LISTEN TO YOUR HEART

LINKING HEART AND BRAIN FOR DEPRESSION

TABITHA A. ISEGER

The work described in this doctoral dissertation was carried out at Research Institute Brainclinics, in collaboration with Utrecht University. The support from Brainresource, neuroCare Group, and neuroConn is acknowledged.

Iseger, Tabitha Amanda
Listen to your heart
Linking heart and brain for depression

ISBN-978-90-830013-1-9
Cover and lay out: Brainclinics Insights, Nijmegen
Printed by GVO, Ede, The Netherlands

Published by Brainclinics Insights,
Nijmegen, The Netherlands
www.brainclinics.com

An electronic version of this dissertation is available at: brainclinics.com

LISTEN TO YOUR HEART

LINKING HEART AND BRAIN FOR DEPRESSION

LUISTER NAAR JE HART

VERBINDEN VAN HET HART EN HET BREIN VOOR DEPRESSIE

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus,
prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

woensdag 27 november 2019 des middags te 14.30 uur
door

Tabitha Amanda Iseger
geboren op 21 mei 1989
te Alphen aan den Rijn

Promotor:
Prof. dr. J. L. Kenemans

Copromotor:
Dr. M. W. Arns

"Follow your heart,
but take your brain with you"

Alfred Adler

TABLE OF CONTENTS

CHAPTER 1:

INTRODUCTION

This chapter is adapted from the following book chapter (submitted): "Repetitive Transcranial Magnetic Stimulation (rTMS) in Depression: Protocols, Mechanisms and New Developments", by Arns, M., Iseger, T. A., Spronk, D. B., Brown, T. & Fitzgerald, P. B.
and provides an introduction on rTMS for depression, the depression network, stimulation methods and target engagement.

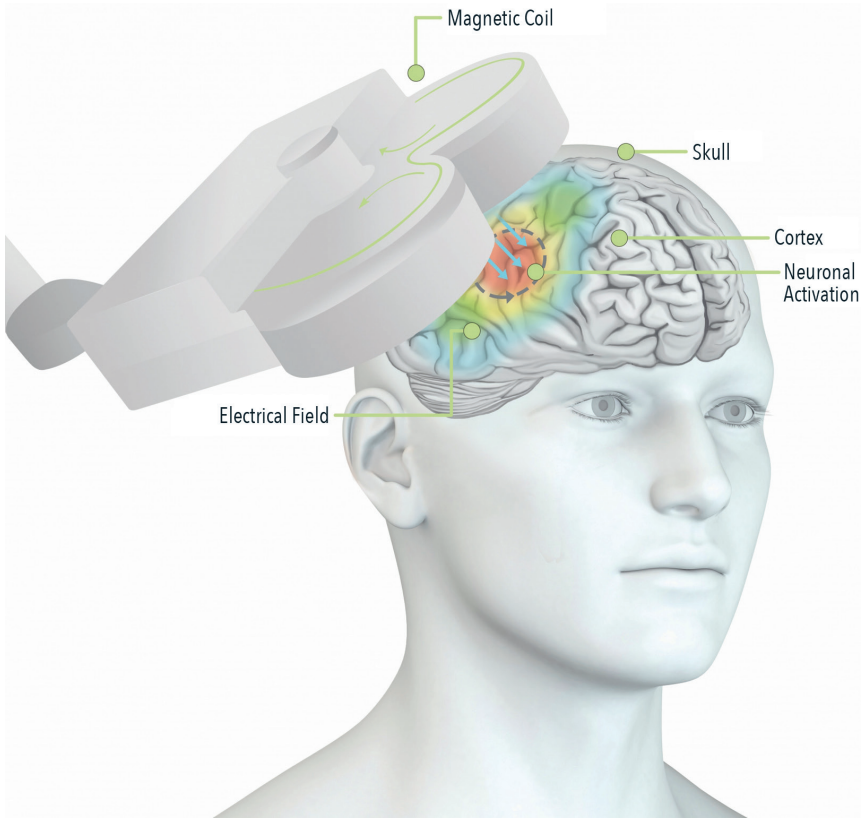


Figure 1: *Visual illustration of the induction of electrical currents in the brain (blue arrows in the brain) through the magnetic pulses applied by means of the coil (grey 8-shaped figure) positioned above the head. The colors on the scalp reflect the electrical field, i.e. where neural activation is most focused. Figure used with permission from neuroCare Group ©*

