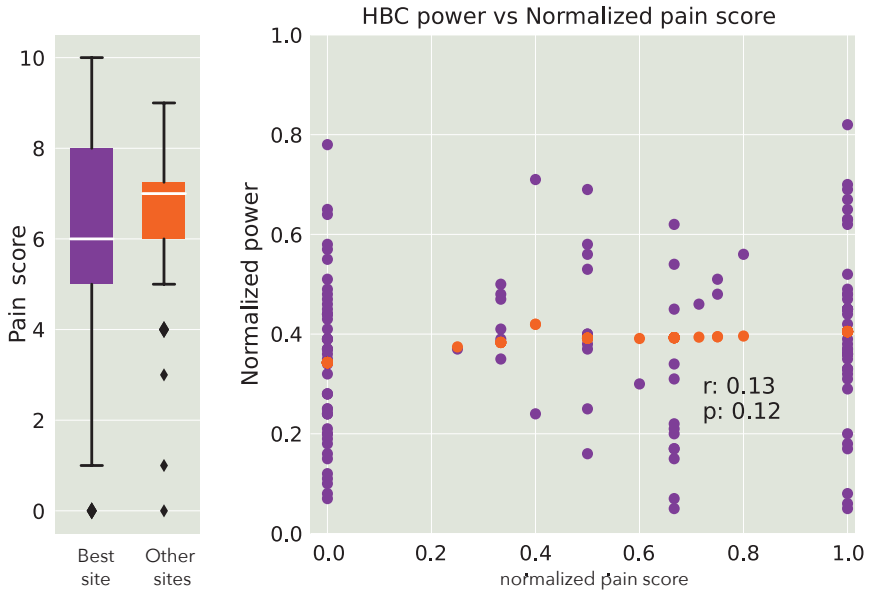


Figure S4.4. (Left) Pain scores of the location with the highest HBC-power vs. the other locations (right). The difference was not significant. (Right) Correlation between normalized pain score and HBC-power

Table S4.1. MT and corresponding FT as %MSO, stimulation intensity (matching 120% MT), and the percentage of difference between the FT and the stimulation intensity. Presented data is left hemisphere data from study 1, assessment 1.

ID	MT as%MSO, (120%MT)	FT (%MSO)	%MSO stimulation intensity above FT	% difference between FT & stimulation
NC3	48 (58)	34	24	242
NC4	73 (88)	64	24	138
NC5	42 (50)	30	20	167
NC6	55 (66)	44	22	150
NC7	55 (66)	42	24	157
NC9	49 (59)	55	4	107
NC10	50 (60)	-	-	-
NC11	49 (59)	35	24	169
NC12	53 (64)	40	24	160
NC13	46 (55)	-	-	-
NC14	70 (84)	70	14	120
NC15	55 (66)	42	24	157
NC16	54 (65)	57	8	114
NC17	73 (88)	66	22	133
NW1	46 (55)	39	16	141
NW2	40 (48)	24	24	200
NW3	35 (42)	20	22	210
NW4	36 (43)	41	2	105
NW5	38 (46)	22	24	209
NW6	31 (37)	15	22	247
NW7	33 (40)	24	16	167
NW8	38 (46)	22	24	209
NW9	34 (41)	17	24	241
NW10	43 (52)	-	-	-
NW11	40 (48)	32	16	150
SYL1	54 (65)	-	-	-
SYL2	52 (62)	38	24	163
SYL3	42 (50)	32	18	156
SYL5	33 (40)	28	12	143
SYL7	50 (60)	-	-	-
SYL9	54 (65)	50	15	130
SYL11	55 (66)	44	22	150
SYL12	45 (54)	38	16	142
SYL13	54 (65)	41	24	159
SYL14	55 (66)	-	-	-
SYL15	50 (60)	58	2	103

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TRANSCRANIAL MAGNETIC STIMULATION INDUCED HEART-BRAIN COUPLING: DIFFERENTIAL ACTIVATION PATTERNS USING SHORT PULSES ON FRONTAL RELATIVE TO MOTOR CORTEX

Based on:

Transcranial Magnetic Stimulation induced Heart-Brain Coupling: Differential Activation Patterns using Short Pulses on Frontal relative to Motor Cortex. Zwienenberg, L., Wendt, K., Hutchinson, H., Sack, A.T., Downar, J., Ali, K., Denison, T., Arns, M. & Stagg, CJ. (*In preparation*).

ABSTRACT

The neurophysiological effects of different transcranial magnetic stimulation (TMS) pulse parameters have mainly been studied on the primary motor cortex (M1). In the absence of such quantifiable readout measures for "silent" brain regions, results from motor cortex have been extrapolated to other brain areas, including prefrontal cortex targeted for treatment of depression. TMS induced Heart-Brain Coupling (HBC-TMS) utilizes heart rate as a readout for prefrontal TMS. We used HBC-TMS to investigate possible differential effects of pulse-width and pulse-shape and compared stimulation thresholds for M1 and prefrontal cortex.

In twenty healthy participants, we performed HBC-TMS using four square waves (95μs, 198μs, 265μs and 400μs) to determine frontal threshold (FT) and HBC with an intensity sweep with increasing intensity until 110% of the motor threshold (MT). We further studied the influence of pulse shape by comparing a sine (240μs) vs. square wave (265μs) pulse.

All pulse lengths showed similar dose-response profiles, and no differences were found when comparing different pulse shapes (sine vs.

square wave). However, shorter pulses induced stronger HBC relative to longer pulses, with medium to large effect sizes. The FT was lower than MT for all pulse widths, with larger differences for shorter pulse widths (Cohen's d ranging from 0.5 to 2.2).

HBC-TMS allows us to study the effects of different pulse parameters directly in prefrontal cortex. We observed no differences between sine and square wave pulse-shapes, but short pulse widths appear to be most efficient at exciting prefrontal brain regions relative to motor cortex. These results suggest that optimizing pulse parameters may be important for stimulation effects, both in terms of response to TMS in depression and decrease of potential side effects due to overstimulation.

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive neuromodulation technique used in both clinical practice and research. Currently multiple TMS devices are FDA approved for the treatment of treatment resistant depression on the basis of being 'substantially equivalent' to a predicate device, which for the treatment of depression is the Neuronetics NeuroStar hardware. However, TMS pulses differ substantially between different hardware platforms. Although, for technological reasons (Goetz et al., 2016), current devices generate damped biphasic sine wave pulses (Peterchev & Riehl, 2021), there are clear differences between the generated pulses in regard to pulse width (Figure 5.1). The predicate device Neuronetics NeuroStar has a short pulse width (185μs) relative to the widely-used Magstim and MagVenture devices (295μs and 300μs respectively) and slightly longer pulse width compared to the Mag&More device (160μs; Figure 5.1).

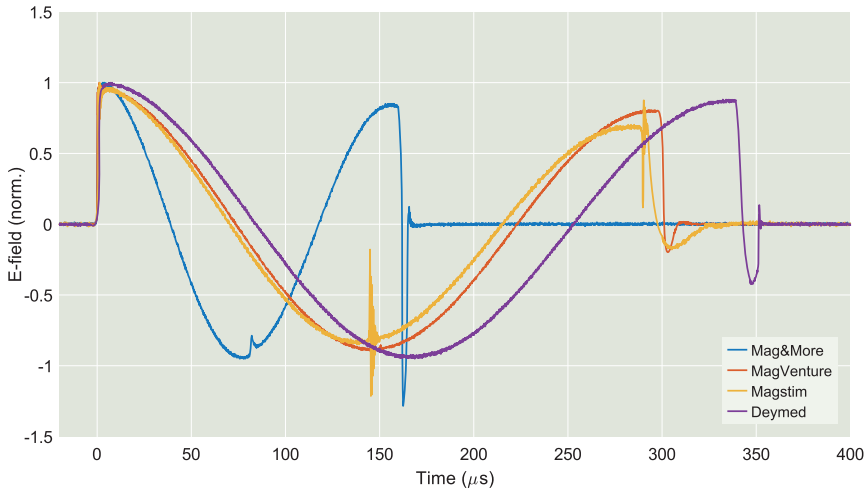

Figure 5.1. E-field measurements of four biphasic pulses: Mag&More (160μs; Apollo device with a PMD70-pCool coil), Magstim (295μs; Magstim Rapid2 device with an D70 Air film coil), MagVenture (300μs; MagVenture MagPro with an MCF-B70 coil) and Deymed (340μs; Deymed XT35 device with a 70BFXLQC coil). All pulses were measured with the same pick-up coil. The Deymed and MagVenture pulse shown are inverted for ease of comparison.

It is therefore not yet clear whether these devices can be truly considered 'substantially equivalent' in terms of their neural effects. We therefore aimed to systematically investigate the neurophysiological effects of repetitive TMS (rTMS) applied with different pulse parameters to the dorsolateral prefrontal cortex (DLPFC), the target site for rTMS treatment for depression.

Over time, devices that can generate varying pulse shapes have been developed. These have been tested primarily on the primary motor cortex (M1) to explore the effects of a number of pulse parameters, including pulse width. Previous M1 studies have demonstrated that longer pulses require a lower percentage of maximum stimulator output (MSO) to induce motor evoked potentials (MEPs) than shorter pulse widths (Peterchev et al., 2008), although more energy overall is delivered to the TMS coil during the longer pulses (Delvendahl et al., 2014). Pulse shape and phase are also important: in M1, square wave pulses tend to induce more inhibitory effects than sine waves (Goetz et al., 2016); and iTBS with monophasic pulses induces greater changes in M1 excitability than biphasic pulses (Wendt et al., 2023).

Such results are useful to understand the neurophysiology of M1. However, neurophysiological findings of M1 do not necessarily extrapolate to other cortical areas of interest such as the prefrontal cortex. Important treatment parameters for DLPFC-TMS, such as stimulation intensity, conventionally rely on the responses of M1 as a proxy, even though this is not the actual target site of the treatment itself. It has previously been largely impractical to investigate possible differential effects of pulse shape and pulse width in the DLPFC, as no objective, convenient readout measure such as visible motor contractions has been available.

Recently, a new method was proposed: TMS induced Heart-Brain-Coupling (HBC-TMS). HBC-TMS stimulates the frontal vagal pathway, which overlaps substantially with the depression network and, critically, includes the DLPFC as a key hub. For an extensive explanation of HBC-TMS, see Dijkstra et al. (2023) and Chapter 4. In short, a brief train of 10Hz rTMS to the DLPFC results in an almost immediate, transient heart rate deceleration which normalizes during the inter-train interval suggesting vagal nerve activation due to stimulation. As such, TMS induces changes in the cardiac rhythm as a function of stimulation cycle time, seen in oscillations in the inter-beat interval. The cycle time of a specific 10Hz protocol (5s on. 11s. off) comprises one TMS train every 16 seconds which results in a specific frequency of 1/16 = 0.0625Hz.

Previous HBC-TMS work showed that motor threshold (MT) and frontal threshold (FT) are correlated, but do not correspond exactly: importantly, the FT is significantly lower than the MT with a large effect size (Iseger et al., 2021; Chapter 4), suggesting differential excitability of prefrontal regions compared to M1.

Here, we used HBC-TMS to characterize dose-response profiles and FT for a range of TMS pulse widths overlapping with the majority of the currently FDA-approved TMS devices, to inform parameter optimization for TMS for depression, to reduce potential side effects - such as headache or syncope - and to better understand differences and similarities between the MT and the FT.

METHODS

PARTICIPANTS

20 healthy adults (mean age 22.5 years (SD 4.11), 9 female) gave their written informed consent to participate in the study. All study procedures were reviewed and approved by the local ethics committee at the University of Oxford (Central University Research Ethics Committee; R86517/RE001). Participants were reimbursed £10 per hour for their time. Exclusion criteria were 1) standard contraindications to TMS including, but not limited to, the presence of intracranial metallic or magnetic hardware and the presence of pacemaker or other stimulators/implants, 2) history of, or current, neurological or psychiatric illness, 3) family history of epilepsy, 4) treatment with any psychotropic medication (e.g. antiepileptics, antidepressants, antipsychotics, psychostimulants, benzodiazepines 5) pregnancy (assessed through participant declaration), and 6) use of medication that slows heart rate.

STUDY OUTLINE

Participants attended two sessions separated by at least a week (mean interval 9.25 days (SD 3.25). In session 1, we established the optimal HBC location for each subject and then we performed four HBC intensity sweeps, testing four square wave pulses of different pulse widths. Session 2 again started with establishing the optimal HBC location, to account for possible HBC variations. Then, using the HBC location from session 1 to ensure replicability, we performed three HBC intensity sweeps with biphasic sine wave pulses. After every sweep, participants were asked to rate painfulness of the stimulation from 0-10 (10 as most painful) on a numerical rating scale.

TMS DEVICES

TMS was applied with a cTMS device (controllable TMS; Rogue Research, CPWT901001-00002, FV 2.9.1), generating square-shaped pulses, and an xTMS device (Ali et al., 2023; Oxford University, Department of Engineering Science) which is able to generate varying

pulse shapes and widths. The cTMS technology is extensively explained in (Peterchev et al., 2008; 2011; 2013 & 2014) and the xTMS in (Sorkhabi et al., 2020; 2022; Wendt et al., 2023). The cTMS device was equipped with a figure-of-eight coil (Rogue Research, 901003-00009). For the xTMS, we employed a 70 mm figure-of-eight coil with a PA current (Magstim Co., P/N 9925-00). In one subject, due to lack of availability of the xTMS device, a Magstim Rapid2 TMS machine with a D70 Alpha flat (uncoated) coil was used for the initial HBC assessment.

CTMS PULSES

Bidirectional negative, square shaped (SQ) pulses with pulse widths of 95μs, 198μs, 265μs and 400μs (SQ95-400 respectively; Figure 5.2 left) were used with an M-ratio of 0.333. This M-ratio, which defines the relative amplitudes of each phase of the pulse, was chosen as the most likely to elicit MEPs across all pulse widths. Pulse width was defined as the total length of the negative (initial; twice because of the bidirectional waves) and positive phase. The duration of the positive and negative phases of the pulse were established so that there would be a linear difference between the different pulses (see the supplemental materials for the pulse characteristics). AP-PA current was used for all pulses

XTMS

The xTMS device uses an H-bridge-based circuit and a model predictive control algorithm to generate TMS pulses by approximating a reference waveform (Ali et al., 2023). To obtain the reference pulse shape and width, electric field waveforms generated by commercially available TMS devices from different manufacturers were measured using a pick-up coil. The induced E-field follows the same waveform as the coil voltage, which varies as the first derivative of the coil current (Peterchev et al., 2021). This relation was used to convert each E-field waveform to its equivalent coil current which was supplied to the model predictive control algorithm of the xTMS as the reference. A 10Hz protocol was generated with each of the pulses (described

below) and stored in a library which could be called during the study sessions using a graphical user interface.

XTMS PULSES

First, we recorded single biphasic TMS pulses from the following devices and measured their pulse widths: 1) a Mag&More Apollo device with a PMD70-pCool coil, 2) a Deymed XT35 device with a 70BFXLQC coil, 3) a Magstim Rapid2 device with an D70 Air film coil and 4) a MagVenture MagPro device with an MCF-B70 coil (Figure 5.1). Pulse width was defined as the total time for which the E-field is non-zero. The approximate pulse widths of the pulses were as follows: Mag&More: 160μs, Magstim: 295μs, MagVenture: 300μs, Deymed: 340μs. To accommodate the full range of sinusoidal pulse widths in common clinical use, we defined xTMS biphasic sinusoidal (SI) pulse waveforms of lengths 160μs, 240μs, 295μs, and 340μs (SI160-340 respectively) for experimental testing (Figure 5.2 right). To create the 240ms pulse, we manipulated the time course of the standard, biphasic Magstim waveform. AP-PA current was used for all pulses.

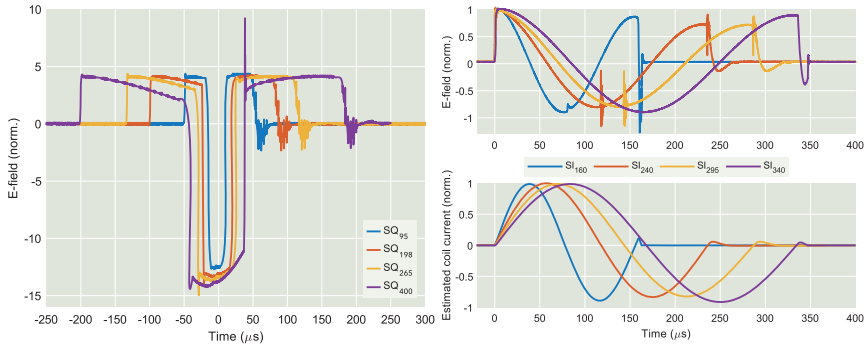

Figure 5.2. Measured E-field of (left) square wave pulses generated by the cTMS device: $SQ_{95\text{-}400}$, and (right) measured E-field and estimated coil current to generate the references for the sine wave pulses generated by the xTMS device: $SI_{160\text{-}340}$.

MOTOR THRESHOLD DETERMINATION

MTs were measured for each waveform in turn with the coil in a 45° angle to the medial-parasagittal plane. MT was defined as the lowest stimulation intensity that, in 4 trials, induced at least 2 visible thumb twitches of the contralateral hand. For participants whose MT was greater than 90% MSO, we defined their HBC sweeps for a MT of 90% MSO, since the HBC protocol increases up to 110% MT. If we were unable to determine the MT for a participant, they were excluded from the primary analyses.

HBC HOTSPOT LOCALIZATION

In session 1 we established the location with the highest average oscillatory power at 0.0625Hz (HBC-power; i.e., over all intensities) with at least two stimulation intensities with HBC (HBC-logpower over 9.5). The coils were positioned over the Beam and 5CM location (described below) of the left hemisphere at an angle of 45° relative to the parasagittal plane (the coil handle pointing posteriorly), which seems to be the optimal angle to stimulate frontal areas (Thomson et al., 2013) and which is used in most depression trials (Opitz et al., 2016).

To determine the optimal HBC location, we used SI295, first stimulating at the Beam location, which was defined using the Beam-F3 algorithm and software (Beam et al., 2009). We then stimulated at the 5CM location, defined as the location 5cm anterior to the MT hotspot in a parasagittal line. The optimal HBC location was then used as the stimulation location for that participant for the duration of the study. If HBC was absent in both locations, the participant was excluded from the study.

HBC STIMULATION PROTOCOL

HBC was assessed on the left hemisphere for all pulses using a 10Hz intensity sweep protocol (trains of 10Hz for 5 seconds with an ITI of 11 seconds; Figure 5.3) as described in Dijkstra et al. (2023) and Chapter 4. Neuronavigation (Brainsight, Rogue Research, version 2.5) was

used for within session site accuracy. The intensity was changed in incremental steps to ensure an intensity sweep as independent from the MT and covering the broadest intensity range as possible (see supplement for details and exact incremental steps per pulse width). Subjects were presented with an intensity sweep of 15 stimulation trains from low to high intensities with the 15th intensity matching 110% MT. Each sweep was preceded by 16 seconds of no stimulation, resulting in a total sweep duration of 256 seconds. A minimum of 5 minutes of rest after each stimulation round was ensured to prevent carry-over effects.

During stimulation, heart rate was measured using a Polar H10 band (Polar Electro 2022) connected through Bluetooth with the Heart-Brain Connect app (version 1.33, 2022 Brainclinics). Subjects were asked to sit still and to not talk during the recordings.

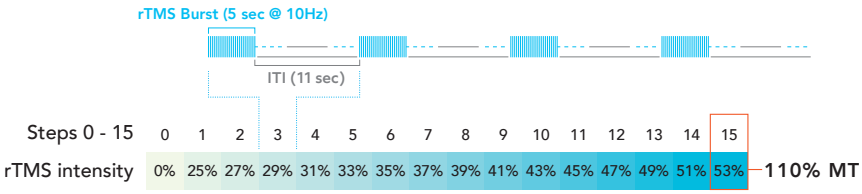

Figure 5.3. Visual overview of the TMS protocol used for the HBC-TMS sweeps.

ANALYSES

The ECG data were analyzed according to the methods described in Dijkstra et al. (2023) and Chapter 4. The time-frequency power of 0.0625Hz was computed using a 10 cycle Mortlet wavelet that was min-max normalized between 0 and 10, and then between 0 and 1 over locations and the computed HBC-power was log-transformed. Because of the between-subject comparisons, we operationalized HBC in this study as HBC-logpower, see Chapter 4 for more information. These analyses were used to create individual HBC reports, which were visually inspected for artefacts. In case of distinct visual deviations in the HBC reports, the best site was determined based on visual inspection (n=1). Given the low frequency of interest, data at the edges (first and last two intensities) are in some cases atten-

uated by the analysis methods. For dose-response analyses, data recorded during intensity 3 to 13 were considered, given the higher signal-to-noise ratio for these intensities. An extended explanation for this is found in the supplemental materials of Chapter 4. The FT determination is extensively described in Chapter 4, but in short, it is the first stimulation round (of at least two consecutive stimulation rounds) with HBC-logpower > 9.5 and shows the lowest intensity of stimulation that activates the frontal-vagal pathway. For the participant and data inclusion flowchart, see Figure 5.4.

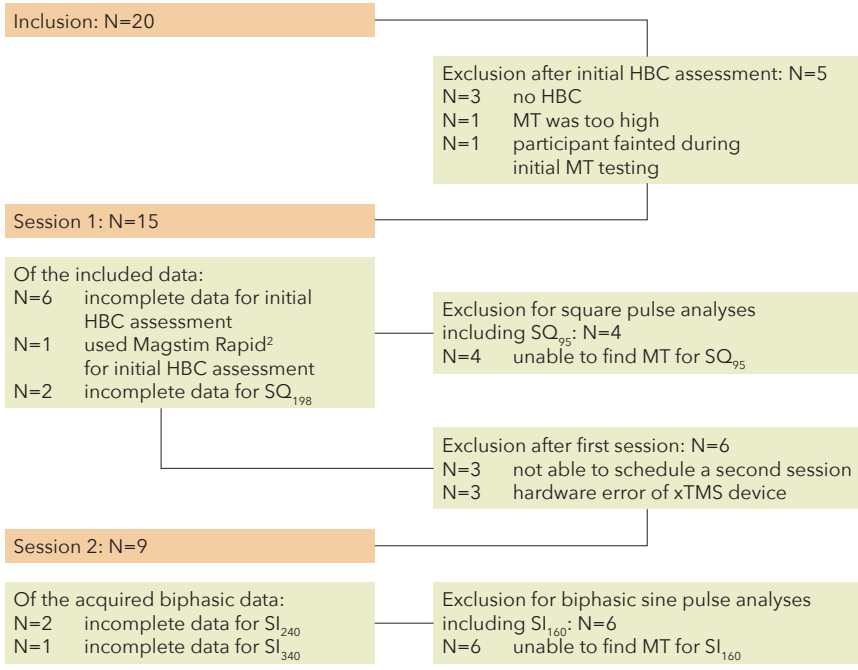

Figure 5.4. Flowchart of data and participant inclusion.

Statistical analyses were performed using SPSS (IBM Statistics, version 27). All ANOVAs were corrected using a Greenhouse Geisser correction if Mauchly's assumption of sphericity was violated. All t-tests were Bonferroni corrected for multiple comparisons as appropriate.

RESULTS

Shorter square pulses resulted in greater HBC than longer pulse-widths
We first wanted to determine whether participants' HBC dose-response profiles were affected by the pulse width of the square pulses. We therefore performed a two-way repeated measures ANOVA with within-subject factors of intensity (11 levels) and pulse width (4 levels; Figure 5.5 left). There was a significant main effect of both intensity ($F(2.257,22.568)=9.736$, $p<.001$, $h_p^2=.493$) and pulse width ($F(3,30)=10.202$, $p<.001$, $h_p^2=.505$), but no significant intensity by pulse width interaction ($F(30,300)=.976$, $p=.506$, $h_p^2=.089$).

Post-hoc paired sample t-tests demonstrated significantly greater mean HBC with SQ_{95} than SQ_{198} ($t(10)=3.398$, $p=.007$, $d=1.024$) and with SQ_{95} than SQ_{400} ($t(10)=4.009$, $p=.002$, $d=1.209$). The difference between SQ_{95} and SQ_{198} was marginally (as a result from the Bonferroni correction) significant with a large effect size ($t(10)=3.154$, $p=.010$, $d=.951$). No significant differences were observed between the other pulses.

As TMS intensity at DLPFC is often determined by the MT, we also wanted to specifically investigate the HBC induced at 100%MT across the range of pulse widths. A repeated measures ANOVA with within-subject factor pulse width (4 levels) showed a significant effect of pulse width ($F(3,30)=4.080$, $p=.015$, $h_p^2=.290$). Post-hoc paired sample t-tests showed medium to large effect sizes (Cohen's d ranging from 0.611 to 1.425), with the exception of the small effect size for SQ_{95} vs SQ_{198} ($d=.406$) and SQ_{265} vs SQ_{400} ($d=.083$; Figure 5.5 right). SQ_{95} and SQ_{400} remained significantly different after Bonferroni correction ($t(10)=4.814$, $p<.001$, d=1.452), again suggesting that the shorter pulse width led to greater HBC.

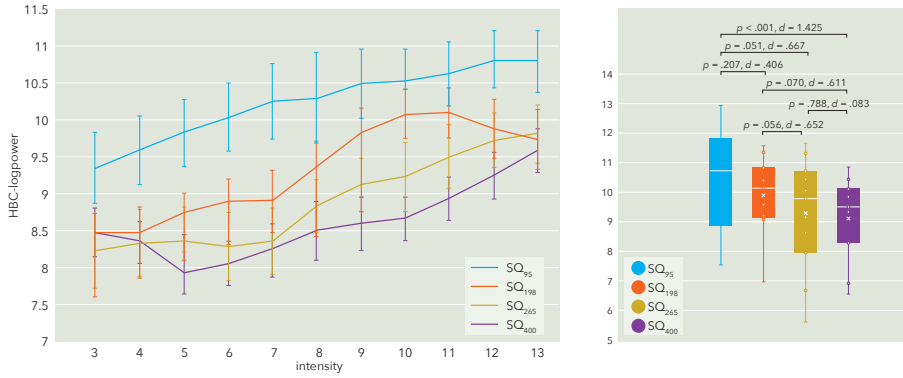

Figure 5.5. Induced HBC-logpower dose-response for four different pulse widths (left) over the eleven increasing intensities and (right) at the stimulation intensity matching 100%MT. Error bars are +-1SE.

Higher frontal and motor thresholds for shorter pulse widths

We next wanted to know whether MT and FT differed across pulse widths. A two-way repeated measures ANOVA with within-subject factors of pulse width (SQ_{95}, SQ_{198}, SQ_{265}, SQ_{400}) and threshold type (MT, FT) was performed to look at differences between the MT and FT (both as %MSO). We found a significant main effect of pulse width ($F(3,18)=519.602$, $p<.001$, $h_p^2=.989$) and threshold type ($F(1,6)=36.361$, $p<.001$, $h_p^2=.858$) and a significant threshold type by pulse width interaction effect, ($F(1.595,9.570)=15.145$, $p<.001$, $h_p^2=.716$). These results suggest that, as expected, FT and MT differ, and that pulse width affects both thresholds differentially. It also suggests that pulse width has a differential effect on the two thresholds, which may have important implications for the use of MT to determine FT across TMS pulse widths.

To further explain this interaction between pulse width and threshold type, we performed post-hoc comparisons of FT and MT for all four pulse widths using paired sample t-tests (Figure 5.6 left). This analysis demonstrated a significant difference between the MT and FT with SQ_{95} ($t(9)=5.653$, $p<.001$, $d=1.788$) and SQ_{198} ($t(7)=6.218$, $p<.001$, $d=2.199$). The other pulses showed no significant differences between the FT and MT but had medium effect sizes.

Finally, we wanted to investigate the nature of the relationship be-

tween MT and FT across pulse widths (Figure 5.6 right). There was a significant correlation between MT and FT at all pulse widths (SQ_{95} (r=.738, p=.015), SQ_{198} (r=.944, p<.001), SQ_{265} (r=.818, p=.004) and SQ_{400} (r=.815, p=.007)), suggesting that while the slope of the relationship between MT and FT varies by pulse width, as expected, MT and FT remain highly related regardless of pulse width.

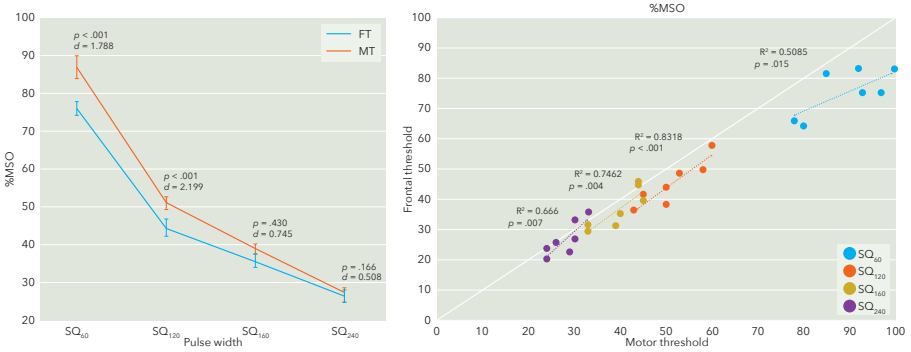

Figure 5.6. MT and FT as %MSO (left) of the four different pulse widths and (right) as a correlation plot showing strong correlations with medium to high explained variance between the FT and MT for all pulse widths. Error bars are +/- 1SE.

Pulse shape has no significant effect on HBC

We wished to investigate whether sinusoidal and square waves had different effects on HBC, for which we used two pulses of approximately the same length with a different pulse shape (SQ_{265} and SI_{240}). We performed a two-way repeated measures ANOVA with within-subject factors of pulse shape (SQ_{265}, SI_{240}) and intensity (11 levels; Figure 5.7 on page 102, left). This analysis demonstrated a significant main effect of intensity (F(10,80)=4.638, p<.001, h_p^2=.367), but no significant main effect of pulse shape (F(1,8)=.336, p=.578, h_p^2=.040) or pulse shape by intensity interaction (F(10,80)=.890, p=.546, h_p^2=.100). Similarly, a paired sample t-test comparing SQ_{265} and SI_{240} (Figure 5.7 right) showed no significant difference in the mean induced HBC (t(8)=-.719, p=.493, d=.240). A comparison of MT and FT for these pulse shapes was not possible, because the %MSO needed to induce MEPs was different resulting from the power differences between the machines.

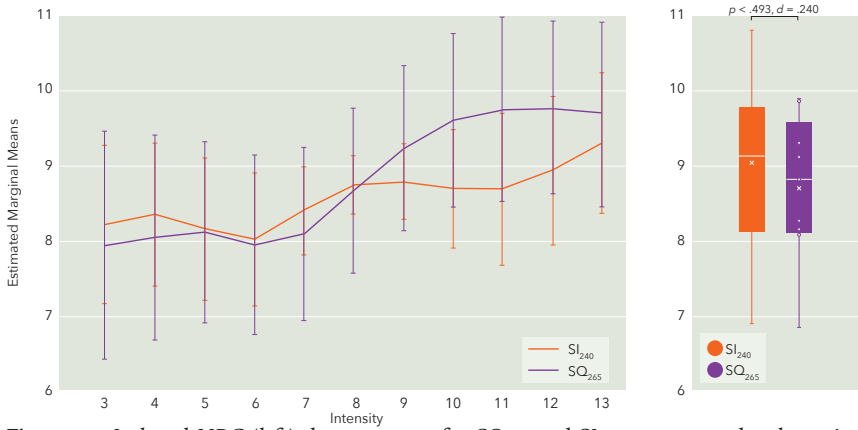

Figure 5.7. Induced HBC (left) dose-response for SQ_{240} and SI_{240} wave over the eleven increasing intensities and (right) for both pulse shapes (square and sine wave). Error bars are +/- 1SE.

Participant comfort

Finally, we wanted to investigate any discomfort resulting from the different pulses. Participants described the longer pulses as dull and flat and the shorter as more pinching, but no pulse was described or rated as painful: all scores were below 6 out of 10 (3.73 to 5.73). To statistically test the perceived discomfort, we performed a repeated measures ANOVA between pulse widths (comparing SQ_{95}-SQ_{400}) and a paired t-test between pulse shape (comparing SQ_{265} vs SI_{240}). These analyses demonstrated a significant main effect of pulse width for square pulses ($F(3,42)=11.518$, $p<.001$, $h_p^2=.803$) with shorter square wave pulses having higher scores than longer pulses. There was no significant difference between the perceived discomfort of SQ_{265} and SI_{240} ($t(8)=.182$, $p=.860$, $d=.061$).

DISCUSSION

This study was designed to investigate the neurophysiological effects of TMS pulse shape and pulse width applied to the DLPFC, a vital question for the optimal use of rTMS in the treatment of depression and other disorders. We investigated differences in induced Heart-Brain Coupling (HBC) of four square wave pulse widths, investigating their dose-response profiles, the induced HBC at MT intensity,

and the relation between the MT and FT. In addition, we studied the influence of pulse shape by comparing induced HBC between a sine and square wave of approximately the same pulse width.

Shorter TMS pulses induced stronger HBC than longer pulse widths
As expected (based on Dijkstra et al., 2023 and Chapter 4), all pulse widths showed similar dose-response profiles, with higher induced HBC at higher stimulation intensities. However, we demonstrated for the first time that shorter pulses induced stronger HBC than longer pulses (Cohen's *d* ranging from .952 to 1.209), with strongest effects for SQ_{95}. While these neurophysiological results may not translate directly into therapeutic effects, this finding is important to understand how different TMS pulse widths effect the DLPFC, and future work should examine whether the stronger HBC elicited by square, short-duration pulses will translate into superior clinical outcomes for therapeutic TMS protocols.

We tested four different pulse widths in the same session, capitalizing on the technical advances available to allow this direct comparison for the first time. However, this raised the potential that any difference seen between the pulse widths might be driven by order effects across the session. Previous studies showed no order effects across TMS runs of this type (Dijkstra et al., 2023). Here, however, we were not able to test for order effects, caused by the use of different machines and the study design. Future studies should counterbalance pulse parameters to account for any potential order effects.

Difference between FT and MT is greater for shorter pulse widths
In line with previous work, we demonstrated that higher stimulation intensities (as %MSO) were required to reach MT and FT for shorter pulses relative to the longer pulses (Delvendahl et al., 2014). Further, FT was lower than the MT for all pulse widths, in line with earlier work (Chapter 4). Interestingly, greater differences were found between MT and FT for the short pulses compared to the longer pulses with effect sizes (Cohen's *d*) of 1.788, 2.199, 0.745 and 0.508 for $SQ_{95\text{-}400}$ respectively. For SQ_{95} and SQ_{198} the FT and MT differed significantly, suggesting that – in comparison to MT - shorter pulses may be more effective at activating DLPFC than longer pulses. This finding has

important clinical implications. A stimulation intensity of 120%MT is most commonly used to treat depression. Our findings indicate that this intensity is 21.5-34%MSO higher than FT, implying that such high intensities may not be required to stimulate the DLPFC. This finding is consistent with previous studies taking scalp-cortex differences between M1 and the DLPFC into account, showing a larger distance to the scalp for M1 compared to the DLPFC (Stokes et al., 2005; Lu et al., 2019), resulting in a higher stimulation intensity needed for M1 activation. These findings are supported by recent work (Chen et al., 2021) where treatment at 80%MT achieved similar therapeutic effects to treatment at 120%MT. Our finding of a pulse width-dependent discrepancy between MT and FT adds to an increasing literature highlighting the importance of using an individualized and localized stimulation intensity, based on the FT rather than MT for optimal treatment response whilst minimizing side-effects.

Pulse shape appears to be less important than pulse width in determining neurophysiological effects

We found no difference in either HBC dose-response profiles or mean induced HBC between square vs. sine wave TMS pulses. The data presented here would therefore suggest that pulse shape is not a major factor driving stimulation effects on HBC. However, due to technical difficulties our sample size for this comparison was small, and we were only able to compare a single pulse width. A more extensive investigation into the effects of pulse shape on the metrics examined here is therefore warranted. Additionally, other waveforms with more pronounced differences may have distinct effects.

Lastly, we found that even though different pulse widths are perceived differently regarding discomfort, with shorter pulses being more pinching and longer pulses more dull, all pulse widths were tolerable and participants reported no painfulness of stimulation. Furthermore, as described in Chapter 4, the sensation of stimulation is not related to the amount of HBC, therefore excluding influence from peripheral effects on the amount of induced HBC.

Future directions

We were able to perform this study as we had access to novel hard-

ware, meaning that to the best of our knowledge, this is the first study to be able to directly compare TMS pulse width and shape on the prefrontal cortex. However, the hardware we used had some limitations, somewhat restricting the approaches used and limiting the data we were able to collect. Firstly, hardware power limit meant we were unable to reach MT for the shorter pulses in some participants, meaning we could not assess HBC in these participants.

In addition, longer pulses require higher energy levels, which caused our coil to overheat. To address this with the xTMS device we swapped coils in between sweeps and wrapped the coils in a flexible cooling pad (Plastipad Infant CSZ-193, Cincinnati Sub-Zero, USA) connected to a temperature-controlled water chiller after use (F250, JULABO GmbH, Germany). For full details of the cooling system, see Rieger et al. (2023). This significantly shortened the waiting time before the coil could be used again.

However, despite these approaches, coil overheating resulted in a small sample size for the xTMS, and we were not able to run all analyses we wished to perform. However, because of the magnitude of the effect sizes, we were able to find significant differences. For the non-significant findings, most effect sizes were medium to high, suggesting the non-significant findings might result from a lack of power. Future studies should aim for larger sample sizes.

Conclusions

HBC seems to be a promising neurophysiological readout for DLPFC-TMS, providing a direct assessment of effects at this target of stimulation, without relying upon M1 or MEPs as indirect proxies. Here, we performed a first study demonstrating the feasibility of using HBC rather than MEPs to characterize the effects of different pulse waveforms and durations. These results highlight the importance of pulse width in the neurophysiological response of DLPFC to TMS. Further work is required to understand whether these neurophysiological differences translate into therapeutic effects, but HBC-TMS offers a potential approach for optimizing stimulation parameters for DLPFC-TMS (such as stimulation site, intensity, or pattern) in individual patients prior to treatment.

SUPPLEMENTAL MATERIALS

1. CTMS PULSE CHARACTERISTICS

Settings of the M-ratio: we looked at M-ratios of 0.5 and 0.333 and searched for the maximum positive deflection within the possible range of stimulation of the machine. With this, we opted for a linear increase over the different pulse widths (Figure S5.1). This led to deflections of:

	SQ_{95}	SQ_{198}	SQ_{265}	SQ_{400}
M=0.5 Negative	35	69	92	140
M=0.5 Positive	25	51	68	100
M=0.333 Negative	35	78	105	160
M=0.333 Positive	25	42	55	80

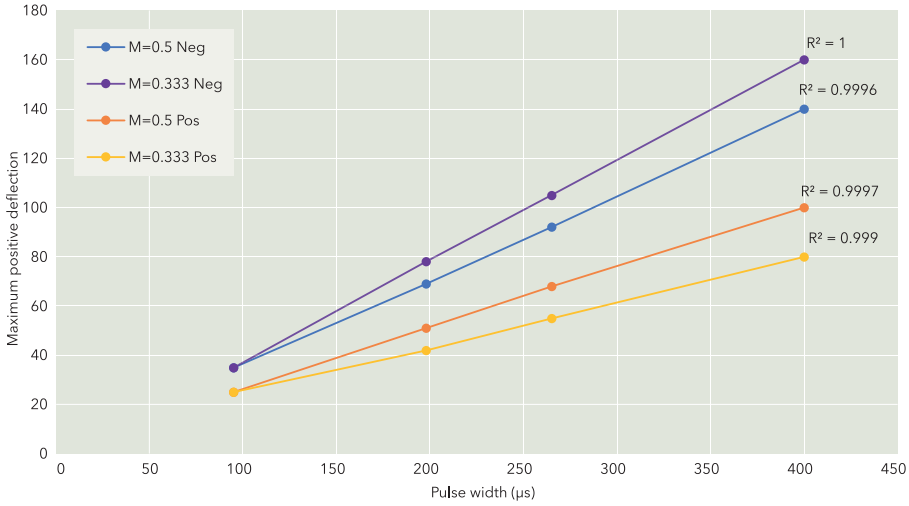

Figure S5.1. Visual overview of the negative deflections matching the maximum positive deflections in a linear manner, for an M-ratio of 0.5 and 0.333.