TMS (transcranial magnetic stimulation) is a non-invasive neuromodulation technique. It has a very direct influence on brain physiology. The basic principle of TMS is the application of short magnetic pulses over the scalp of a subject with the aim of inducing electrical currents in the neurons of the cortex. A typical TMS device consists of a stimulator that can generate a strong electrical current, and a coil in which the fluctuating electrical current generates magnetic pulses. If the magnetic pulses are delivered in the proximity of a conductive medium, e.g. the brain, a secondary current in the conductive material (e.g. neurons) is induced (Figure 1, facing page). In the practice of TMS, a subject is seated in a chair and an operator positions the coil above the scalp of the subject, and the TMS pulses are applied.

Anthony Barker and his colleagues at the University of Sheffield were the first to develop a TMS device, introducing a new neuromodulation technique in neuroscience. The new application, demonstrated first by these researchers, was the induction of a motor evoked potential (e.g. activating the muscles abducting the thumb) by means of applying a

TMS pulse over the primary motor cortex (Barker et al., 1985).
Initially, TMS was used mainly in studies on motor conductivity through investigating the temporal aspects and amplitude of the evoked motor responses after stimulating the motor cortex. Continuing progress on the technical aspects of TMS devices soon made it possible to deliver multiple pulses within a short time period, i.e. repetitive TMS (rTMS). With the development of rTMS, researchers were able to induce changes that outlasted the stimulation period (Pascual-Leone et al., 1999). This has led to a considerable extension of the possible applications of TMS. Currently, rTMS is used for an increasing variety of applications such as the study of pathophysiology of diseases, the investigation of the contribution of certain brain regions to particular cognitive functions and, most relevant for this chapter, the treatment of psychiatric diseases - most specifically, depression.

The potential of repetitive TMS in the treatment of psychiatric disorders was suggested for the first time relatively soon after the development of the first TMS device in 1985. In a study on motor conductivity, changes in mood in several healthy volunteers who received single pulses over the motor cortex were described (Bickford et al., 1987). Following this initial observation, the technical progress and the increasing availability of TMS devices has led to the further investigation of rTMS in the treatment of depression. Apart from being the first investigated psychiatric application, it is also the most well investigated psychiatric application. Major depression has a disabling effect on daily activity, indicating that effective treatment is crucial. Treatment with antidepressant medication is the most common and first line treatment for many individuals. However, a significant percentage of patients are treatment resistant (TRD) meaning that they do not sufficiently respond to antidepressant medication (Keller et al., 2000; Kirsch et al., 2008; Rush et al., 2006). Some of these patients proceed to electroconvulsive therapy (ECT). Despite remarkable clinical results (Husain et al., 2004), ECT is a controversial and unpopular treatment option due to the required induction of a seizure and associated side-effects such as memory complaints (Robertson and Pryor, 2006).
Following initial positive results with depression, and due to its painless and non-invasive administration, rTMS has been proposed as a

'better' alternative to ECT (Paus and Barrett, 2004) or as an alternative for patients who may not be willing to undergo ECT, or for whom ECT may not be suitable. In order to compare efficacy of these treatments, rTMS and ECT have been jointly investigated in several studies (Rosa et al., 2006; Eranti et al., 2007). Of the several studies performed Eranti et al. (Eranti et al., 2007) observed a great advantage for ECT. However, others (Pridmore et al., 2000; Grunhaus et al., 2003; Rosa et al., 2006) found comparable efficacy rates for ECT and rTMS in the treatment of depression. Furthermore, Eranti et al. (Eranti et al., 2007) included patients with psychotic depression whereas the other studies only involved non-psychotic depression (Pridmore et al., 2000), suggesting that rTMS may not be indicated for the treatment of depression with psychotic features. This notion was further supported by a meta-analysis from Berlim and colleagues (Berlim et al., 2013) comparing ECT and rTMS studies in the treatment of depression. They found an overall effect size of 1.42 favoring ECT, which dropped to a small effect size of 0.3 when studies that included psychotic depression were excluded.