We then compared the MT for the different pulses (Figure S5.2) and asked for the tolerability (0-10; 10=perfectly tolerable) of the pulses. This resulted for 3 people in:

		Pulse width			
ID 1		SQ_{95}	SQ_{198}	SQ_{265}	SQ_{400}
	MT 0.5	-	44	35	26
	MT 0.333	88	53	39	28
	Tolerability 0.5	-	9	8	8
	Tolerability 0.333	10	9	9	9
ID 2	MT 0.5	-	45	34	-
	MT 0.333	94	54	39	-
	Tolerability 0.5	-	8	8	-
	Tolerability 0.333	9	9	10	-
ID 3	MT 0.5	-	48	37	29
	MT 0.333	90	49	42	33
	Tolerability 0.5	9	9	9	9
	Tolerability 0.333	8	8	9	9

The cTMS machine could not get to the level of MT for the 0.5 M-ratio with the pulse width of 95µs for all three pilot participants (max intensity=85.5%). All pulses were reported to be tolerable, but the 0.333 a little more (quote: 'it takes the sharp edges off'). Based on this, we decided to use the M-ratio of 0.333.

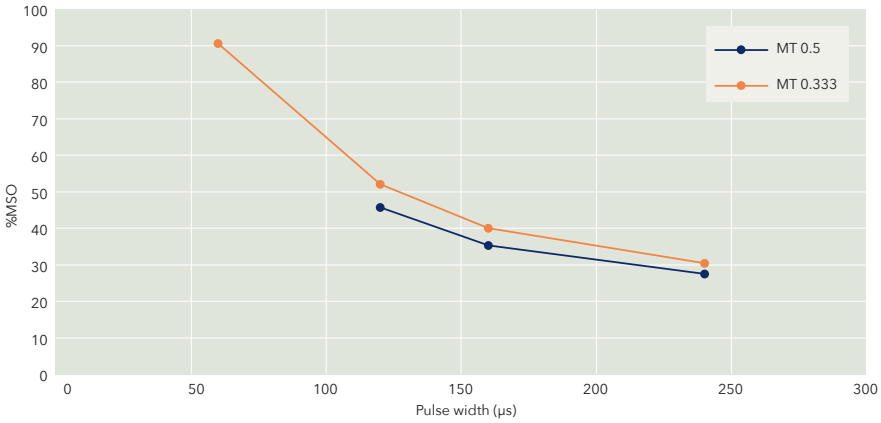

Figure S5.2. *Average stimulation intensity (%MSO) at which the MT was found for the four different pulse widths with an M-ratio of 0.5 and 0.333.*

2. INCREMENTAL STEPS PER PULSE WIDTH

The incremental steps per pulse width were calculated based on the stimulation range of the specific machine (= possible %MSO intensity to use; min-max), of the conventional machine (= Deymed / Magventure device) and the conventional incremental steps used.

In a formula: range of bandwidth / range of conventional bandwidth * conventional bandwidth increment.

	Min	Max	Range	Proposed increments
SQ_{95}	45	95	50	2.0%
SQ_{165}	25	60	35	1.4%
SQ_{265}	20	45	25	1.0%
SQ_{400}	15	35	20	0.8%
SI_{160}	80	115	35	1.4%
SI_{240}	55	100	45	1.8%
SI_{300}	40	90	50	2.0%
SI_{340}	25	85	60	2.4%
Conventional	40	90	50	2.00%

6

INDIVIDUAL ALPHA FREQUENCY AND HEART-BRAIN COUPLING AS PROSPECTIVE STRATIFICATION BIOMARKERS FOR TRANSCRANIAL MAGNETIC STIMULATION FOR MAJOR DEPRESSIVE DISORDER: A FEASIBILITY STUDY

Based on:

Individual Alpha Frequency and Heart-Brain Coupling as Prospective Stratification Biomarkers for Transcranial Magnetic Stimulation for Major Depressive Disorder: A Feasibility Study. Zwienenberg, L., Geijselaars, M., Dijkstra, E., Rouwhorst, R., van Dijk, H., van Oostrom, I., van der Vinne, N., Sack, A.T. & Arns, M. (*In preparation*).

ABSTRACT

The individual alpha peak frequency (iAF) based Brainmarker-I and Heart-Brain Coupling (HBC) are proposed stratification biomarkers for different transcranial magnetic stimulation (TMS) protocols and stimulation targets, aiming to optimize TMS treatment outcome for depression. However, they were never prospectively tested in clinical practice. Here, we examined the feasibility and effectiveness of using these biomarkers in clinical practice and analyzed the relationship between the two cross-modal biomarkers, also exploring the relationship between HBC and a wider EEG frequency spectrum.

Prospective EEG and HBC-TMS stratification was assessed in a multi-site, open label, naturalistic sample assessing depressed patients (2022-2024; n=117). Patients with a synchronized iAF (sync marker; 9.6-10.4Hz) were stratified to 10Hz left DLPFC-TMS and those with a high Brainmarker-I decile score to 1Hz right DLPFC-TMS, after which a 2-point HBC assessment determined the stimulation site (Beam or 5CM). Patients with a low Brainmarker-I decile score underwent a 4-point HBC assessment to determine both the stimulation protocol and site. Remission rates at session 25 and

treatment progression, assessed with the Beck Depression Inventory (BDI) at baseline, session 10, 15 and 25 were compared between a retrospective sample receiving treatment as usual (TAU, 2014-2022; n=39) and a subsample of the prospective stratification dataset (n=22). Additionally, the effects of having HBC or a sync marker on early treatment response (session 15) was assessed. Furthermore, the relationship between the two biomarkers was investigated. EEG oscillatory power in the 0-40Hz frequency band was studied, comparing patients with and without HBC at F3 and F4.

Implementation of Brainmarker-I and HBC based stratification appeared feasible. The remission rate increased from 25.6% to 40.9%, resulting in an NNT of 7. However, the baseline BDI of the prospective sample was higher compared to TAU baseline scores and no interaction effect was observed regarding treatment progression and the two groups ($F(1,57)=.218$, $p=.884$). No differences were found regarding early treatment response between patients with and without HBC ($F(2,79)=.086$, $p=.918$) or sync marker ($F(2,35)=1.064$, $p=.356$). No relationship was found between the various iAF and HBC metrics. Delta EEG power at 0.8Hz at F4 appeared to be higher for patients with HBC compared to those without HBC ($t(104)=2.1$, $p=.038$).

Prospective Brainmarker-I and HBC based stratification is feasible, low-cost and easy to implement in clinical practice. The iAF and HBC appear to be independent biomarkers: the iAF representing temporal and HBC spatial information. This suggests that combining both biomarkers for prospective stratification may result in synergistic effects. The relationship between delta oscillations and heart rate modulations may be of interest for future research. Although the results indicate that EEG and HBC-TMS based prospective stratification towards evidence based TMS protocols is feasible and promising, its clinical effectiveness needs to be further assessed in future studies, preferably comparing stratified vs. non-stratified treatment outcomes in adequately sized samples.

INTRODUCTION

Transcranial magnetic stimulation (TMS) targeting the dorsolateral prefrontal cortex (DLPFC) is an evidence-based treatment for difficult-to-treat depression (DTD; Donse et al., 2017) with remission rates of 30-50% (Carpenter et al., 2012; Blumberger et al., 2018). This means that up to 70% of treated patients remain depressed. There is a growing field of interest to optimize TMS treatment outcome. A promising method to optimize treatment effects of already existing treatments is using biomarker-based stratification. Stratification aims to assign patients to subgroups based on their biomarker feature, which is used to guide them to the treatment they are most likely to respond to as compared to an alternative, proven effective treatment (Arns et al., 2022; 2023).

Klooster et al. (2023) reported on a robust EEG biomarker for stratification to different TMS treatment protocols: the individual alpha peak frequency (iAF). The iAF is an individual's most prominent frequency within the alpha band (7-13Hz) and is considered a trait-marker as it appears stable within a person (Grandy et al., 2013). Between-subject, however, the iAF differs considerably. Individuals with an iAF close to 10Hz are more likely to respond to the 10Hz TMS stimulation protocol (Corlier et al., 2019; Roelofs et al., 2020) compared to individuals with larger iAF proximity to 10Hz. Building upon that, Voetterl and colleagues (2022; 2023) independently replicated these findings and investigated Brainmarker-I, which is the age and sex normalized iAF represented as decile scores (0-10). Brainmarker-I appears promising as a stratification biomarker, with the synchronization (sync) marker (iAF between 9.6-10.4Hz) representing the highest likelihood of response to 10Hz TMS (normalized positive predictive value (nPPV) of +29%) and high decile scores (6-10) to 1Hz stimulation (nPPV of +14%). However, incorrect allocation results in decreased chances of response to treatment. For treatment with 10Hz L-DLPFC TMS this is indicated by nPPVs of -11% for the low decile score and -17% for the high-decile score group (Figure 6.1; (unpublished) results from Voetterl et al., 2023). Individuals with a low decile score (0-5) generally have a lower chance of responding to TMS treatment with 1Hz and 10Hz stimulation. However, they are

predicted to better respond to electroconvulsive therapy (ECT; Voetterl et al., 2023), and 18/20Hz left DLPFC (L-DLPFC) TMS (Voetterl et al., 2024), as compared to individuals with higher decile scores.

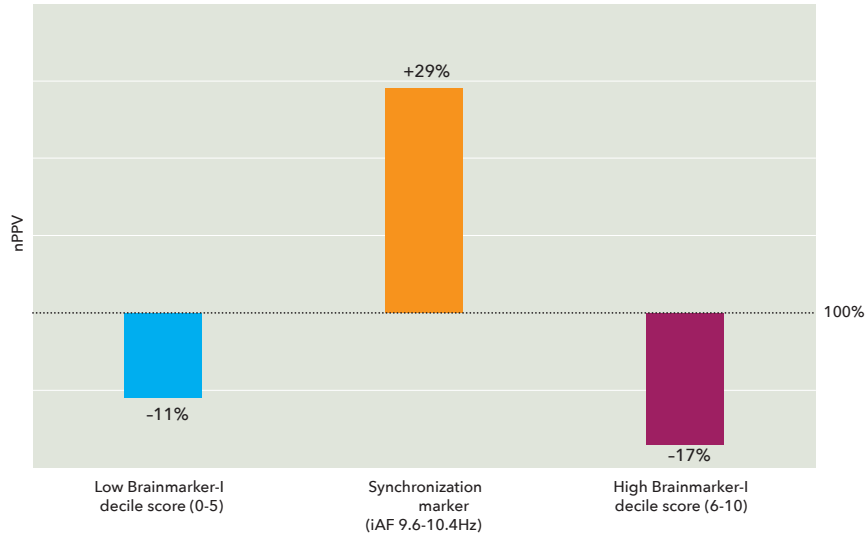

Figure 6.1. *Normalized positive predictive values (nPPVs) for 10Hz L-DLPFC TMS for patients with low decile (blue), sync marker (orange; iAF between 9.6-10.4Hz) and high decile (burgundy) Brainmarker-I scores. The figure is based on (unpublished) data from Voetterl and colleagues (2023).*

For TMS treatment, the evidence-based stimulation sites 'Beam' and '5CM' are commonly used, which both rely on scalp measures, with no difference in clinical efficacy between these two targets (Trapp et al., 2023). However, a shift is being made from scalp measures to network-based targeting, using functional magnetic resonance imaging (fMRI) to locate the hotspot at the scalp level to enter the desired, deeper lying, brain network. Stronger anti-correlation between the stimulation target and the subgenual anterior cingulate cortex (sgACC) are found to be related with better treatment outcomes of TMS for depression (Fox et al., 2012; Siddiqi et al., 2021; Elbau et al., 2023). fMRI is, however, expensive and often not accessible for clinical use, therefore, it is important to find low-cost, quick and accessible ways to implement network-based targeting. Recently, Dijkstra et al. (2024) showed that they were able to determine (with an accuracy of 86%) which site was negatively anti-correlated to the

sgACC when stimulating an anti-correlated, correlated and neutral prefrontal site, using Heart-Brain Coupling TMS (HBC-TMS). HBC-TMS, extensively explained in Dijkstra et al. (2023), measures the frequency of heart rate oscillations during DLPFC-TMS. In short, the frontal-vagal network theory suggests that adequate prefrontal TMS targeting results in heart rate decelerations through trans-synaptic activation of the vagal nerve via the sgACC (Iseger et al., 2017; 2020; 2021). The inter-train-interval (ITI) results in heart rate normalization, resulting in an heart rate oscillation frequency that matches the stimulation protocol. The site with the highest HBC is hypothesized to be the prefrontal site with the strongest anti-correlation to the sgACC, which is hypothesized to be the best entry into the depression network.

Even though the cross-modal biomarkers, iAF and HBC, seem promising for stratification, the feasibility and effectiveness of implementation for prospective stratification was never tested. To this end, in this study, we prospectively implemented the iAF based Brainmarker-I and HBC stratification biomarkers in clinical practice. We furthermore investigated the relationship between the iAF and HBC to determine whether their underlying mechanisms of actions – the iAF being a temporal and HBC a spatial biomarker - are similar or independent of each other. Lastly, we explored the relationship between HBC and a broader EEG frequency spectrum.

METHODS

We designed this study as an open-label, naturalistic study, mimicking real-world practice and optimizing the translatability of the study outcome to real world settings.

Participants

We included non-psychotic adult DTD patients that underwent at least 10 TMS treatment sessions and who did not undergo TMS or ECT treatment before. The primary diagnosis of non-psychotic DTD was confirmed by a psychiatrist or specialized clinical psychologist, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM; version IV or V) criteria, and a score of >13 on the Dutch

21-item Beck Depression Inventory Second Edition (BDI-II-NL; Beck et al., 1996). All patients provided written informed consent to use their TMS treatment and EEG data for scientific purposes.

Prospective stratification data was obtained from treatments performed at Synaeda Psycho Medisch Centrum (Leeuwarden and Drachten; Synaeda for short) from April 2022 to April 2024 and at neurocare clinics (Nijmegen, Arnhem and Eindhoven) over the period of July 2022 to November 2023. Only patients with available EEG and HBC assessment data and a baseline BDI score were included. Retrospective data of patients receiving TMS treatment as usual (TAU) was obtained from treatments performed at Synaeda in the period of 2014 to April 2022.

Administration of treatment effects
All patients were sent a BDI questionnaire at baseline and every 5th (Synaeda) or 8th (neurocare) treatment session. The BDI scores closest to session 10 and 15 were chosen to assess treatment effect at that moment in time, resulting in BDIs of session 10 and 15 for Synaeda and 8 and 16 for neurocare. In case of missing data, we replaced it with the last available BDI score (last observation carried forward (LOCF) method).

TAU vs. EEG and HBC based stratification
Patients receiving TAU did not undergo an EEG and HBC assessment before the start of their treatment. TAU started with 1Hz stimulation (1200 pulses, 120%MT) on the right Beam location with a treatment evaluation between session 10 to 20. After evaluation, the 1Hz protocol was continued in case the BDI score had decreased by ≥40%. In case the symptom reduction was smaller, a switch to bilateral stimulation was made, adding 10Hz stimulation to the left Beam (1000 1Hz pulses followed by 1000 10Hz pulses, 120%MT).

Prospectively stratified patients underwent an EEG and HBC assessment before the start of TMS treatment (details on both assessments are described below). These appointments were scheduled in consultation with the patient, either on the same or on different days, assessing EEG before HBC-TMS. Patients were stratified based on

Brainmarker-I as proposed by Voetterl et al. (2023; visualized in Figure 6.2a), supplemented with the results of the HBC assessment (described below). In short, given that patients with low Brainmarker-I decile scores (0-5; blue range in Figure 6.2b) have a generally lower chance of responding to TMS treatment and no preference for either the 1Hz or 10Hz protocol, they were stratified to a 4-point HBC assessment to determine the stimulation protocol and site. Patients with a sync marker (iAF between 9.6-10.4Hz; orange range in Figure 6.2b) were stratified to the 10Hz protocol targeting the L-DLPFC, whereas patients with high decile scores (6-10; burgundy range in Figure 6.2b) were stratified to the 1Hz right DLPFC (R-DLPFC) protocol, after which a 2-point HBC assessment followed on the left or right hemisphere, respectively. Patients were assigned to a stimulation protocol and site until session 25, after which protocol switches could be made in case of non-response. A flow diagram of the stratification protocol is shown and described in Figure 6.2. on the following page.

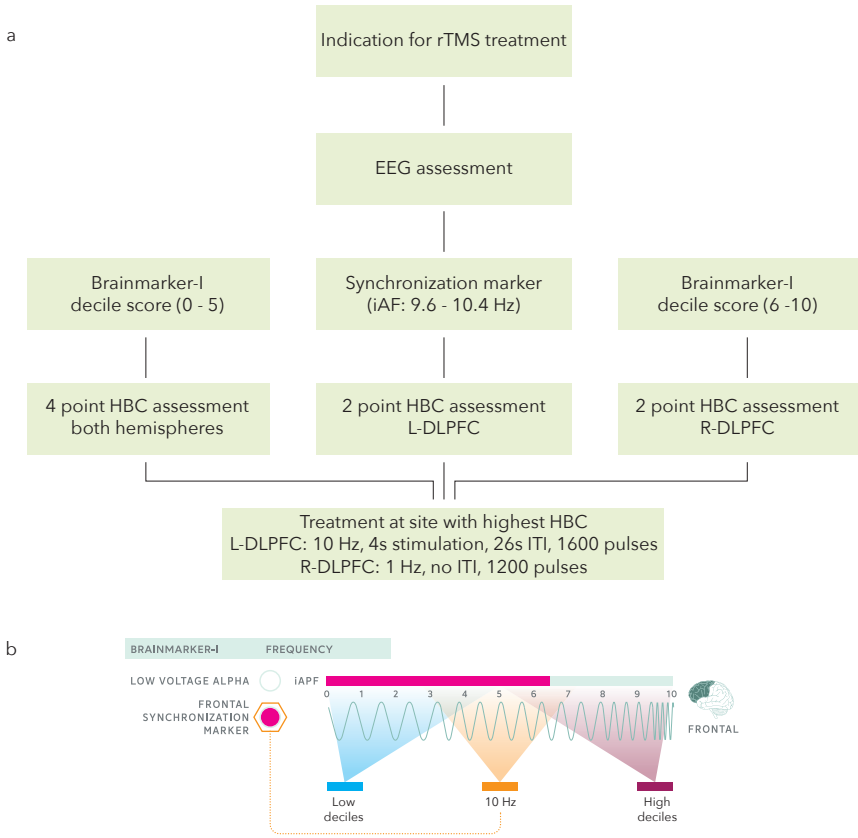

Figure 6.2. *(a) Decision tree used for Brainmarker-I and HBC informed prospective treatment stratification. In the first step, resting state eyes closed EEG was assessed, and a Brainmarker-I report was created for each individual patient which stratified them to a 2- or 4-point HBC assessment as shown and explained in (b). For the second step, the HBC assessment was performed on the EEG stratified hemisphere at the Beam and 5CM location. The stimulation site for treatment was the site with the highest HBC. (b) Example of an individual EEG report. A filled, pink dot on the left denotes either that the patient has low voltage alpha (LVA) or that their iAF falls into the frontal synchronization (sync) range (9.6-10.4Hz; depicted above). The iAF is depicted as Brainmarker-I decile scores from 1 (relatively slow) to 10 (relatively fast). Low deciles (0-5; blue) indicate stratification to a 4-point HBC assessment, sync (orange) indicates 10Hz L-DLPFC treatment stratification and a 2-point HBC assessment on the L-DLPFC and high deciles (6-10; burgundy) indicate 1Hz R-DLPFC treatment and a 2-point HBC assessment on the R-DLPFC. As visualized, the synchronization range overlaps with the decile scores, depending on the age of the individual. The synchronization range is leading, that is, if an individual falls into that range, they are assigned to the synchronization group. Otherwise, the decile score indicates assignment to either low- or high-decile subgroup.*

EEG data collection and processing

EEGs were recorded from 28 EEG channels based on the 10-10 electrode international system (FP1, FP2, AFz, F7, F3, Fz, F4, F8, FC1, FC2, FC5, FC6, T7, C3, Cz, C4, T8, CP1, CP2, CP5, CP6, P3, P7, Pz, P4, P8, O1, O2; Neoprene-Cap, Compumedics) using a sampling rate of 2048Hz. Data were recorded with Curry (version 9), using the Compumedics Grael amplifier. The ground was located at FPz and data was Cz referenced. The same preprocessing methods were used as described in Van Dijk et al. (2022), adjusting for the different amplifier. In short, automatic artifact detection and removal were performed using a custom-built Python package (Hunter et al., 2007; Virtanen et al., 2020; Harrit et al., 2020; Team T Pandas Development, 2020) and were in accordance with de-artifacting as described by Arns et al. (2016), with full code available online (http://www.brainclinics.com/resources). Data were offline re-referenced to linked mastoids. Horizontal eye movements were recorded with cap electrodes FHR11 and FHL11, approximately 1.5 cm lateral to the outer canthus of each eye, and vertical eye movements were recorded with bipolar electrodes placed 3mm above the middle of the left eyebrow and 1.5 cm below the middle of the left bottom eyelid. An electrode was placed at C7 to measure ECG which was offline referenced to M1. Skin resistance was kept at <10 kΩ for all electrodes. The EEG assessment consisted of a 3-minute eyes closed recording.

iAF and Brainmarker-I

The iAF and sync marker were calculated as described by Voetterl et al. (2022). In short, the iAF was determined by computing the Fast Fourier Transform (FFT) of the preprocessed, artefact-free data. Subsequently, each individual's iAF at F3, Fz and F4 was determined by identifying the highest peak within the frequency range of 7-13Hz. For Fz, a Brainmarker-I decile score (0-10) was obtained, after age and sex normalization. The sync marker is based on the iAF at F3, which approximately matches the left Beam location (see below) and is considered synchronized to the 10Hz TMS protocol if it falls in the range of 9.6-10.4Hz. If no alpha peak was found, reflected by low voltage alpha (LVA) at F3, Fz was used and vice versa.

HBC data collection and processing

The HBC assessment comprised of sequential stimulation of both the Beam and 5CM location on both the left and right DLPFC while measuring heart rate. Beam sites were defined using the Beam-F3 algorithm and software (Beam et al., 2009), therefore corresponding to approximately the F3 and F4 EEG sites. The 5CM site is defined as the site 5cm anterior to the scalp position of the motor hotspot in a parasagittal line. TMS was applied with a DuoMag XT-100 system (Deymed Diagnostic; DuoMag 70BFX LQC coil). Stimulation parameters (Figure 6.3) followed a specific 10Hz protocol (5s on, 11s off) with an intensity sweep of 15 stimulation trains from low to high intensities defined as 2% maximum stimulator output (MSO) steps, with one stimulation train applied per intensity, and with the 15th intensity matching to the individual's 120%MT. The starting intensity was thus defined as 28%MSO below 120%MT. The stimulation was preceded by 16 seconds of no stimulation, resulting in a total duration of 256 seconds of stimulation per round. Heart rate was measured using a Polar H10 band (Polar Electro 2022) connected through Bluetooth with the Heart-Brain Connect app (version 1.33, 2022, Brainclinics).

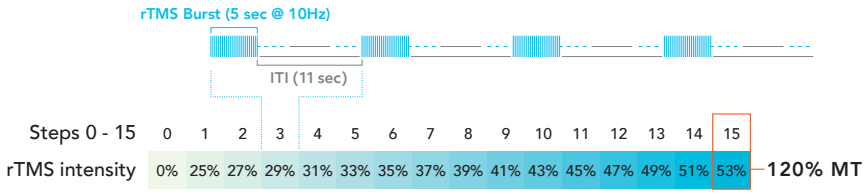

Figure 6.3. *Visual overview of the HBC assessment stimulation protocol.*

HBC stimulation protocol and coil positioning

Stimulation started at the left Beam with stimulation rounds alternating between hemispheres to prevent cross-over effects and the same targeting method (Beam/5CM) was always used consecutively (e.g., Beam left – Beam right – 5CM left – 5CM right). In case of a 2-point assessment, the stimulation started at the Beam location on that hemisphere. In case of <1cm distance between the Beam and 5CM location on a hemisphere, only the Beam location on that hemisphere was stimulated and the order of stimulation was adjusted accordingly. The TMS coil was positioned over the Beam and 5CM sites at an angle of 45° relative to the parasaggital plane. MT was defined

as the lowest stimulation intensity that, in 4 trials, induced 2 visible thumb twitches of the contralateral hand. For an HBC assessment on both hemispheres, the average MT was used to determine the stimulation intensity, for one-sided stimulation the regular MT sufficed.

ECG data analysis
ECG data were analyzed according to the methods described in Dijkstra et al. (2023). The time-frequency power of 0.0625Hz was computed using a 10 cycle Morlet wavelet that was min-max normalized and normalized between 0 and 1 over all measurements within the subject. Additionally, in the current analysis the computed HBC-power was log-transformed to obtain a parametric distribution that could be compared between measurements (within and between subjects, see supplemental materials of Chapter 4). These analyses were used to create individual HBC reports, which were visually inspected for artefacts. In case of artefacts and deviations, the raw ECG data was corrected in KubiosHRVScientific (version 4.1.0, 2023, Kubios Oy) by manually scoring the R-peaks (n=18). The optimal site in the HBC report was established based on a combination of the HBC-power values, HBC-logpower values and visual inspection, aiming for sites with HBC-logpower values above the cut-off value of 9.5 (described in Chapter 4).

STUDY ANALYSES

Feasibility
We expected the EEG and HBC-TMS based prospective stratification to be feasible, as both methods were designed to be implemented in clinical practice. The feasibility of implementation was assessed by patient and clinician experiences.

TAU vs. prospective stratification
Furthermore, we expected the prospective stratification sample to report increased remission rates at session 25 in comparison to the group receiving TAU, since treatment selection was tailored to the individual based on Brainmarker-I and HBC outcomes.

The primary outcome measure to assess TAU vs. prospective treat-

ment differences was remission at session 25. Remission is often perceived as more clinically relevant than response and was defined as a BDI score of ≤12. Session 25 was chosen to allow for enough treatment sessions to establish an effect, but also for clinicians to change to another treatment protocol in case of non-response to the stratified protocol within a reasonable number of treatment sessions.

To assess the clinical relevance of prospective Brainmarker-I and HBC based stratification, a Repeated Measures ANOVA was performed that compared BDI score over time (baseline, session 10, 15 and 25) between TAU and the prospective sample. A Cohen's d effect size was calculated for the BDI difference at session 25. Furthermore, the effect of stratification was depicted as the number needed to treat (NNT).

Effects of sync marker and HBC on early treatment response
For the analyses of iAF and HBC effects on early treatment outcome the hypotheses require further explanation. *Without stratification*, we expected – based on prior research, as described in the introduction and visualized in Figure 6.1 – better 10Hz TMS treatment outcomes for the sync marker group as compared to non-sync marker group. For patients showing actual HBC, we expected generally better treatment outcomes compared to those without HBC, because of the hypothesized DLPFC-sgACC functional connectivity. However, since we performed stratification both ways, we hypothesized that *with stratification* the differences would be straightened out. This would result in more similar treatment outcomes for all groups. For example, a patient *without* a sync marker receiving 10Hz stimulation (these were only low decile score patients in this study, since high decile score patients were stratified to 1Hz R-DLPFC TMS) would normally be expected to improve less (-11%) than patients *with* a sync marker receiving 10Hz (+29%). But when their HBC was highest at a left sided treatment site – indicating 10Hz treatment -, we expected them to improve more, leading to no or smaller group differences.

Our primary measure for analyses regarding the effects of the two biomarkers on treatment outcome was early treatment response, assessed with BDI scores up to session 15. For these analyses, session

15 was chosen since switching to a bilateral TMS treatment protocol (therefore stepping away from the stratified treatment protocol) was often carried out in case of non-response at session 15. Remission rates were not expected to be high at session 15, resulting in small sample sizes for the remitter groups, therefore we investigated response rates. To assure that we were solely assessing the effect of the biomarkers of interest, we performed early treatment response analyses on protocol completers (up to session 15). The analyses comparing sync vs. no-sync marker were assessed in the patient group receiving 10Hz TMS, since, based on prior research, better treatment outcome for 10Hz TMS stimulation was expected for patients with a sync marker in comparison to those without sync marker. The HBC analyses were performed on the total protocol completers sample.

To this end, a Repeated Measures ANOVA assessed whether there were differences in treatment progress (BDI score at baseline, session 10 and 15) for patients with and without actual HBC at their stimulated site. A second Repeated Measures ANOVA assessed the same for patients with and without sync marker (sync vs. no-sync marker; 10Hz stimulation only as described above). Since our goal was to determine that there is no treatment outcome difference between patients with/without HBC and with/without sync marker, a non-significant interaction effect in the Repeated Measures ANOVA was complemented with Bayesian alternatives. This was done for testing evidence of absence of influence of HBC and/or the sync marker on treatment outcome. To this end, a Bayesian repeated measures ANOVA framework (based on work by Jeffreys (1961) and Rouder et al. (2009)) was performed. For the Bayesian analyses, we analyzed the data with JASP (JASP Team, version 0.19.1).

HBC analyses, performing between-subject comparisons, were performed using HBC-logpower (as described in Chapter 4) - hereafter called HBC for short. The threshold for someone showing Heart-Brain Coupling (actual HBC) was operationalized as an HBC logpower value ≥ 9.5 over any stimulation intensity at their stimulated site. Mean HBC was calculated over intensities 3-13. Given the low frequency of interest, data at the edges (first and last two intensities) are in some cases attenuated by the analysis methods. Therefore, data

recorded during intensity 3 to 13 were considered, given the higher signal-to-noise ratio for these intensities. See the Supplemental materials of Chapter 4 for an extensive explanation.

Relationship between iAF and HBC
The relationship between the iAF and HBC was investigated by One-Way ANOVAs comparing the iAF at F3 and F4 between the patients with and without HBC at the corresponding Beam site. A Pearson correlation was calculated between the mean HBC at F3/F4 and the corresponding iAF. Lastly, mean HBC at F3/F4 was compared to the frontal excitability threshold (FT) at the corresponding site with a One-Way ANOVA between patients with and without sync marker. The FT is extensively described in Chapter 4, but in short, it is the first stimulation round (of at least two consecutive stimulation rounds) with actual HBC and shows the lowest intensity of stimulation that activates the frontal-vagal pathway.

Relationship between EEG power and HBC
For a broader, exploratory investigation of the relationship between HBC and EEG power in the 0-40Hz frequency band, EEG power was calculated using a Fast Fourier Transformation (FFT) with a Hanning window of 20% using Brain Vision Analyzer (BVA; 2.2.0.7383, Brain Products GmbH). 5 second segments were preprocessed using automatic artifact rejection (AR) on all EEG channels (segments with an amplitude >150μV were excluded). n=1 was excluded, since all segments were removed after AR. The power spectrum of F3 and F4 was compared between patients with and without HBC at the corresponding Beam site using independent sample t-tests for all frequencies. As these latter tests were exploratory analyses designed to generate hypotheses for future studies, the results were not corrected for multiple comparisons.

RESULTS

A total of 125 patients met the inclusion criteria of which 8 patients were excluded for various reasons: deviations from the standard HBC assessment (*n*=5), unusable EEG data (*n*=1) and deviations to their number of treatment sessions (*n*=2). This resulted in a total of 117 included DTD patients (mean age 46.29 (SD 14.99), 54.7% female).

Table 6.1. Demographic, EEG and HBC assessment and treatment outcome features of the total included patients, protocol completers up to session 15 and the prospective and TAU sample of Synaeda only.

	Total prospective	Protocol completers	Prospective Synaeda	TAU sample Synaeda
n	117	82	22	39
Mean age in years (SD)	46.29 (14.99)	43.57 (14.0)	44.73 (14.98)	49.8 (10.2)
Females	54.7%	55.4%	72.7%	69%
Synchronization marker	23.9%	30.1%	18.2%	-
Low voltage alpha	6.0%	4.8%	0%	-
HBC at stimulated site	56.4%	63.9%	59.1%	-
Stimulation at Beam*	74.4%	74.7%	68.2%	-
10Hz DLPFC-TMS	47.9%	47.0%	40.9%	-
Baseline BDI score (SD)	33.79 (9.2)	34.8 (9.6)	28 (10.0)	32.8 (9.1)
Response session 15	27.4%	27.7%	31.8%	28.2%
Remission session 15	18.8%	15.7%	36.4%	17.9%
Response session 25	33.6%	34.1%	40.9%	35.9%
Remission session 25	29.3%	29.3%	40.9%	25.6%
Response post treatment	50.4%	51.2%	50.0%	51.3%
Remission post treatment	35.9%	36.6%	36.4%	30.8%

** Note: this value is not representative of the distribution between Beam and 5CM sites being the best HBC site, since only Beam sites were stimulated if the distance between Beam and 5CM location was <1cm. Synchronization marker: individual alpha frequency (iAF) between 9.6-10.4Hz, HBC; Heart-Brain Coupling, TAU; treatment as usual.*

In the total prospective sample, a higher baseline BDI (and subsequent BDIs) score was found for the sync marker group compared to the non-sync marker group ($F(1,107)=6.908$, $p=.010$, Cohen's $d=.567$). When repeating the analysis while covarying for the baseline BDI scores, the subsequent BDI scores were no longer significantly different. The HBC group was significantly younger than the non-HBC group ($F(1,115)=12.921$, $p<.001$, Cohen's $d=.675$).

In the specific analysis groups (TAU vs. prospective stratification, sync vs. no-sync in the 10Hz sample and HBC vs. no-HBC in the protocol completers sample), no significant differences were found regarding age, sex and baseline BDI, although baseline BDI appeared marginally higher with a medium effect size for TAU as compared to the prospective sample ($F(1,59)=3.670$, $p=.060$, Cohen's $d=.505$).

Feasibility

Although no metric data collection and therefore statistical analysis was performed, both patients and clinicians reported to be satisfied with the new protocol. They understood the concept of stratification between evidence-based protocols and locations and that actual improvement was unknown beforehand. Patients mentioned that they feel seen and taken seriously by assessing the EEG and administering the HBC measurement. The practical implementation of the logistics in a clinical setting proved feasible in being part of treatment as usual, as no problems were encountered with the implementation. This is supported by data showing that 74% of patients were treated with the protocol they were stratified to. Observed deviations to the stratification protocol were a caused by improvements to the EEG and HBC algorithm over the course of the study, which resulted in stratification to a different treatment protocol than the original algorithm predicted (e.g. a different Brainmarker-I decile score or another location showing highest HBC metrics). Training of EEG and HBC technicians appeared sufficient for the assessments to be performed independently.

Van der Vinne et al. (2019) reported various waiting times from EEG-assessment to outcome in their EEG biomarker feasibility study. In some cases, this waiting time was too long to use the report for treatment decisions. In current study, we used a similar, but improved EEG analysis pipeline. This generated an individual EEG report within 15 minutes after the assessment, which solved the problem reported by Van der Vinne and colleagues. This improvement made it easier for clinicians to include the EEG and HBC results in their treatment decisions. As a consequence, it was possible to perform an EEG informed HBC assessment immediately after the EEG assessment, which $n=56$ (47.9%) of patients in this study received without reported issues.

The waiting times for an HBC report were similarly short, as the Heart-Brain Connect app generates an outcome immediately after finishing measurements. Therefore, HBC-outcomes could be reported or used immediately. This makes it possible to implement in clinical practice, even when clinics can start treatment quickly after diagnosing depression. For this study, however, we created additional individual HBC reports as described in the methods section above, to have a better understanding and control of the data. The HBC app does not show data artifacts, and it is not possible to update the outcome based on adjusted R-R interval data in Kubios, whereas this is possible when using the HBC reports. The report corrects missing or unreliable data of individual patients. However, using the app will never lead to problems on a patient level, since either option (corrected or non-corrected) will always lead to an evidence based TMS protocol.

TAU vs. prospective stratification

The remission rate at session 25 in the retrospective sample was 25.6% and increased to 40.9% in the prospective sample, which resulted in an NNT of 7.

The Repeated Measures ANOVA comparing BDI scores at baseline, session 10, 15 and 25 for TAU vs. prospective (Figure 6.4, following page) showed a significant reduction in BDI score over time ($F(1,57)=14.118$, $p<.001$). No main effect of Group ($F(1,59)=1.661$, $p=.203$) and no Time*Group interaction effect was found ($F(1,57)=.218$, $p=.884$). The effect size of the BDI difference at session 25 (BDI in dataset 1A: 24.72 vs. in 1B: 22.05) was Cohen's $d=.181$.

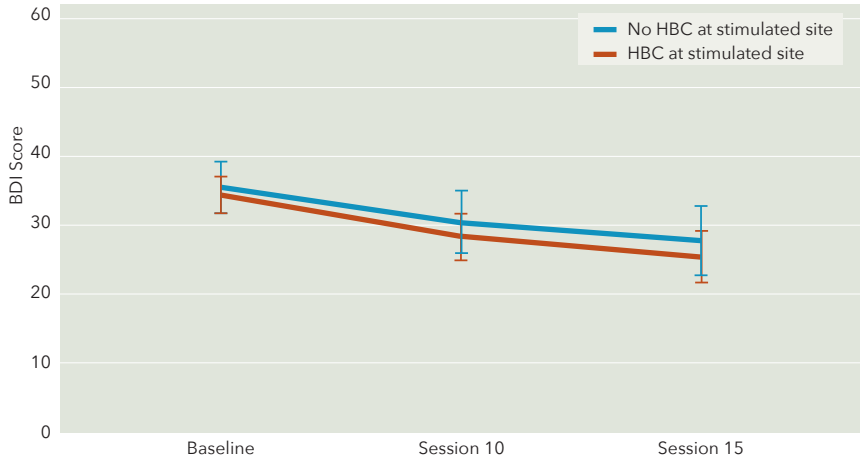

Figure 6.4. Repeated Measures ANOVA comparing BDI scores at baseline, session 10, 15 and 25 for treatment as usual and prospective EEG and HBC based stratification. Error bars are +/- 95%CI.

Effect of HBC on early TMS treatment response

A repeated measures ANOVA with within-subject factor time (BDI at baseline, session 10 and session 15) and between-subjects factor HBC (with/without; Figure 6.5) showed a significant main effect of time ($F(2,79)=26.630$, $p<.001$), no main effect of HBC ($F(1,80)=.496$, $p=.483$) and no significant time*HBC interaction effect ($F(2,79)=.086$, $p=.918$).

The Bayesian repeated measures ANOVA with within-subject factor time (BDI at baseline, session 10 and session 15) and between-subjects factor HBC (with/without) showed a $BF_{01}=24.216$ for the time*HBC interaction effect, indicating that the null hypothesis (no difference between HBC vs. no HBC) is 24 times more likely to be true compared to the alternative hypothesis.

Effect of sync marker on early treatment response to 10Hz TMS

A repeated measures ANOVA with within subject-factor time (BDI at baseline, session 10 and session 15) and between-subject factor sync marker (with ($n=25$)/without ($n=13$); Figure 6.5) showed a significant main effect of time ($F(2,35)=13.585$, $p<.001$), no significant main effect of sync marker ($F(1,36)=2.743$, $p=.106$) and no significant time*sync marker interaction effect ($F(2,35)=1.064$, $p=.356$).

The Bayesian repeated measures ANOVA with within-subject factor time (BDI at baseline, session 10 and session 15) and between-subjects factor sync marker (with/without) showed a BF_{01}=7.251 for the time*sync marker interaction effect, indicating that the null hypothesis (no difference between sync vs. no sync marker) is 7 times more likely to be true compared to the alternative hypothesis.