The early reports of rTMS as an antidepressant treatment modality consisted of pilot studies with small numbers of subjects. In these early studies arbitrary stimulation parameters over various and non-specific brain regions were applied (Hoflich G, Kasper S, Hufnagel A, Ruhrmann S, Moller HJ 1993). A report by George and colleagues (George et al., 1995) showed robust improvements in depressive symptoms in two out of six patients. This study marked the start of the serious pursuit of rTMS as a potential treatment option for depressed patients. Subsequently, a reasonably large number of open label as well as randomized sham-controlled studies were performed. Most studies found a moderately favorable treatment effect for rTMS over sham using various designs (Padberg et al., 1999; Garcia-Toro et al, 2001; Rossini et al., 2005; Avery et al., 2006; O'Reardon et al., 2007; Mogg et al., 2008; Fitzgerald et al., 2003; Fitzgerald et al., 2006), which has been confirmed by several meta-analyses (d=0.39 and d=0.63) (Schutter, 2009; Schutter, 2010), two independent multicenter randomized controlled trials (O'Reardon et al., 2007; George et al., 2010) as well as the published rTMS guidelines (Lefaucheur et al., 2014).
After more than 20 years of research, rTMS is increasingly consid-

ered an acceptable intervention for treatment resistant depression (TRD). Most notably, the above-mentioned two independent multicenter randomized controlled studies (O'Reardon et al., 2007; George et al., 2010) and rTMS guidelines (Lefaucheur et al., 2014) have been instrumental in this process. rTMS is currently approved by the FDA in the United States for TRD, both for 'regular rTMS' as well as 'deep-rTMS' (discussed below). In addition, a growing number of outpatients as well as hospitalized depression patients are being treated in clinical settings worldwide. Repetitive TMS treatment for depression is now being reimbursed by insurance companies in more and more countries e.g. the US and the Netherlands.

In this chapter, a comprehensive overview of rTMS in the treatment of depression will be provided. In the first section various rTMS protocols will be reviewed in terms of the different stimulation parameters of interest. Subsequently, potential neural mechanisms associated with antidepressant outcomes will be reviewed. Finally, new developments in the field are addressed.

PROTOCOLS

The clinical and physiological effects of rTMS have been found to depend on the frequency, intensity and duration of stimulation (O'Reardon et al., 2007; Avery et al., 2006; Padberg et al., 2002; Fitzgerald et al., 2006). The most important distinguishing parameters for rTMS protocols in depression are the stimulation frequency and the stimulation location. These will be discussed at length by reviewing literature that used diverse choices for these parameters. Some other relevant parameters (intensity, number of trains, inter train interval and number of sessions) will be briefly described. In Figure 2 (facing page), some of the characteristics of an rTMS stimulation protocol are illustrated.

Progress in the development of technical aspects of TMS devices and advancing insights have led to a continuing progression of experimental and innovative protocols. Some more recently developed pro-