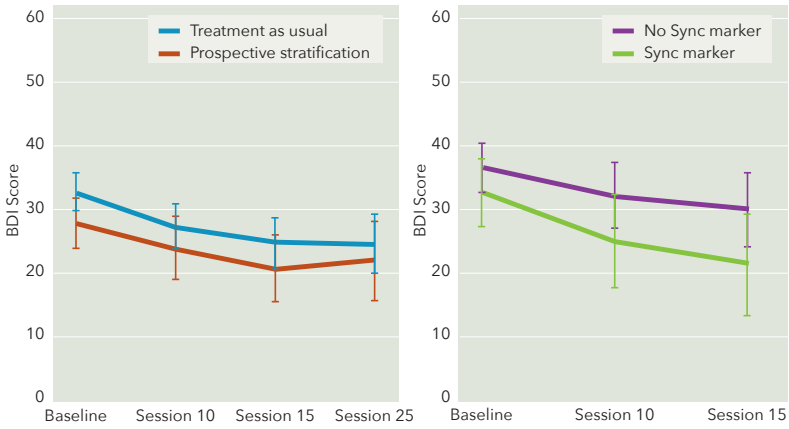

Figure 6.5. Mean BDI scores over time (baseline, session 10 and session 15) for patients with and without (left) HBC and (right) sync marker. HBC data is compared for the whole (per protocol) dataset with age as a covariate. Sync marker data is compared for protocol completers receiving 10Hz TMS stimulation. Error bars are +/- 95CI.

Relationship between iAF and HBC

No difference was found between the iAF of patients with and without HBC on the corresponding site, both at F3 ($F(1,96)=.422$, $p=.518$, Cohen's $d=.132$) and at F4 ($F(1,92)=.239$, $p=.626$, Cohen's $d=.101$). No correlation was observed between the iAF and mean HBC at F3 ($r=.115$, $p=.302$) and F4 ($r=.137$, $p=.218$) and there was no observed difference between the sync and no-sync marker groups regarding the mean HBC ($F(1,96)=.982$, $p=.324$, Cohen's $d=.211$) or the FT ($F(1,53)=.060$, $p=.808$, Cohen's $d=.082$).

Relationship between EEG power and HBC

The power spectra comparing EEG power from 0-40Hz for F3 and F4 are shown in Figure 6.6. Independent sample t-tests comparing the power for the HBC vs. no-HBC group (Figure 6.6B) showed no significant differences for F3 and F4 in the alpha frequency band. A

significant difference was found in the delta range for F4 at 0.8Hz ($t(104)=2.1$, $p=.038$) with a higher power for the patients showing HBC on F4. Topographical plots of the t-values for all EEG electrodes are shown in Figure 6.6C.

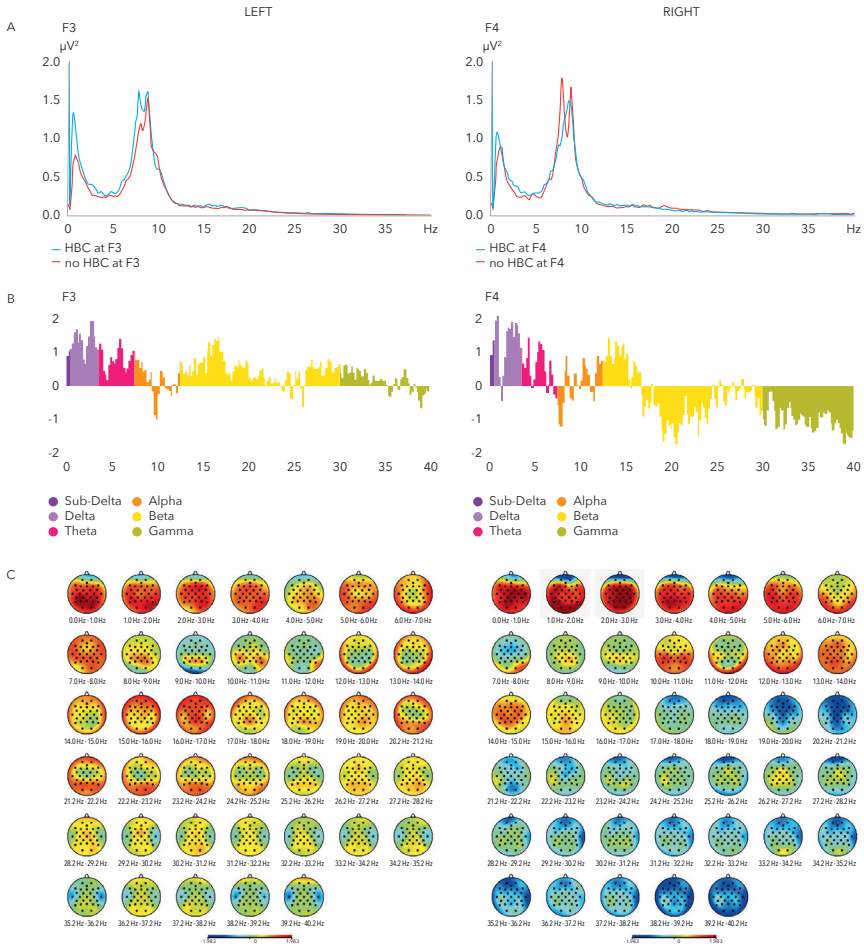

Figure 6.6. *A) Fast Fourier Transform (FFT) analysis on F3 (left) and F4 (right) comparing the HBC (blue line) with the no-HBC group (red line) in the 0-40Hz frequency range (0-30Hz shown in Figure). B) T-test values of independent t-tests comparing the EEG power of the FFT of 0-40Hz between patients with HBC vs without HBC on F3/F4 shown as power bars for F3 (left) and F4 (right) and C) Topographic plots of the t-values as seen in B but visualized for all EEG electrodes; each head displays a frequency band of 1Hz.*

DISCUSSION

The aims of the current study were to evaluate the feasibility and effectiveness of implementing Brainmarker-I and HBC based prospective stratification in clinical practice for TMS for depression, to examine the relationship between the two cross-modal prospective stratification biomarkers; the iAF and HBC, and to exploratory investigate the relationship between a broader EEG frequency spectrum and HBC.

The implementation of prospective EEG and HBC based stratification showed, based on experience and feedback, feasible and successful regarding satisfaction of patients and clinicians.

The analyses investigating the relationship between the iAF (including the sync marker) and HBC (mean HBC and the FT), showed no correlations, and the sync- and non-sync marker group did not significantly differ regarding mean HBC and their FT. These results therefore suggest that the iAF and HBC are independent biomarkers. Therefore, it is important to investigate their separate effects on treatment outcome. In a future stratification approach, they could be implemented independently of each other and may have synergistic utility to improve the treatment effectivity. This is supported by research investigating the effect of stratification based on HBC only on treatment outcome, where an nPPV of +10% was reported after implementation (Dijkstra et al., in preparation). Voetterl and colleagues (2023) reported nPPVs of +29% and +14% for the synchronization and high decile group respectively, after retrospective implementation of Brainmarker-I based stratification. Future studies should determine the synergistic effect of prospective iAF and HBC stratification in a larger sample, preferably compared to a non-stratified control group.

A reasonable explanation for the independent effects of the iAF and HBC could be the underlying source they are representing. The iAF informs us about the oscillatory rhythm of the neuronal excitability in the alpha range, whereas the site with highest HBC shows us where the prefrontal to sgACC connectivity is suggested to be highest. This suggests that the iAF is more of a temporal biomarker, in-

forming us when to stimulate – or at what rhythm (Pantazatos et al., 2023), whereas HBC seems to be a spatial biomarker, informing us where to stimulate. Combining them - e.g. by assessing the iAF at the individual's optimal HBC site - increases the information obtained from the individual brain. The individual iAF could then be used to determine the stimulation frequency to obtain synchronized stimulation. Hence, an individual with an iAF of 8Hz would be stimulated with a TMS frequency of 8Hz, aiming for stimulation at the optimal phase of the oscillation (the trough and rising phase; Wischnewski et al., 2022; Sack et al., 2023). This may increase the chances of optimal stimulation, potentially resulting in increased remission rates. Although this is very promising and brain state dependence research informs us about brain functioning and possibly extends our knowledge about the working mechanisms of TMS for depression, this subject is still in its infancy. As this is optimization of treatment (personalized psychiatry) and not stratification to already proven effective protocol, more supporting research is needed to apply this in clinical practice.

Furthermore, patients with HBC showed a higher delta power (0.8Hz) compared to patients without HBC. This finding between delta wave activity and heart rate values is in line with findings of Patron et al. (2019) who found a relationship between delta waves prior to the R-wave of the ECG and vagal control over the heart. Future studies could investigate this relationship further by studying the temporal dynamics of this delta signature, more specifically whether a higher delta power implies higher cardiac modulation. For clinical purposes it would be very efficient when an EEG marker could indicate HBC, maybe in the future reducing the number and type of measurements needed to stratify patients.

Regarding treatment outcome of TAU vs. prospective stratification, an increased remission rates was observed from 25.6% to 40.9%, resulting in an NNT of 7. However, no difference between the two groups was found regarding treatment progression. This observed, but not statistically significant, improvement might have been driven by the marginally significant (p=.060) difference regarding baseline BDI scores for TAU and the prospective sample, with higher BDI

scores in the TAU group. The response rates in these groups, both at session 15 and 25, further imply no group differences regarding treatment outcome. However, the sample sizes of both groups were small, resulting in a lower power than necessary to detect small improvements which are expected with treatment optimization (instead of comparing to a sham control group). Future prospective biomarker stratification studies should aim for a total sample size of approximately 200 participants (conform power analyses as performed in Van der Vinne et al., 2021), to achieve adequate statistical power. Furthermore, the design of the study, a naturalistic setting in which TAU and prospective data are compared, resulted in the lack of randomization. Preferably, patients were randomly assigned to either TAU or the stratified TMS protocol, which would exclude influences on treatment effects outside the intervention, such as general differences within periods of time and differences in treating clinicians. However, both clinics implemented the stratified based TMS treatment as their new TAU and therefore this was not feasible. Additionally, the prospective sample underwent an EEG and HBC assessment before the start of treatment and extended explanation about the stratification method, which could have influenced the expectations of patients.

As described in the introduction and methods section, individuals with an iAF close to 10Hz appear to have a higher chance of responding to 10Hz TMS treatment compared to those with an iAF further away from 10Hz (Figure 6.1). This was independently replicated in four studies (Corlier et al., 2019; Roelofs et al., 2020; Voetterl et al., 2022; 2023). Therefore, we expected, without stratification, the sync marker group to respond better to 10Hz TMS treatment in comparison to non-sync marker patients. Regarding HBC, without stratification, we expected better treatment outcomes for those with HBC as compared to those without HBC, since HBC is hypothesized as suggestive of a DLPFC-sgACC connectivity which is related to improved treatment outcome. However, with stratification, all individuals were stratified to their individual most optimal treatment protocol and site, aiming to decrease group differences, thus straightening the differences that were found in earlier iAF and HBC studies. Therefore, we expected similar treatment outcomes for the different sub-

groups. Our findings indicate that, regardless of having a sync marker or HBC or not, after stratification, all patients have similar chances of early response to TMS treatment. HBC stratification thus appears to be effective. However, the analyses regarding the effects of the iAF and HBC on early treatment response appeared difficult to interpret, since it was a two-way stratification with no isolated effects. Furthermore, the sample sizes were small and since we cannot compare our data to non-stratified treatment outcome measures, it is not possible to state whether we actually improved treatment outcome for the low decile score group. The same holds true for the HBC vs. no HBC group: since we cannot compare to a non-stratified group, we don't know whether the stratification for the no-HBC groups was successful based on the iAF or whether there are simply no differences between the groups. Lastly, investigating early treatment response instead of full-term treatment outcome might have also influenced the results. However, as described above, HBC only stratification and multiple out-of-sample independent iAF replications remain promising and future studies should take these study limitations into account, for example by prospectively randomizing stratified (based on iAF and HBC only and a combination of both) and non-stratified patients to assess the effects of both biomarkers.

Dijkstra et al. (in preparation) presented a nPPV of 110% for early treatment response after prospective HBC-TMS stratification only. Even though this is promising as even small improvements may be clinically relevant (Arns et al., 2022), we could improve effects even further. For clinical stratification, thus assuring that patients are always receiving a proven effective treatment, it is crucial to decide between evidence-based target sites. However, using this method, we might have missed the optimal sgACC-DLPFC connected site. If we were to determine the location with the highest HBC for each individual in a wider grid on the DLPFC, letting go of the evidence-based locations, we may find a more optimal site – showing higher HBC (as data in Figure 3.1C confirms). This could decrease noise in our current measurements and improve treatment outcomes further.

Another potential way to optimize HBC-TMS further is developing an age-dependent HBC cut-off value. As shown in this study, and

which is consistently found (as described in Chapter 4 and Dijkstra et al., 2023), people with HBC are significantly younger than people without HBC. There is a high likelihood that older patients did show frontal-vagal pathway activation, however with lower power values, therefore the activation was not identified as HBC. HBC-TMS will likely benefit from similar age (and sex) adjustments as are used with Brainmarker-I. However, large sample sizes are required for this development, which are currently not yet available.

In our baseline analyses comparing group differences, we found a significant difference in our total sample dataset between the sync vs. no-sync marker groups regarding baseline BDI score. As this was never reported by prior iAF research, and also not found in the per protocol subgroup, we investigated this using a larger and independent dataset (n=196; Donse et al., 2017). This analysis did not show the same results, suggesting our finding should be considered a spurious finding.

Conclusion
EEG and HBC based prospective stratification for TMS treatment for depression appears feasible and promising. The two assessed biomarkers, the iAF and HBC, appeared to be independent biomarkers. Therefore, they provide complementary - temporal and spatial - information about intended TMS stimulation. A relationship between EEG delta power and HBC was found, which should be investigated further in future studies.

Even though the clinical effectiveness of prospective stratification and the effects of using both biomarkers on treatment outcome needs to be investigated further using a larger sample size, comparing a non-stratified and stratified group, the method used in this study is promising and could be implemented more broadly in clinical practice, as it is already implemented in the described outpatient clinics. The stratification process always results in patients being treated with one of four evidence-based protocols and locations which all result in similar clinical outcomes when no stratification would have taken place (Fitzgerald, 2020; Trapp et al., 2023). This demonstrates that no harm can be done when implementing

this stratification procedure as described in this study. Implementation of the stratification method within, for example, a randomized controlled trial would result in obtaining further knowledge on the effects of the used biomarkers on treatment outcome, while opting for immediate improved treatment outcomes.

7

DISCUSSION

The previous chapters explored the heart-brain connection as a means to optimize antidepressant treatment using transcranial magnetic stimulation (TMS), with a particular focus on three key biomarkers: the heartbeat evoked potential (HEP), Heart-Brain Coupling (HBC), and the individual alpha peak frequency (iAF). The upcoming chapter will summarize the key findings of this thesis and follows up on the case study of Elien. It will then address several important topics, including patient-centered care, the heart-brain connection in psychiatry, the use of electroencephalogram (EEG) to determine the optimal TMS stimulation site, the role of the iAF in TMS outcomes, and directions for future research.

KEY FINDINGS

As outlined in Chapter 1, depression is a highly debilitating and potentially life-threatening condition. Despite the availability of evidence-based treatments, such as psychotherapy and antidepressant medications, response rates remain limited, ranging between 30-50%. Furthermore, individuals who do not respond to two proven treat-

ments face significantly lower chances of success with subsequent interventions, a condition known as difficult-to-treat depression (DTD), often resulting in chronic symptoms. Optimizing treatment selection processes for DTD is therefore crucial. One validated and effective non-invasive neuromodulation therapy for DTD is TMS. The response and remission rates for TMS are comparable to other – including first-line - antidepressant treatments, yet the different mechanisms of action across treatments suggest heterogeneity within the depressed patient population. This variability is reflected in TMS treatment for depression, where consistent outcomes are observed across different stimulation protocols, targets, intensities, and other parameters, underscoring the need for individualized treatment approaches.

Stratified psychiatry is a promising method for tailoring treatment selection to the individual. In this approach, biomarkers are used to match patients to the treatment most likely to be effective based on their specific biomarker profile, while avoiding treatments less likely to yield positive outcomes. Importantly, treatment selection in stratified psychiatry is always grounded in proven, effective therapies, thereby excluding the risk of doing harm.

One promising biomarker for clinical application is heart rate, as depression is frequently associated with cardiovascular abnormalities, such as elevated heart rate, reduced heart rate variability, and increased blood pressure. Given that 20% of heart-brain communication is efferent (from the brain to the body), and 80% is afferent (from the body to the brain), both pathways are assessed for their potential as stratification biomarkers to further our understanding of both depression and TMS. Another relevant biomarker is the iAF, measured through EEG, which shows promise for stratifying patients into different TMS protocols.

Chapter 2 investigates the afferent pathway assessing the HEP. Baseline HEP amplitudes of 1008 depressed patients (treated with escitalopram, sertraline or venlafaxine), 336 healthy controls and 196 depressed patients treated with TMS were assessed. The primary finding was that the HEP amplitude (at EEG site Cz) of patients remitting to venlafaxine treatment was significantly lower compared to non-remitters. For TMS

treatment, an opposite pattern was observed, indicating the potential of HEP for stratifying patients between venlafaxine and TMS as distinct treatment options.

The subsequent chapters assess the afferent pathway through the frontal vagal network. Heart rate decelerations were found during TMS stimulation using NCG-TMS in healthy participants. In Chapter 3, we validated NCG-TMS in thirty-three depressed patients. Findings of site-specificity and inter-individual variations were replicated with similar cortical topography for heart rate decelerations (mostly found at sites F3/F4 and FC3/FC4) and accelerations.

A novel implementation – HBC-TMS – measuring induced heart rate oscillations (decelerations during TMS stimulation followed by normalization between stimulation trains) instead of pure heart rate decelerations, is replicated, validated and optimized in Chapter 4. Forty-five healthy participants underwent two HBC-TMS assessments and ten underwent five. Findings of site-specificity and inter-individual differences were robust, with clear dose-response effects. Furthermore, a method was developed to compare between-subject effects, which also made it possible to establish an individual frontal threshold (FT) – indicative of 'actual HBC'. The FT appeared significantly lower compared to the MT and was found to be stable over time. Participants with higher HBC values showed more consistent HBC over time.

An example of wider research implications of HBC-TMS is explored in Chapter 5. Here, the impact on the frontal-vagal activation of different TMS pulse parameters – pulse length and shape – are assessed in twenty healthy participants. Shorter pulses appeared more efficient than longer pulses, particularly on the DLPFC (inducing HBC) as compared to the motor cortex (activating motor evoked potentials). Contrary, pulse shape appeared to have no major effect on induced HBC.

Chapter 6 bridged the gap between research and clinical practice by exploring the feasibility and effectiveness of prospective stratification in a cohort of 117 patients with depression. Patients were stratified into two distinct TMS stimulation protocols (1Hz or 10Hz) based on their Brainmarker-I score in combination with HBC-TMS outcomes

to determine the stimulation site (Beam or 5CM). Prospective stratification proved feasible and promising. The relationship between the individual alpha peak frequency (iAF) and HBC was also examined, revealing that these are independent biomarkers. Additionally, a higher delta power was reported in the group exhibiting HBC at site F4, suggesting a potential relationship between HBC and delta oscillations.

Follow-up Elien.

After several treatment options on top of the already described ones - AD switches, the use of benzodiazepines, AD augmentation with lithium and creative therapy - Elien's depressive symptoms did not improve. She was considered to have a difficult-to-treat depression. Elien was signed up for TMS treatment, accompanied by cognitive behavioural therapy. At this point, she was taking nortriptyline, lithium and lorazepam daily, feeling depressed, exhausted, completely drained and hopeless for the future.

As Elien participated in the prospective stratification study (Chapter 6), she underwent an EEG and HBC assessment before the start of her TMS treatment. She had an iAF within the synchronization frequency, therefore she was stratified to the 10Hz L-DLPFC stimulation protocol. She appeared to have low heart-brain coupling, but the location with the highest HBC was found to be the left Beam.

At the start, she did not expect too much of the treatment. In session 6, however, she and her husband had experienced that one of her crying spells was noticeably shorter than she was used to. During treatment, her mother experienced a brain infarct and as a cause of that they identified some potentially cancerous spots on her MRI scan. Elien felt resilient enough not only to go through this process, but also to support her mother during the process, which she was not able to do before. The TMS clinicians started noticing differences in her posture: she seemed more present and open. At session 10, her BDI questionnaire showed a score at remission-level, matching her mood.

Elien progressed quickly and had a wish to lower the dosage of her medication. She tried quitting before, but her symptoms increased as she lowered the dosage. Over a few weeks during TMS treatment, she completely quit taking lorazepam, without experiencing any problems and consistently feeling better. Happy and motivated, she started lowering her lithium dosage too with supporting TMS sessions once a week. She was able to quit taking lithium without any side-effects or symptom increases.

Through TMS treatment, Elien was thus able to recover from most of her symptoms in only 10 sessions and subsequently quit 2 types of medication during the course of only 25 sessions. After years of thinking she had to do everything people asked of her, and everything perfectly, she finally believed and experienced that she is good enough being who she is.

Three months after the last treatment session, she still feels really good and went on a family holiday to the South of France, which she enjoyed immensely. She enjoys being a mother and wife and although she loved her job at the fashion store where she worked for almost 20 years, she decided to switch jobs, something she always wanted to do, but never dared to. Instead of sadness and fear controlling her life, Elien is back in control of life herself. She hopes that many others, like her, will benefit from TMS for depression.

Although Elien is only one example, her case represents many other – from very similar to completely different - patient stories. One thing all patients have in common is that they are desperate to find a treatment option that works for them to relieve their symptoms, sooner rather than later. With that purpose, depression is extensively studied. Research focuses on a wide variety of topics; from the etiology of depression, to brain responses to specific interventions, to the best way to assess symptoms (Wang et al., 2021). It is, however, challenging and it often takes years, if not decades, to implement research findings into clinical practice. This is especially true for more fundamental research – which often focuses on such small details that the clinical implications are difficult to establish - and for randomized clinical trials (RCTs).

RCTs assign subjects randomly to an experiment and control group, therefore excluding non-intentional effects (Hariton & Locascio, 2018). This allows for the assessment of purely the intervention of interest. Therefore, RCTs are considered the gold-standard of research. There are, however, some important limitations to RCTs, especially regarding the application and translatability of research findings into clinical practice. The long duration of performing RCTs – and publication of the results (Ross et al., 2012) – is one limitation, which increases the amount of time needed for obtaining and sharing new knowledge with the world. Furthermore, RCTs often apply strict in- and exclusion criteria, for example the use of (specific) medications or having multiple mental disorders (comorbidities; Kostis & Dobrzynski, 2020). These result in low internal and external validity, decreasing translatability to the clinic, in which increasing numbers of comorbidity are reported (Nordgaard et al., 2023). Although RCTs remain crucial for investigating causal relationships, they assess treatment efficacy and not effectiveness. Therefore, there is a need for more clinically applicable research methods, so that patients, like Elien, can benefit from newly acquired knowledge sooner – as depression research is originally intended.

As explained in the introduction, and prospectively implemented in Chapter 6, biomarker-based stratification is one method to apply re-

search findings early on for direct patient benefit (Arns et al., 2022; 2023). For the following paragraph, I will be using the case of Elien to explain stratification further, although any specific case could have been used. TMS treatment, of which the protocol stratification was based on HBC-TMS and the iAF based biomarker Brainmarker-I, resulted in a fast remission of depressive symptoms for Elien. This allowed for a supported way of quitting two types of medication during an average length TMS treatment. Elien's biomarker profile showed she had the highest chance of responding to 10Hz left (L) DLPFC-TMS, which, inherently to the stratification method, means she had the lowest chance of responding to 1Hz right (R) DLPFC. It is unknown what these reduced chances were, since Voetterl and colleagues (2023) did not report on expected reduced effects of stratifying to the worst treatment option. As we did not test this, we do not know how Elien would have responded to the other stratification protocol. However, following the theory of stratification: the best-case scenario for 1Hz R-DLPFC stimulation would result in a similar treatment outcome as she experienced with 10Hz L-DLPFC TMS, whereas the worst-case scenario would be a similar outcome as the one-size-fits-all prescription practice – thus a 30% chance of remission, but also 70% chance of non-remission. The latter would then result in another failed treatment option, longer lasting depression symptoms and an increased chance of depression severity and chronicity. The question that remains is how ethical it is to not use stratification when the information is available, therefore not treating to the best of our knowledge. The results of the study described in Chapter 6 show promise for the improved clinical effectiveness of stratification. It is, however, important, potentially even more important than in other clinical research, to perform (multiple) interim-analyses to check whether the protocol does not stratify to the one treatment that a patient is least responsive to – resulting in lower remission rates than expected. To assess the robustness of certain stratification biomarkers before implementation, multiple retrospective out-of-sample validations, as performed by Voetterl and colleagues (2022; 2023; 2024), are of added value. Additionally, to study the actual effects of stratification, prospective stratification may be combined with RCTs (e.g. comparing a stratified group with a non-stratified control group). This increases the potential of ob-

taining clinically relevant, but also scientifically based, treatment information. Lastly, stratification allows for studying real-world data, including effects on heterogenous patient samples as seen in the clinic. Stratified psychiatry is thus not just a method to implement recent research findings in clinical practice. The group-level findings and individual cases inform us further on the effects of stratification on treatment. For this, treatment outcome measures are assessed in relation to the biomarkers. The stratification approach is therefore one that could be studied in clinical practice more often than is cur-

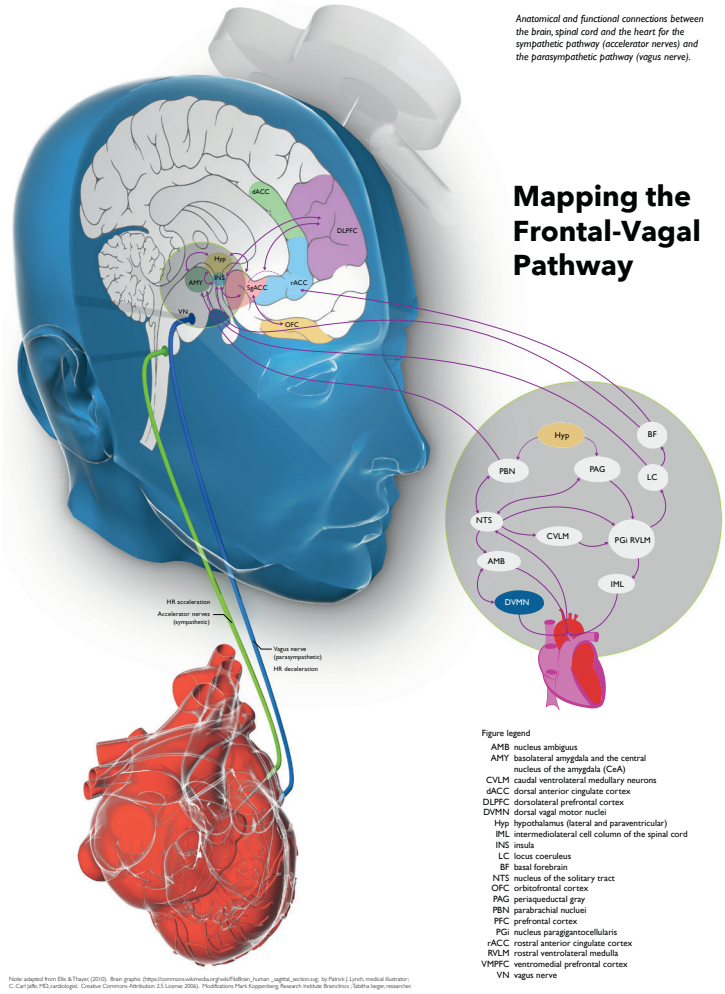

Figure 7.1. Visual overview of sympathetic and parasympathetic heart-brain connections.

rently done, therefore obtaining even more, clinically relevant, information while patients are already treated according to latest knowledge.

THE HEART-BRAIN DIALOGUE: TALKING AUTONOMIC REGULATION

The heart-brain connection is a continuous dialogue between two intrinsically important organs. This continuous communication suggests a crucial relationship that holds important information about bodily functioning. Candia-Rivera and colleagues (2022) showed that emotional arousal cannot be exclusively explained by changes in cardiac parameters or EEG power, but the relation between the two provides crucial additional information. Furthermore, they investigated the temporal dynamics of the heart-brain connection in emotional response and showed that the heart initiates an emotional response. They suggest that emotions are a cause of the afferent input received by the brain instead of the interpretation of the peripheral changes. A recent study provides further evidence for oscillatory coupling between the heart and brain, with larger bottom-up influences as compared to top-down influences (Sargent et al., 2024). This further connotates the importance of a functional heart-brain connection, which may be even more important in individuals with psychiatric disorders. A well-regulated autonomic system, which sends adaptive signals between the heart and brain, thus allows for better emotional self-regulation, enabling patients to process stress and emotional experiences more effectively. Throughout this thesis, we focused on the heart-brain connection through the frontal-vagal pathway (visualized in Figure 7.1) to find biomarkers for TMS treatment for depression. However, psychiatry has increasingly recognized that not only depression, but executive functioning in general (Kimhy et al., 2013) and other mental health conditions, including – but not limited to - anxiety (Sharma et al., 2011), posttraumatic stress disorder (PTSD; Dennis et al., 2016), autism spectrum disorder (ASD; Owens et al., 2021), attention-deficit hyperactivity disorder (ADHD; Geiss et al., 2023), Tourette syndrome (Hawksley et al., 2015), Parkinson's disease (Chen et al., 2020) and epilepsy (Uhlmann & Fröscher, 2016) involve not just neurochemical imbalances in the brain but also dysregulation of the autonomic nervous system, which governs heart-brain

communication.
Psychophysiological coherence – a state in which the heart, brain and nervous system operate in harmony – is an emerging research field. Coherence reflects optimal bodily homeostasis: the synchronization of physical, mental and emotional systems (Anurag et al., 2023). Coherent heart rhythms, perceived as a high heart rate variability (HRV), are indicative of a well-balanced autonomic nervous system (ANS). HRV reflects the fluctuations of the time intervals between consecutive heartbeats. As Shaffer and Ginsberg (2017) wrote: "the heart is not a metronome": a good working nervous system is constantly responding to the changing environment. A higher variability between heartbeats is thus indicative of an adequately changing sympathetic/parasympathetic activation. Billman (2020) said that disruptions of homeostasis seem to be the cause of all physiologic and psychologic diseases. This suggests that restoring the homeostasis results in relieve of symptoms, which offers therapeutic avenues that go beyond traditional psychological and pharmacological treatments. Treatment examples include biofeedback, HRV training, VNS and mindfulness practices. These practices have in common that they require conscious alterations of autonomic functioning, changing the afferent signals that are sent to the brain (Sinha et al., 2020). Mindfulness training may, for example, alter baseline HRV (Kirk & Axelsen, 2020) and improve resilience against stress (Oh et al., 2022), contributing to better outcomes in various psychiatric disorders. Thus, restoring ANS functioning results in relieve of psychiatric symptoms.

Using NCG-TMS and HBC-TMS, we found similar effects for DLPFC-TMS. Through the vagal nerve, DLPFC-TMS seems to influence the sympathetic/parasympathetic balance by increasing parasympathetic activity. As an effect, TMS stimulation has shown to lower heart rate, increase HRV and lower blood pressure, although these effects were restricted to the time during stimulation only and no longer lasting effects were found (Iseger et al., 2020; Lee et al., 2023).

Furthermore, over the course of a TMS session (especially with 10Hz and iTBS on the DLPFC), both the brain and heart are oscillating in the same frequency inducing heart-brain coherence (although the

frequency depends on the stimulation protocol used, as described in the introduction). The primary purpose of TMS, as discussed in the introduction of this thesis, is to modulate brain activity in regions associated with mood and cognition to restore functional network connectivity, however it is possible that (even only short-term) optimization of autonomic functioning and inducing coherence play a role in the relief of mental health symptoms. This, in combination with the hypothesis that all (psychiatric) disorders develop as a cause of ANS disruptions - might be an explanation for the effectiveness of the same target site for various psychiatric disorders, for example when stimulating the DLPFC for depression, anxiety (Rodrigues et al., 2019), posttraumatic stress disorder (Edinoff et al., 2022), the negative symptoms of schizophrenia (Lorentzen et al., 2022), and addiction (alcohol, tobacco and cocaine; Antonelli et al., 2021). Furthermore, the effects of TMS on various other, over the years increasingly studied, target sites, like the ventromedial PFC (vmPFC) and the orbitofrontal cortex (OFC), may be explained in a similar way. As visualized in Figure 7.1, the vmPFC and OFC are part of the frontal-vagal pathway as well. Although on the group level these frontal stimulation sites (approximately Fp1/Fp2) resulted in increased heart rate (Chapter 3; Iseger et al., 2021), there were individuals for which these sites resulted in heart rate decelerations – suggestive of the most optimal stimulation site - as can be seen in Figure 3.1C (Chapter 3). However, only little information about cardiovascular effects during stimulation at these target sites is currently available and conclusions about ANS influence cannot yet be made.

Improvements in autonomic regulation are thus shown to be effective for various psychiatric diseases. This raises the question whether these dysregulations are common for all patients – and potentially even the cause for their symptoms, however presenting itself through various different symptoms that we clustered as psychiatric classifications as they are commonly reported in these specific clusters. If this is indeed true, the question arises whether we could use the heart-brain connection – and the resulting biomarkers – to determine the origin of autonomic dysregulation. This approach might be useful for transdiagnostic stratification – moving towards precision psychiatry. Prior studies supported the idea that distinct neuronal

circuits regulate different physiological systems, as indicated by various cardiovascular measures (Berntson et al., 1997; Critchley et al., 2003). Different heart rate-related biomarkers could thus inform on various dysregulations of the ANS, like interoceptive awareness (the nervous system's interpretation of bodily signals). Research, such as the review article by Khalsa and colleagues (2018), have shown that altered interoception is associated with various psychiatric disorders, including – but not limited to - anxiety, depression, eating disorders and somatic symptom disorders. These conditions often involve disruptions in the ability to accurately perceive and interpret bodily sensations, which can impact emotional regulation and decision-making. Behavioral assessments of interoception, such as the often used heart rate perception task (Schandry, 1981), have become essential tools for investigating these sensory-processing abnormalities. The HEP, however, is a measure assessing afferent influence – from the heart to the brain - and is found to be an objective biomarker for interoceptive awareness. As mentioned in the introduction, the physiological processes underlying the HEP are still unknown. However, there is evidence that the HEP does not originate from pure cardiac influences (Kumral et al., 2022). Kumral and colleagues hypothesized – note that this has not yet been proven - that the specific localization of the altered HEP to the right insula, is an argument against the influence from physiological noise or brain damage, as these would probably induce more diffuse signals. HEP deviations therefore would indicate heart-brain dysregulations. Deviations to HEP amplitudes might thus not only indicate autonomic nervous system dysregulations, but moreover that these dysregulations happen at the level of the insula/somatosensory cortex. Lower and higher HEP amplitudes thus indicate decreased and increased interoception, respectively. Different treatment options can potentially normalize a decreased or increased interoceptive awareness. Our findings described in Chapter 2 may imply that venlafaxine treatment normalizes the HEP through increasing interoception, whereas DLPFC-TMS stimulation may decrease interoception, although no conclusion can be drawn based on this data. At present, there is no evidence supporting the former, but findings have been reported that address the latter (Pollatos et al., 2016). Subsequently, it would be beneficial to gain further insight into the mechanisms of action for both treatments

by examining post-treatment HEP amplitudes. Biomarkers, like the HEP and HBC-TMS, may thus help find the crux of the autonomic dysregulation and can be used to determine the best target site to influence the ANS which could then be treated with the most appropriate treatment.

BRAIN WAVES LOCALIZING THE ENTRANCE INTO THE FRONTAL-VAGAL PATHWAY

EEG is a widely accessible and well-researched method for identifying biomarkers in psychiatry. As discussed in the introduction and Chapter 6, the iAF has proven to be a robust stratification marker for distinguishing between different TMS protocols. The potential of EEG-based stratification to tailor TMS treatment protocols—and even antidepressant medications (Van der Vinne et al., 2021), although that is outside the scope of this section—may lead to more patients undergoing EEG assessments prior to treatment. As emphasized throughout this thesis, and particularly in the preceding paragraph, the ANS plays a crucial role in depression and is often dysregulated in affected individuals. HBC-TMS provides an accessible method to optimize target localization by focusing on the frontal-vagal pathway, addressing the top-down regulation of the ANS. However, given that the heart-brain connection operates as a bidirectional system, it should also be possible to determine the optimal stimulation site by evaluating the bottom-up pathway—specifically by analyzing brain wave activity with EEG.

Delta waves (0.5–4 Hz) are predominantly observed during deep sleep and are associated with restorative functions. Theta waves (4–7 Hz) typically emerge during early stages of drowsiness and rapid eye movement (REM) sleep, while alpha waves (7–13 Hz) reflect a state of relaxed wakefulness. Beta waves (13–30 Hz) are associated with increased alertness, and gamma waves (>30 Hz) are present during focused concentration (Stern & Engel, 2013). Vagal tone can modulate these slow-wave activities through parasympathetic regulation. A recent study has demonstrated oscillatory coupling between heart and brain rhythms across all frequency bands except gamma (Sargent et al., 2024). However, parasympathetic regulation is most prominent in

the delta, theta, and alpha bands, as these oscillations correspond to resting states, whereas sympathetic activation increases in beta and gamma states. Consequently, these slower brain oscillations appear as the most promising markers for vagal modulation. Cardiac vagal control, assessed via HRV, reflects the influence of the parasympathetic nervous system on cardiac regulation. Greater vagal control, indicated by higher HRV, is associated with better recovery from stressors and a more effective return to homeostasis (Thayer & Lane, 2009; Mezzacappa et al., 2001).

Vagus nerve stimulation (VNS) is commonly employed to investigate the relationship between vagal control and brain activity. VNS – as the name implies - directly stimulates the vagus nerve, thereby activating the parasympathetic system. Its cortical effects are typically assessed using EEG. Although VNS has not been shown to alter cortical excitability (Mertens et al., 2022), multiple studies indicate its influence on brain oscillations. Evidence of VNS's effect on alpha oscillations remains inconsistent: Sharon et al. (2020), for example, reported that VNS attenuates alpha oscillations, but these findings were not replicated in a subsequent direct replication study (Lloyd et al., 2023). In our study described in Chapter 6, we examined the relationship between the iAF and HBC. Our results align with the non-replication study, since we found no correlation between alpha peak frequency and HBC at specific EEG sites. Exploratory analysis in our study revealed higher delta power (0.8 Hz) at EEG site F4 in patients exhibiting HBC. Previous research has linked high vagal tone with decreased delta power preceding the R-wave of the ECG (Patron et al., 2019). These authors propose that delta oscillations in the prefrontal cortex may trigger increased cardiac vagal control, acting as both a "go" and "brake" mechanism on cardiac rhythm. VNS, when used to treat drug-resistant epilepsy, has been shown to significantly affect not only delta oscillations (Ricci et al., 2020) but also the theta band (Candia-Rivera et al., 2022; Lanzone et al., 2022). However, the observed theta-band effects may be related to the emotional paradigms of the studies, as theta oscillations have been repeatedly linked to emotional processing (Krause et al., 2000; Balconi & Pozzoli, 2009), instead of pure cardiac vagal control.

Given the limited research on this topic, it is premature to make definitive recommendations for future biomarkers. However, delta oscillations appear to be the most promising EEG frequency for investigating vagal modulation, particularly in identifying EEG sites that best represent the entrance into the frontal-vagal pathway. Further research into the relationship between delta oscillations and vagal control, such as assessing the temporal dynamics of the delta signature or exploring whether increased delta power correlates with enhanced cardiac modulation ability, may prove valuable.

TMS AS A NEURAL PACEMAKER

An idea proposed in this thesis is that TMS stimulation delivered at the iAF could enhance treatment outcomes. This hypothesis is based on multiple, independently replicated studies (Corlier et al., 2019; Roelofs et al., 2020; Voetterl et al., 2023; 2024) showing that patients with an iAF close to 10Hz are more likely to respond favorably to 10Hz TMS. This suggests that TMS protocols aligned with an individual's neural oscillations might lead to improved therapeutic effects by optimizing neural entrainment. iAF-based TMS is theoretically appealing because it aligns with the brain's natural oscillatory rhythms, allowing stimulation at the optimal phase of the oscillation. Research on the motor cortex has shown that the phase of neural oscillations (such as the peaks and troughs of the mu rhythm) determines neuronal excitability and responsiveness to external stimulation (Wischnewski et al., 2022). MEPs were found to be larger when stimulation is timed at the trough (and the rising phase) compared to the peak (and the falling phase). These findings support broader theories of brain-state dependence in neurostimulation (Sack et al., 2023).