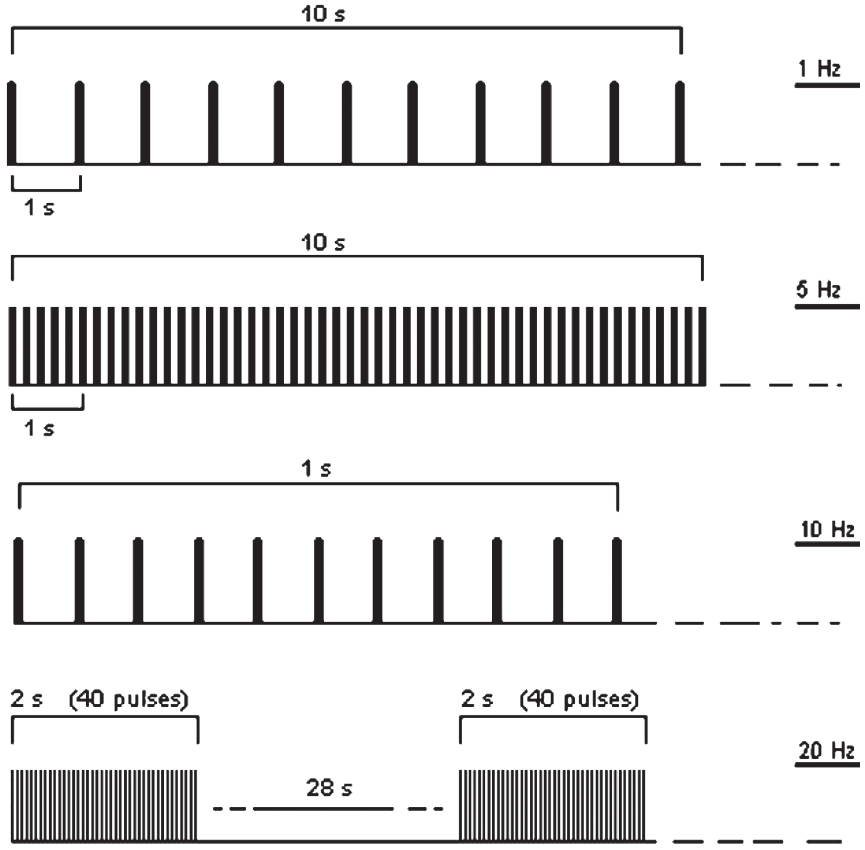


***Figure 2** : Examples of 10 s of rTMS at 1 Hz (first trace) and at 5 Hz (second trace); 1 s of rTMS at 10 Hz and an example of 20 Hz application (trains of 2 s interleaved by a pause of 28 s). Figure taken and adapted from Rossi et al. (Rossi et al., 2009).*

tocols investigated in the treatment of depression, such as theta burst stimulation and deep TMS stimulation, are discussed in the section 'new developments'.

STIMULATION FREQUENCY

The stimulation frequency refers to the number of pulses delivered per second, as can be programmed on the TMS device. Examination of stimulation frequencies in rTMS studies in depression reveals that, at first glance, two types of studies can be discerned: studies per-

forming high frequency (also referred to as fast) rTMS (HF-rTMS) and studies in which low frequency (also referred to as slow) rTMS (LF-rTMS) parameters are applied. HF-rTMS usually includes frequency parameters of 5Hz or above, whilst LF-rTMS incorporates stimulation frequencies of 1Hz or below. HF-rTMS is usually applied to stimulate the left dorsolateral prefrontal cortex (DLPFC), whilst LF-rTMS is mostly applied to inhibit the right dorsolateral prefrontal cortex (see 'stimulation location' for a more elaborate overview). In addition to studies applying solely HF-rTMS or LF-rTMS, combined approaches have also been proposed and investigated.

HIGH FREQUENCY RTMS

Most rTMS studies in depression have applied a left-hemisphere high-frequency stimulation protocol (O'Reardon et al., 2007; Avery et al., 2006). To date, HF-rTMS protocols have mostly used stimulation frequencies of 10 Hz (but this has varied from 5 to 20 Hz). The largest study (O'Reardon et al., 2007) reported significantly better clinical results in an active rTMS group in comparison to the sham group, as measured by the Hamilton Rating Scale for Depression (HAM-D) scale and the Montgomery Asberg Depression Rating Scale (MADRS). This was a randomized study in which 301 medication-free patients were treated with 10 Hz stimulation frequency. In a trial by George and colleagues (2010) these results were independently replicated in a sample of 190 patients. Apart from these large multi-center studies, numerous single site studies applying stimulation frequencies of 10 Hz have been performed. These have shown response rates (defined as more than 50% decrease on the depression scale) between 30-50% (Avery et al., 2006; Garcia-Toro et al., 2001; Mogg et al., 2008; O'Reardon et al., 2007; Padberg et al., 1999; Rossini et al., 2005; George et al., 2010). Most of these studies have been performed in treatment resistant patients. In a study by Fitzgerald and colleagues (Fitzgerald et al., 2006), patients who did not respond to a protocol with frequencies of 1 or 2 Hz (LF-rTMS) were assigned to either a 5Hz or 10Hz HF-rTMS protocol. No significant differences in response to a 5 or 10 Hz protocol were shown. Due to the limited number of studies no definitive conclusions can be drawn, but results suggest that in

general HF-rTMS (including 5, 17 or 20 Hz stimulation frequencies) do have an antidepressant effect. However, some reports have shown differential effects of various stimulation parameters, including a report of 9 Hz rTMS tending to be less beneficial than 10 Hz (Arns 2010). To summarize, the most optimal stimulation frequency is not yet known, but 10 Hz rTMS has been most frequently investigated and is therefore most commonly used in clinical settings.