The data from our study, detailed in the supplement of Chapter 6, did not show a significant difference in early treatment response between individuals with synchronized iAF (9.6-10.4Hz) and non-synchronized iAF (low deciles scores; slow iAF) who received 10Hz TMS to the DLPFC. The synchronized group received 10Hz TMS based on their iAF, whereas the low-decile score group received a stimulation protocol (either 1Hz or 10Hz) based on the site showing the highest HBC. The unsynchronized group receiving 10Hz TMS therefore had most HBC at

a left hemispheric site. Without stratification, based on prior research, we would thus expect the synchronized group to perform better in comparison to the unsynchronized group. However, with stratification, the results show no difference. This is a potential cause of successful stratification, although we could not conclusively state this since no comparison could be made with non-stratified treatment outcomes. However, if the hypothesis of neural entrainment as a cause of a stimulation intensity matching the iAF were true, these findings would have still resulted in enhanced treatment outcomes for the 10Hz iAF group. The findings of a recent double-blind randomized controlled trial (George et al., 2023) support these findings. They showed that there was no significant difference in clinical outcomes between patients receiving individual alpha-synchronized TMS (the first pulse of the stimulation train was synchronized to the iAF) and those receiving unsynchronized (random) stimulation. In fact, the unsynchronized group had better treatment outcomes with higher remission rates (46% vs. 13%). This might be due to the limited synchronization to the first pulse only, leaving subsequent pulses potentially unsynchronized or even more synchronized with the inferior phase. Treatment effects of continuous phase synchronization - warranting for stimulation of the actual inferior phase of the oscillation – should be investigated for potential treatment improvements, as the hypothesis of endogenous stimulation is promising.

Another hypothesis about the neural mechanisms of TMS is akin to the concept of deep brain stimulation (DBS) functioning as a "neural pacemaker". TMS may similarly modulate brain waves, with high-frequency TMS pushing slower brain waves, like those seen in depression, toward an optimal alpha frequency (~10Hz; described in the introduction), while low-frequency TMS might reduce fast-wave hyperactivity. This theory aligns with findings from Voetterl et al. (2023; 2024), where patients with low Brainmarker-I scores (indicating slower alpha oscillations) responded better to higher-frequency TMS (18/20Hz), whereas those with higher Brainmarker-I scores responded better to lower-frequency (1Hz) TMS. This could potentially be explained by changes in brain plasticity and synaptic functioning driven by TMS. At the synaptic level, TMS pulses can either inhibit or excite brain areas by modulating synaptic efficacy, reflecting long-term potentiation (LTP) or

long-term depression (LTD). Research by Vlachos and colleagues (2012) demonstrated that magnetic stimulation increases the size and number of synapses, while Brown et al. (2022) confirmed that TMS-induced LTP enlarges dendritic spines. Conversely, LTD is associated with a decrease in spine size (Zhou et al., 2004), reflecting weaker synaptic connections. High-frequency TMS is generally thought to induce LTP, while low-frequency protocols are associated with LTD. However, the effects of TMS also depend on the state of the synapses before stimulation (Abraham & Bear, 1996).

The overall effect of TMS may be explained by the (homeostatic) metaplasticity theory (extensively explained by Thomson and Sack, 2020 and Downar and colleagues, 2024) which suggests that synapses function optimally in the middle of their dynamic range – at homeostasis. If a synapse is already maximally excited, further excitatory stimulation might result in inhibitory (LTD) effects, and vice versa. Thus, TMS does not produce purely excitatory or inhibitory effects but moves synaptic activity towards a balanced state. This implies that, over time, any TMS protocol could lead to clinical improvement, though some patients may take longer to reach this optimal balance. Patients with a lower iAF may benefit more quickly from excitatory TMS (inducing LTP), while those with a higher iAF might respond better to inhibitory TMS (inducing LTD), as these stimulations help bring synaptic activity back to homeostasis (~10Hz). Therefore, the iAF may serve as a useful indicator of underlying synaptic changes related to neuroplasticity.

FUTURE RESEARCH DIRECTIONS

As most research, we were able to find answers to some of the research questions, but over the course of this thesis many more questions arose, accompanied by future research ideas of which some are presented in the following paragraph.

Heart-brain coherence through breathing as an add-on to TMS treatment
Breathing influences the ANS. During inhalation, heart rate increases, while during exhalation it decreases, a phenomenon known as respiratory sinus arrhythmia (RSA; Ben-Tal et al., 2012). This pro-

cess is regulated not only by the ANS but also by baroreceptors in the arteries (Pham et al., 2021). RSA has long been associated with HRV (Grossman & Taylor, 2007; Pham et al., 2021). Gao et al. (2023) demonstrated that after eight weeks of mindfulness-based breathing training, there was a significant correlation between the individual alpha peak frequency (iAF) and heart activity. The optimal breathing rate for maximizing HRV—and thus achieving the best balance between sympathetic and parasympathetic activity—varies between individuals (Shaffer & Meehan, 2020; Hasuo et al., 2021). This so-called resonant breathing frequency (RF) typically ranges from 4.5 to 7 breaths per minute. Breathing at one's RF has been shown to increase HRV, reduce blood pressure, and improve mood (Steffen et al., 2017), while also decreasing perceived stress and enhancing cognitive function in young adults (Chaitanya et al., 2022). A recent meta-analysis of RCTs further revealed that breathwork in general—not limited to RF breathing—improves stress levels and mental health (Fincham et al., 2023). Given that both RF breathing and TMS induce heart rate oscillations and affect brain activity, it would be valuable to explore the potential benefits of incorporating RF breathing exercises between TMS sessions. Moreover, future research could extend beyond breathing frequency to investigate the effects of breath regularity, depth, and rhythm, as these factors also appear to impact the ANS (French et al., 2024).

HBC throughout the course of TMS treatment

Elien demonstrated an iAF around 10Hz (sync marker) yet exhibited low HBC at the stimulated site. This data, in contrary to expectations, suggests that HBC, and by extension the hypothesized activation of the DLPFC-sgACC connectivity at the treatment site, may not be crucial for the therapeutic response to TMS. This discrepancy might be explained by the design of the study, in which HBC was only assessed before the initiation of treatment and not prior to each session. This may have resulted in a different stimulation site as compared to the initial HBC-TMS assessment. Since HBC-TMS outcomes are site-specific (as discussed in Chapters 3-6), and manual target site measurements can vary between sessions (as outlined in Chapter 4), this may lead to variations in coil placement. Therefore, it is possible that during TMS treatment sessions, Elien demonstrated higher

HBC as compared to the initial HBC-TMS assessment. This highlights the need for further investigation of HBC-TMS throughout the course of treatment.

Another hypothesis posits that TMS may restore the DLPFC-sgACC connection through repetitive activation of the frontal-vagal pathway in patients that do not show baseline HBC. To test these hypotheses, it would be useful to assess HBC at baseline, during and after treatment and compare pre- and post-treatment HBC levels for both remitters and non-remitters.

The FT as a method to define TMS stimulation intensity
As briefly touched upon in the introduction, the TMS stimulation intensity has changed over time from 80%MT to 120%MT. Research using different stimulation intensities still show similar treatment outcomes, suggesting that either stimulating at 80%MT is sufficient for TMS treatment effects or there are inter-individual differences regarding the optimal stimulation intensity. HBC-TMS has potential as a direct and reliable neurophysiological readout for activation of the frontal-vagal pathway, therefore showing that the stimulation intensity is sufficient for reaching the brain, as shown in Chapter 4. The FT appeared significantly lower compared to the MT. Future studies should focus on investigating whether using the FT to determine individual (optimal) treatment intensity improves TMS treatment outcomes. As studies are already treating patients with stimulation intensities in a range from 80-120%MT, resulting in similar treatment outcome values, this could be applied immediately without doing harm.

Brain-gut axis: microbiome, oscillations
Throughout this thesis, we examined the heart-brain connection via the vagus nerve. It is important to note that the vagus nerve does not solely link the brain to the heart; it also connects to the stomach, gut, and various other organs, excluding the adrenal glands. Recent studies on depression have increasingly highlighted the significance of the brain-gut axis. Research in this area has shed light on the complex relationship between gut health and mental well-being, particularly regarding depression (Liu et al., 2023). Future research should

further investigate the role of the microbiome in influencing mood and cognitive functions, with a particular focus on how microbial diversity and composition may affect neuroinflammatory pathways and neurotransmitter synthesis. Additionally, exploring the potential of TMS to impact the gut oscillations and the microbiome offers an innovative avenue for understanding the interactions between brain activity and gastrointestinal function. By combining microbiome analyses with TMS studies, researchers can clarify the mechanisms through which gut health affects depression and potentially identify microbial biomarkers that may predict therapeutic responses. This comprehensive approach not only deepens our understanding of the biological foundations of depression but also opens new avenues for developing treatment strategies that target both the microbiome and gut-brain signaling pathways.

LISTENING TO THE BODY AS A WHOLE, INTERCONNECTED SYSTEM TO FURTHER OPTIMIZE ANTIDEPRESSANT TREATMENTS

This thesis adds on to the knowledge about the heart-brain connection in depression. We looked at afferent and efferent biomarkers, measured with EEG and ECG. First of all, we – researchers - should always keep in mind why we are doing research, which is obtaining knowledge about depression and antidepressant treatments to optimize treatment effectiveness. Research should implement newly obtained knowledge as soon as possible, so that patients – like Elien – can benefit from our work as soon as possible, as originally intended.

As can be seen in the findings of all studies within this thesis – and plenty more in the world of biomarker research – there are no (robust) biomarkers that are independent of the rest of the body. The whole body is an interconnected system which continuously communicates with all its parts. The heart and the brain are, although immensely important, just two parts within this system. This relationship between the heart and brain highlights the potential for non-invasive interventions like TMS to work in concert with physiological processes to optimize brain functioning. The evolving understanding of heart-brain interactions and their impact on brain plasticity suggests that a more nuanced, integrated approach to

TMS—one that considers both intrinsic neural dynamics and broader bodily rhythms—may hold promise for enhancing therapeutic outcomes. Ultimately, while the optimization of TMS using HBC-TMS and the iAF for stratification has yet to be fully validated, these emerging insights point to the potential for a more adaptive, holistic approach that incorporates the brain's real-time oscillatory state and its interactions with other physiological systems. As technologies like (closed loop) EEG-TMS – allowing for brain-state dependent stimulation - continue to evolve, the field of non-invasive brain stimulation, including TMS, may similarly shift toward more personalized, responsive treatments that adjust dynamically to an individual's brain state and physiological needs.

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ENGLISH SUMMARY

Approximately 11% of the global population will experience depression at some point in their lives. Characterized by persistent sadness, loss of energy and interest, appetite and sleep disturbances, and in severe cases, suicidal thoughts, depression is a debilitating and potentially life-threatening disorder. Fortunately, effective treatment options, such as psychotherapy and antidepressants, exist with a success rate of 30-50%. However, this also means that up to 70% of treatments may fail, and after unsuccessful attempts with one or two treatments, the likelihood of success with future options significantly diminishes. This condition is referred to as difficult-to-treat depression (DTD), often resulting in chronic symptoms.

Early identification of the most effective treatment for each individual is therefore critical. Precision psychiatry - or personalized medicine - is emerging as a promising approach. It involves the use of biomarkers – biological characteristics such as genetic and brain activity profiles – to guide treatment decisions for specific biomarker subtypes. Despite its potential, current limitations, such as challenges in translating research findings into clinical practice, remain. Stratified psychiatry is a more pragmatic approach, also using biomarkers to individualize treatment selection. For this, patient subgroups that are more likely to respond to an effective treatment that is approved for their disorder are identified based on their biomarker profile, also considering predicted non-response to alternative treatments. Stratification options are always limited to evidence-based treatments only, herewith preventing the risk of doing harm by potential off-label prescriptions.

One proven effective treatment for DTD is transcranial magnetic stimulation (TMS), a non-invasive neuromodulation technique. TMS success rates are comparable to other antidepressant treatments (as described above), even for patients unresponsive to prior treatment. The dorsolateral prefrontal cortex (DLPFC) has been the primary brain region targeted in TMS for depression. When administering DLPFC-TMS, a TMS coil is placed over the DLPFC, inducing magnetic pulses that are sent through the skull to change neuronal activity

in the underlying brain area. To define the DLPFC at scalp level, a wide variety of methods is available – from manual scalp measures to E-field modeling using magnetic resonance imaging (MRI). In clinical practice, scalp measures like the 5CM and Beam method are the most commonly used as these are quick, easy, inexpensive and accessible methods. Furthermore, various stimulation protocols and pulse parameters, as well as stimulation intensities, are extensively studied. Even though there are clear differences, these methods all result in similar treatment outcomes on the group level. This suggests a heterogeneity within the treated population. Biomarkers could enhance treatment outcomes by identifying subgroups that respond best to specific TMS stimulation protocols and target sites.

One biomarker of interest for the treatment of TMS for depression is heart rate. Depression is characterized by a higher prevalence of cardiovascular disorders and a higher heart rate, lower heart rate variability, and higher blood pressure – suggestive of a dysregulated autonomic nervous system - as compared to non-affected individuals. The heartbeat evoked potential (HEP) – which reflects the brain's processing of heart signals – differs between those with depression and those without depression. Additionally, heart rate decelerations during DLPFC-TMS stimulation, which engages the frontal-vagal pathway, is another potential biomarker. Through activation of the frontal-vagal pathway, the vagal nerve – part of the parasympathetic nervous system and a direct connection between the brain and heart – is activated, resulting in almost immediate heart rate decelerations during TMS stimulation. Neuro-Cardiac Guided TMS (NCG-TMS) measures these decelerations, while Heart Brain Coupling TMS (HBC-TMS) – a novel method - assesses the heart rate's oscillation frequency during TMS stimulation. Both methods aim to identify the optimal stimulation site based on heart-brain connectivity, specifically targeting the subgenual anterior cingulate cortex (sgACC). DLPFC-sgACC connectivity has been related to treatment outcome, therefore the site with the highest HBC is hypothesized to be the best treatment site.

Another potential biomarker is the individual alpha peak frequency (iAF): an individual's most prominent brainwave frequency within

the alpha band (7-13 Hz), measured via electroencephalogram (EEG). The iAF has shown promise as a stratification tool for tailoring TMS protocols.

In **Chapter 2** we investigated the HEP as a potential stratification biomarker between TMS and various antidepressant medications (escitalopram, sertraline, and venlafaxine). The main finding was that remitters to venlafaxine treatment had a significantly lower HEP amplitude than non-remitters. Opposite effects were found for TMS treatment, suggesting the HEP is a potential stratification biomarker for choosing between treatment with TMS and venlafaxine.

Chapter 3 describes a validation study using NCG-TMS. We replicated heart rate deceleration findings during TMS stimulation in depressed patients, which were previously found in healthy controls. Similar topographies at group level were found, with strongest heart rate deceleration at F3/F4 and FC3/4, confirming the often-used 5CM (that roughly corresponds to FC3/4) and Beam-F3/4 sites in a data driven way. These effects are found to be site-specific with inter-individual-differences.

HBC-TMS is validated, replicated and extended in **Chapter 4**. We established a method to determine the frontal threshold (FT). The FT indicates activation of the frontal-vagal system, therefore indicating that the TMS pulse intensity is strong enough to enter the brain and influence neuronal firing. This threshold is significantly lower than the one found on the motor cortex – the motor threshold (MT). As treatment intensity is related to the MT – treatment stimulation intensity if commonly 120%MT – the found FT indicates that we might be overstimulating in treatment. The FT appeared reliable over time, and participants showing more HBC had more stable HBC-TMS outcomes (regarding which stimulation site showed highest HBC) compared to those with lower HBC values. Additionally, we confirmed that heart rate effects are not caused by the sensation of stimulation.

Chapter 5 describes the use of HBC-TMS to assess the effects of different TMS pulse parameters on the induced HBC. We measured whether the brain responds differently to various pulse lengths and

shapes (sine or square waves). Pulses were generated using two TMS devices that are both able to generate different pulse parameters: the cTMS and xTMS device. The study showed that shorter pulses are more efficient at inducing HBC than longer ones, with bigger differences for the DLPFC relative to the motor cortex. Pulse shape did not affect the capability of inducing HBC.

In the final chapter, **Chapter 6**, we applied EEG and HBC based stratification in clinical practice. Before TMS treatment, we determined each patient's iAF – and Brainmarker-I decile score - with EEG and performed an HBC assessment. Brainmarker-I was used to stratify patients to the laterality of the treatment protocol (e.g. right-DLPFC or left-DLPFC) – and HBC-TMS determined stratification to the anterior-to-posterior gradient (e.g. 5CM vs. Beam). The study showed that Brainmarker-I and HBC based stratification is feasible, low-cost and easy to implement in clinical practice. The iAF and HBC appear to be independent biomarkers: the iAF representing temporal and HBC representing spatial information. This suggests that combining both biomarkers may have synergistic effects. There were no treatment outcome differences for patients with or without HBC or a synchronization marker (iAF between 9.6-10.4Hz). This may be suggestive of successful stratification, since the group differences that were expected (based on prior research) without stratification appear to be straightened by our prospective stratification. Lastly, we found a relationship between delta power and HBC at F4, with patients with HBC showing a higher delta power compared to those without HBC.

CONCLUSION

This thesis has an overarching goal of improving antidepressant treatment, focusing on TMS for depression. Stratified psychiatry, using biomarkers, shows potential as a method of tailoring treatment indications to the individual and implementation in clinical practice appears feasible. The heart-brain connection provides valuable information to this end, and it can also be used to optimize treatment parameters and gain insight into underlying mechanisms in depression and antidepressant treatments. The iAF combined with HBC-TMS, although in need of further replication, are suggested to be effective

as a prospective stratification biomarker. Using biomarkers in stratified psychiatry, we are bridging research and real-world applications for immediate patient benefit.

NEDERLANDSE SAMENVATTING

Ongeveer 11% van de wereldbevolking krijgt te maken met een depressie. Depressie is een slopende en mogelijk levensbedreigende aandoening die zich uit in aanhoudende somberheid, verlies van energie en interesses, verstoringen in eetlust en slaap en in ernstige gevallen het hebben van suïcidale gedachten. Gelukkig zijn er effectieve behandelingen beschikbaar, zoals psychotherapie en antidepressiva, met een slagingspercentage van 30-50%. Dit betekent echter ook dat tot 70% van de behandelingen niet slaagt. Na één of twee onsuccesvolle behandelingen neemt de kans op succes met toekomstige behandelopties aanzienlijk af. Deze toestand staat bekend als moeilijk te behandelen depressie (difficult-to-treat depression; DTD), wat vaak resulteert in chronische symptomen.

Het is dus cruciaal om vroeg in het behandelproces de meest effectieve behandeling voor elk individu te bepalen. Precisiepsychiatrie lijkt een veelbelovende methode om dit voor elkaar te krijgen. Hierbij wordt gebruikgemaakt van biomarkers – biologische kenmerken zoals genetische profielen of hersenactiviteit – om te helpen beslissen welke therapie het beste past bij een individuele patiënt. Ondanks dat precisiepsychiatrie veelbelovend is, zijn er beperkingen, zoals de moeilijkheid om onderzoeksresultaten te vertalen naar de klinische praktijk. Gestratificeerde psychiatrie is een meer pragmatische aanpak die biomarkers gebruikt om subgroepen van patiënten te identificeren die waarschijnlijk beter reageren op een al goedgekeurde behandeling voor hun aandoening. Hierbij wordt ook rekening gehouden met een verminderde effectiviteit van andere behandelopties. Het maken van keuzes tussen wetenschappelijk bewezen behandelingen verkleint het risico op het maken van foute behandelkeuzes.

Een bewezen effectieve behandeling voor DTD is transcraniële magnetische stimulatie (TMS). Hierbij worden de hersenen vanaf de buitenkant van het hoofd gestimuleerd met magnetische pulsen. De succespercentages van TMS zijn vergelijkbaar met andere antidepressieve behandelingen, zelfs voor patiënten die eerder niet reageerden op behandeling. Bij depressie wordt meestal de dorsolaterale prefrontale cortex (DLPFC) gestimuleerd, een gebied aan

de voorkant van het hoofd. Tijdens de TMS behandeling wordt een magnetische spoel op dit gebied geplaatst, waarmee magnetische pulsen door de schedel worden gestuurd om de activiteit van neuronen (hersencellen) in dit gebied te beïnvloeden. Het idee is dat deze stimulatie, wanneer meerdere pulsen voor langere tijd achter elkaar worden gegeven (repetitive TMS; rTMS), de communicatie van hersennetwerken verbetert, waardoor depressieve symptomen verminderen. Een voorbeeld van zo'n stimulatieprotocol is het 10 Herz (Hz; 10 pulsen per seconde) protocol, waarbij 4 seconden wordt gestimuleerd op 10Hz gevolgd door 26 seconden rusttijd (inter-train interval; ITI), dit gaat voor zo'n 20-30 minuten door (afhankelijk van het aantal pulsen dat je wilt geven).

Er zijn verschillende methoden om de DLPFC op het hoofd te lokaliseren, van handmatige hoofdmetingen tot op MRI-gebaseerde 'E-field'-modellen. In de klinische praktijk worden meestal handmatige meetmethoden zoals de 5CM en Beam methode gebruikt omdat deze snel, gemakkelijk en goedkoop zijn. Verder wordt de effectiviteit van verschillende stimulatie protocollen, puls vormen en intensiteiten uitgebreid onderzocht. Hoewel de uitvoering van deze opties duidelijk verschilt, blijken deze methoden op groepsniveau te leiden tot vergelijkbare behandelresultaten. Dit wijst erop dat er verschillen zijn tussen individuen met een depressie in hoe ze reageren op de behandeling. Biomarkers kunnen helpen om subgroepen te identificeren die op specifieke TMS-protocollen en stimulatielocaties reageren en op andere niet, waardoor de behandeling sneller en beter kan werken.

Een belangrijke biomarker voor TMS-behandeling bij depressie is de hartslag. Depressie gaat vaak gepaard met een hogere hartslag, lagere hartslagvariabiliteit en hogere bloeddruk, wat kan wijzen op een verstoord autonoom zenuwstelsel. Het signaal dat de hersenen genereren door de hartslag – de heartbeat evoked potential (HEP) – verschilt bijvoorbeeld tussen mensen met en zonder depressie. Tijdens TMS-behandeling kan de hartslag vertragen door activering van het frontaal-vagale netwerk. Onderdeel van het frontaal-vagale netwerk is de nervus vagus, een zenuw die de hersenen direct met het hart verbindt. Deze hartslagvertragingen kunnen worden gemeten met

Neuro-Cardiac Guided TMS (NCG-TMS). Tijdens de pauzes tussen stimulatie (de ITI) normaliseert de hartslag weer. De frequentie van het op- en neer gaan van de hartslag tijdens een TMS protocol kan met Heart Brain Coupling TMS (HBC-TMS) worden gemeten. Deze methode is robuuster dan NCG-TMS omdat het invloeden van bijvoorbeeld de ademhaling uitsluit. Beide technieken helpen de optimale stimulatielocatie te bepalen, wat is gebaseerd op de verbinding tussen de DLPFC en een dieper gelegen hersengebied (de subgenuale anterieure cingulate cortex; sgACC) die ook onderdeel is van het frontaal-vagale netwerk. Een sterkere connectiviteit tussen deze gebieden (DLPFC-sgACC) is geassocieerd met betere TMS behandelresultaten.

Een andere potentiële biomarker is de individuele alfa piek frequentie (iAF). Dit is de meest dominante hersengolffrequentie in het alfabereik (7-13 hersengolven per seconde), gemeten met het electroencefalogram (EEG; een methode om de hersenactiviteit van mensen in kaart te brengen). Uit eerder onderzoek blijkt dat de iAF een stabiele biomarker is die kan helpen patiënten toe te wijzen aan verschillende TMS-protocollen.

In **Hoofdstuk 2** hebben we onderzocht of de HEP kan dienen als een biomarker om te bepalen of patiënten beter reageren op TMS of op antidepressiva (drie typen: escitalopram, sertraline en venlafaxine). De belangrijkste uitkomst van het onderzoek was dat patiënten die herstelden met venlafaxine een significant lagere HEP-amplitude hadden dan patiënten die niet herstelden. Voor TMS vonden we tegenovergestelde effecten, wat suggereert dat de HEP kan helpen bij het maken van een keuze tussen behandeling met TMS of venlafaxine: mensen met een lagere HEP kunnen worden toegewezen aan venlafaxine behandeling, terwijl degenen met een hogere HEP beter zullen reageren op een behandeling met TMS.

Hoofdstuk 3 beschrijft een validatiestudie van NCG-TMS. We bevestigden dat de hartslagvertragingen tijdens TMS stimulatie die eerder bij niet-depressieve mensen waren gevonden, ook aanwezig zijn bij depressieve patiënten. De sterkste hartslagvertragingen werden gemeten op de EEG-locaties F3/F4 en FC3/4. Deze locaties komen overeen met veelgebruikte TMS stimulatielocaties zoals de 5CM en

Beam locaties. De hartslageffecten zijn locatie-specifiek en verschillen per individu.

Hoofdstuk 4 repliceert, valideert en optimaliseert de methode HBC-TMS. We ontwikkelden een methode om de frontale drempel (frontal threshold, FT) te bepalen. Of de intensiteit van het TMS-apparaat hoog genoeg staat om de hersenen te bereiken wordt nu vaak bepaald op basis van de motor cortex (motor threshold; MT). De FT bleek lager te zijn dan de MT, dus onze uitkomsten suggereren dat de gebruikelijke intensiteit voor TMS-behandeling mogelijk te hoog is. Het gebruiken van de FT voor het bepalen van de behandelintensiteit, en dus een lagere behandelintensiteit, kan mogelijk bijdragen aan het verminderen van bijwerkingen. De FT bleek stabiel te zijn over tijd en patiënten met hogere HBC-waarden hadden meer stabiele HBC-TMS uitkomsten in vergelijking met patiënten met lagere HBC, wat suggereert dat de HBC-TMS methode robuust is.

In **Hoofdstuk 5** onderzochten we de effecten van verschillende TMS-puls vormen (vierkant of sinusgolf) en puls lengtes op HBC. Kortere pulsen bleken effectiever in het induceren van HBC dan langere pulsen. Deze verschillen waren groter op de DLPFC dan op de motor cortex. De vorm van de puls had geen invloed op het vermogen om HBC te induceren.

Hoofdstuk 6 beschrijft hoe we EEG- en HBC-gebaseerde stratificatie toepasten in de klinische praktijk. Voorafgaand aan de TMS-behandeling bepaalden we de iAF van elke patiënt en voerden we een HBC-meting uit. Patiënten werden toegewezen aan een TMS protocol (linker- of rechter DLPFC) en stimulatie locatie (5CM of Beam) op basis van hun iAF en HBC-resultaten. Zowel patiënten als behandelaren waren tevreden met de implementatie van de stratificatie methode en de toepassing in praktijk bleek goed te doen. We vonden dat de iAF en HBC onafhankelijke biomarkers zijn. In toekomstig onderzoek moeten beide biomarkers dus individueel beoordeeld worden voor hun effecten op het behandeleffect. Verder zijn er aanwijzingen dat het toevoegen van stratificatie kan leiden tot verbeterde behandelresultaten, maar dit moet nog verder onderzocht worden. Tot slot vonden we een verband tussen delta golf activiteit

en HBC op EEG-locatie F4: patiënten met HBC hadden meer delta power dan mensen zonder HBC.

CONCLUSIE:

Dit proefschrift richt zich op het verbeteren van antidepressieve behandelingen, met de focus op TMS voor depressie. Gestratificeerde psychiatrie, met behulp van biomarkers, heeft de potentie om de therapie, met bewezen behandelingen, te individualiseren en lijkt goed toepasbaar in de klinische praktijk. De hart-brein connectie biedt waardevolle inzichten en kan helpen om de TMS behandeling te optimaliseren. Hoewel verdere replicatie nodig is, lijkt de combinatie van iAF en HBC een effectieve biomarker voor het stratificeren van patiënten. Door biomarkers te gebruiken in gestratificeerde psychiatrie kunnen we de kloof tussen onderzoek en praktijk overbruggen, zodat patiënten direct profiteren van de nieuwste inzichten.

IMPACT OF RESEARCH

As outlined throughout this thesis, depression is a highly debilitating and, at times, life-threatening condition. Despite the availability of various treatment options, the overall efficacy at the group level often remains limited. This shortfall may be partially attributed to the heterogeneity of individuals with depression, which stems from the current classification system and a limited understanding of the underlying mechanisms of antidepressant treatments. To improve therapeutic outcomes, it is essential to tailor interventions to individual patients, enabling early remission in the treatment process. One promising approach to achieving this is through stratified psychiatry, requiring robust biomarkers that are both simple and cost-effective for use in clinical practice. Moreover, it is critical to bridge the gap between research and practice, ensuring that biomarkers tested retrospectively are also prospectively validated.

In this thesis, one potential stratification biomarker explored was the HEP, which reflects the brain's processing of heartbeat signals. The HEP was investigated for its ability to differentiate between responses to three antidepressants—escitalopram, sertraline, and venlafaxine—as well as TMS treatment. Crucially, the HEP predicted treatment outcomes differently for venlafaxine and TMS, supporting its role in stratifying patients after two failed first-line treatments (e.g., pharmacotherapy (with an SSRI) and psychotherapy). This stratification approach would allow clinicians to select either venlafaxine or TMS as the next step, moving away from current trial-and-error methods and facilitating more individualized treatment, with the aim of achieving earlier treatment response.

Importantly, we validated the presence of efferent heart-brain coupling effects—measured as heart rate decelerations during DLPFC-TMS stimulation—in patients with depression. This validation strengthens the bridge between research and clinical practice and proved relevance of the novel implementation of HBC-TMS for depression treatment protocols. By assessing heart rate oscillations instead of decelerations, the robustness of the method was improved. The original method was developed to compare target sites within a

patient. For research purposes we optimized this method so between subject analysis could be performed. This method also allowed for establishing a reliable frontal excitability threshold, which, after further validation and replication, could be used to determine optimal TMS stimulation intensity for individual patients, potentially reducing side effects.

To further illustrate how HBC-TMS can be used to optimize and individualize DLPFC-TMS treatment, we examined whether varying pulse parameters, such as shape and width, differentially affect induced HBC. Shorter pulses were found to induce greater HBC compared to longer pulses, with stronger effects observed in the DLPFC than in the motor cortex. These findings underscore the importance of differentiating between stimulation of the DLPFC and the motor cortex. Therefore, research conducted on the motor cortex should be validated in the context of DLPFC stimulation, and HBC-TMS, while still requiring validation and replication, shows promise as a simple and accessible method to gather this information.

In the final chapter - Chapter 6 -, we bridged the gap between research and clinical practice by assessing the feasibility and impact of implementing HBC-TMS. This was complemented by the robust EEG stratification biomarker, the iAF, for prospective stratification to TMS treatment protocols and stimulation sites. This study demonstrated the feasibility of prospective stratification using two cross-modal biomarkers, and the results suggest that stratification may be successful. Moreover, the study revealed that iAF and HBC are independent metrics, which is a valuable insight for future research and raises the question whether assessing HBC using alternative EEG metrics is possible. Delta power, in particular, emerges as a promising approach, with its temporal dynamics potentially providing further insights into the relationship between brain oscillations and vagal control. Most of all, it shows that HBC-TMS is a method that can be – and is already in two Dutch clinics – implemented into clinical care.

In conclusion, this thesis explored the crucial two-way connection in the human body - the heart-brain connection - as a potential

source of biomarkers for use in stratified psychiatry, with the goal of enhancing the effectiveness of TMS treatment for depression. The novel methods and research questions investigated here represent significant contributions to the ongoing effort to optimize TMS treatment. While further validation and replication are needed, the biomarkers identified in this work hold promise for future stratification approaches.


URBANSOLID
you can sleep when you are dead!
Revolucionário
EPH
Perdi as minhas Cartas de Amor Será que me vais Continuar a Amar?
Save the whales fuck the planet be the queen. (Coimbra, 2023)
Isto de escrever não Parece Se me ater a curar Ou destruir
~Rupp Kaur~
QUÍMICA FELICIDAD REFUGIO
5-12-22
ARIANEART

ABOUT THE AUTHOR

Lauren Zwienenberg was born on April 24, 1996, in Enschede, The Netherlands. After completing her secondary education at Het Assink Lyceum in Haaksbergen in 2014, she pursued her passion for psychology by enrolling at the University of Groningen. In 2017, Lauren earned her Bachelor's degree in Psychology.

Before starting her Master's program, Lauren took a gap year during which she conducted research at the University Medical Center in Groningen (UMCG) at the Clinical Genetics Department. There, she focused on studying how rapid exome sequencing—an additional genetic test during pregnancy to identify ultrasound abnormalities—affected parents' decision-making processes and their levels of anxiety. This experience deepened her interest in research, but she decided to pursue a Master's degree in Clinical Neuropsychology in 2018 to explore the clinical aspects of the field as well.

For her Master's degree, Lauren completed a research internship in the Clinical Neuropsychology and Developmental Psychology Department at the University of Groningen, under the supervision of Stefanie Enriquez-Geppert and Diede Smit. Over six months, she collected EEG data from individuals with subjective executive functioning problems. Her Master's thesis focused on identifying effective strategies during neurofeedback training. She graduated in August 2019 and continued working as a research assistant in the neurofeedback study. She also joined Yoki Mertens' research on the neurobiology of post-traumatic dissociation, assisting with MRI scans before and after TMS stimulation targeting the temporoparietal junction in healthy participants.

In January 2020, Lauren began her PhD journey at Synaeda - an outpatient clinic focusing on adult psychiatry - in collaboration with Brainclinics Foundation, an independent research institution. In 2021, she affiliated with Maastricht University. After two years of full-time researching, she started practicing as a psychologist – performing intakes, diagnostics and numerous treatments. Most notably, she specialized in the TMS treatment, becoming a certified TMS

technician and supervisor, performing HBC-TMS assessments and treatments.

Throughout her five-year PhD journey, she conducted - and underwent as part of training new colleagues - multiple EEGs and trained several new colleagues to become TMS practitioners.

Over the years, Lauren has presented her work at several national and international conferences and has published a number of her research findings. She hopes to continue contributing to the field with many more publications in the future.

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* contributed equally to this work as joint first authors

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POSTER PRESENTATIONS

Heart-Brain Coupling TMS: from Motor Threshold to Frontal Threshold & the Effects of Different Pulse Parameters. *BeNe brain stimulation symposium, 16 & 17-11-2023, Nijmegen, The Netherlands.*

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Op 10-10-2019 liep ik Synaeda binnen voor een sollicitatiegesprek voor een PhD over rTMS of EEG, want er stonden twee posities open. Daar zaten Nikita en Vera en na een goed gesprek kreeg ik uiteindelijk te horen dat ik voor een tweede gesprek naar Brainclinics in Nijmegen mocht gaan. Daar zaten Martijn en Hanneke op me te wachten. Ik verliet de boel met een knalrood hoofd van de spanning. Voordat ik de trein in Groningen weer uitstapte werd ik al gebeld: Nikita vertelde me dat ik was aangenomen op het rTMS project als ik de functie zag zitten. Het feest kon beginnen! En wat een avontuur is het geweest. De afgelopen 5 jaar heb ik heel erg veel gehad aan een heleboel mensen die langs zijn gekomen in mijn leven, waarvoor dank. Een paar (veel!) mensen wil ik graag even uitlichten:

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onderzoek, waar ik heel veel van geleerd heb. Naast het inhoudelijke werk ben ik je ook dankbaar dat je zo'n fijn team van onderzoekers vanuit het hele land bij elkaar hebt gebracht. Samen hebben we veel congressen bezocht, heerlijke etentjes gehad, goede feestjes op externe locaties, maar ook borrels op het dakterras en in de kelderbar gehad. Daarnaast vergeet ik nooit wat je me vroeg bij de sollicitatie: 'Dan ben je na een aantal jaar klaar met je PhD en dan weet je heel veel, maar dan kan je niks, en dan?'. Nou, dat gaan we nu uitvinden!

Na een proefperiode van 6 maanden was het tijd om een universiteit te zoeken om bij aan te sluiten. Dit werd – omdat het topografisch zo handig lag - de universiteit van Maastricht met **Alexander Sack** als promotor. Alex, over de jaren heen heb ik mega veel geleerd van jouw feedback en denkwijze en heb ik je leren kennen als ethisch, kritisch en meedenkend. Je was altijd goed bereikbaar en ik vond het erg fijn om jouw kennis mee te kunnen nemen in het hele proces. Ondanks dat ik niet veel bij de universiteit ben geweest, heb ik me altijd super welkom gevoeld. Heel erg bedankt voor alles en fijn dat de samenwerking tussen Synaeda en jullie onderzoeksteam voortgezet wordt!

Hanneke van Dijk (AKA Chanoeka), zowel als co-promotor, maar ook als collega heb ik zo ontzettend veel aan jou gehad. We hebben fysiek relatief weinig samengewerkt, maar de donderdagen die ik naar Nijmegen kwam en de reizen die we hebben gemaakt, waren altijd heel gezellig. Voor jou waren de donderdagen niet de meest productieve dagen, vanwege alle 'Hanneke ...?'-vragen, maar voor mij waren deze super leerzaam en helpend. Ik vond het heel waardevol dat ik mijn twijfels altijd bij jou kwijt kon en je daar uitgebreid de tijd voor nam om ze te bespreken. Jij leerde me niet alleen praktisch onderzoek doen, maar ook dat ik hierin mijn gevoel mag volgen. We blijven collega's, dus ik hoef je gelukkig nog niet te missen, en we zullen nog heel wat avonturen samen beleven.

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voor Synaeda gegeven verkleed als 'echte' onderzoekers (met labjas en al) en konden we in Nijmegen zelfs een studio delen waarbij eigenlijk de hele studio van glas was. Ik vind het heel fijn hoe jij mijn enthousiasme waardeert, maar hier ook een natuurlijke tegenhanger in bent en mij met beide benen op de grond houdt. Hoe jij het onderzoeksteam zoals het nu is hebt weten op te zetten naast al je andere taken (en het leven) vind ik bewonderenswaardig. Ik weet zeker dat we de komende jaren nog prachtig werk zullen neerzetten om zoveel mogelijk mensen te kunnen (blijven) helpen!

Op locatie **Fonteinland** heb ik vele uren mogen doorbrengen met een geweldig team. Ik zou met alle liefde alle namen noteren, maar over de jaren heen zijn het zó ontzettend veel (verschillende) collega's geweest, dat ik het niet ga proberen. Lieve collega's, als echte tukker in Friesland hebben jullie me met open armen ontvangen. Ik heb me altijd heel erg gewaardeerd gevoeld, ook al was mijn functie anders dan die van de rest en wist niemand nou eigenlijk echt goed hoe ik mijn tijd vulde. Ik heb heel veel gelachen, nog veel meer gekletst en ook een beetje gehuild. Ik voel me helemaal thuis bij jullie en verwacht dat we nog heel veel leuke momenten samen gaan beleven. Jullie hebben stuk voor stuk mijn PhD-tijd onvergetelijk gemaakt, waarvoor oneindig veel dank.

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De man die sowieso een superman badge verdient: **Mark Koppenberg**. Alle figuren, tabellen, de binnenkant én de buitenkant van dit boekje heeft hij mooi gemaakt! Mark, heel erg veel dank voor alle geduld die je had met de honderden versies van de figuren die ik je vroeg te maken. Jij zorgt ervoor dat onze resultaten begrijpelijk worden. Dat is onmisbaar. Natuurlijk ook dank voor alle leuke, hilarische momenten: op de Brainclinics borrel op het dakterras, maar misschien nóg wel meer in de groepsapp/mail waar de meest creatieve antwoorden altijd vanuit jou kwamen.

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