LOW FREQUENCY RTMS.

In addition to the HF-rTMS studies, several LF-rTMS studies have been performed (Klein et al., 1999; Januel et al., 2006; Fitzgerald et al., 2003). For example, in a large sham-controlled study, Klein et al. (Klein et al., 1999) showed in 70 patients that 1 Hz rTMS showed a significantly larger improvement in depression scores compared to the sham group. In the largest controlled study on LF-rTMS in depression, 130 patients were initially assigned to a stimulation protocol of either 1 or 2 Hz (Fitzgerald et al., 2006). Of the 130 patients enrolled, approximately 51% could be classified as responders after 10 days of treatment. Although LF-rTMS is a more recently developed protocol and is less well studied, it appears to have beneficial effects comparable to those of HF-rTMS.
In order to systematically investigate if HF or LF-rTMS is more beneficial, protocols were directly compared (Isenberg et al., 2005). In a double-blind, randomized, sham-controlled study, 60 treatment resistant patients were divided into three groups; one received HF-rTMS trains to the left prefrontal cortex at 10 Hz, the second group received five LF-rTMS trains at 1 Hz to the right prefrontal cortex and the third group received sham treatment. The clinical results showed that the groups treated with HF-rTMS and LF-rTMS had a similar reduction in depressive symptoms and, for both groups, treatment response was better than within the sham group (Fitzgerald et al., 2003). In another study with a similar aim, 27 subjects were assigned to either HF-rTMS (10Hz) or LF-rTMS (1Hz) rTMS. It was concluded that both treatment modalities appeared to be equally efficacious (Fitzgerald et al., 2009), also supported by a large open-label study (Donse et al., 2018). Schutter (2010), based on a meta-analysis

of all randomized controlled LF-rTMS studies in depression, suggested that LF-rTMS might even be more beneficial than HF-rTMS. However, direct comparisons of the effect sizes of HF and LF-rTMS did not show a statistically significant difference due to overlapping confidence intervals. More research with larger samples is required to confirm these findings and demonstrate if LF-rTMS and HF-rTMS are similarly efficacious, or if LF-rTMS is more efficacious than HF-rTMS. Aside from the comparison of clinical effects, it appears that LF-rTMS is better tolerated, i.e., patients reported less headaches. It may also minimize the risk of inducing adverse events like seizures (Rossi et al., 2009).

Although the vast majority of studies has focused on low frequency stimulation applied to the right and high frequency stimulation applied to the left dorsolateral prefrontal cortex, it is to be noted that in a few studies other combinations were used. In a study with 219 depressed patients, no effects of laterality and frequency were found while all patients showed a reduction in their depression symptoms (Fitzgerald et al., 2010). This may implicate that low frequency stimulation applied to the left may also have antidepressant effects, thus questioning the traditional model of laterality and frequency in depression, with respect to the DLPFC.

COMBINED HF AND LF-RTMS PROTOCOLS.

These aforementioned studies provide evidence that active HF-rTMS and LF-rTMS are more effective in the treatment of depression compared to sham. However, HF-rTMS and LF-rTMS are not necessarily incompatible with each other. In recent years, add-on, bilateral, sequential and priming protocols have been postulated and investigated. Add-on protocols involve the combination of one protocol with another protocol e.g. when patients do not respond to LF-rTMS after several sessions, they can proceed to HF-rTMS treatment. In the aforementioned study by Fitzgerald et al. (Fitzgerald et al., 2006) in which LF-rTMS was investigated, non-responders to the low frequency protocol were subsequently treated with HF-rTMS. A subset of these LF-rTMS non-responders did respond to HF-rTMS. Hence, it is likely that different protocols act through different mechanisms

and that different patient groups are receptive to different approaches. It could also be argued that subjects in the add-on protocol received more sessions, and possibly needed longer to respond to treatment. Thus, the full extent of the increase in response rate might not solely be attributed to the change in stimulation frequency.
A second variant is the sequential stimulation protocol in which within one session both HF-rTMS and LF-rTMS protocols are applied. Overall no superiority has been found of sequential bilateral rTMS (Fitzgerald et al., 2006), except for Blumberger and colleagues (Blumberger et al., 2012) who found added value of this approach, albeit their unilateral HF rTMS did not differ from sham. Finally, Pallanti et al. (2010) found that a 'simple' unilateral protocol was more effective compared to sequential bilateral rTMS (Pallanti et al., 2010). Nevertheless, it remains relevant to further explore combination protocols and compare them to traditional unilateral protocols, also looking at this option as an escalation option to increase the 'dose', i.e. when patients do not respond well enough in 10-15 sessions unilateral rTMS. Since number of stimuli were kept the same at 420 stimuli for both unilateral and bilateral protocols (Pallanti et al., 2010), adding the contralateral hemisphere and thereby doubling the number of pulses, could still have added value by increasing number of pulses, which has in our experience been found to sometimes provide benefit (unpublished observation).

REAL-LIFE OUTCOMES AND DURABILITY

Several large open label studies have addressed the long-term effects of rTMS. In a large multicenter study with 307 treatment resistant MDD patients (failure of on average 2.5 anti-depressant treatments of adequate dose and without satisfactory benefit), Carpenter and colleagues (2012) reported clinician-assessed response rates (CGI-S) of 58.0% and remission of 37.1%. Patient-reported response rate ranged from 56.4 to 41.5% and remission rate ranged from 28.7 to 26.5%. The authors note that these outcomes demonstrated response and adherence rates similar to research populations (Carpenter et al., 2012). Another large open label study in 1132 patients demonstrated similar effects to the Carpenter et al. (2012) study with 46% response and

31% remission rates (Fitzgerald et al., 2016). In an extension of the Carpenter et al. (2012) study, good long-term effects were observed (Dunner et al., 2014). The majority of the patients (62.5 %) continued to meet response criteria at a 12 month follow-up. Similar results were reported by Donse and colleagues (2018) who conducted a combined rTMS and psychotherapy study while also investigating long term effects in 196 patients with TRD. Combining rTMS and psychotherapy resulted in a 66% response and a 56% remission rate at the end of treatment with 60% sustained remission at a six-month follow-up visit. The authors noted that, interestingly, early symptom improvement (at session 10) was highly predictive of response, and may therefore be used to guide rTMS plus psychotherapy continuation, or escalation to sequential HF and LF rTMS (Donse et al., 2018). As such, it seems that within a naturalistic setting, rTMS can be considered an effective treatment similar to that found in a research setting. Additionally, in combination with psychotherapy, response and remission rates may have the potential to increase further and sustain durable effects.

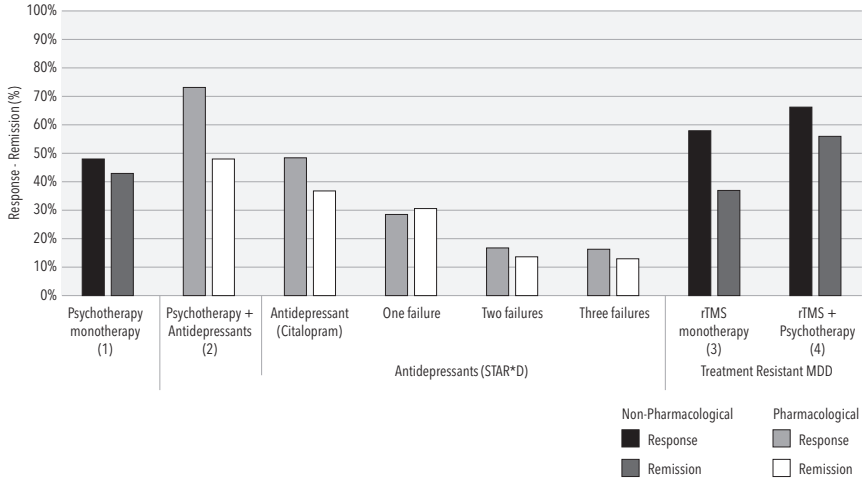


Figure 3 *Response and remission rates of various antidepressant treatments. From left to right: Psychotherapy monotherapy (Cuijpers et al., 2014), Psychotherapy and antidepressants (Keller et al., 2000), Antidepressants as first line, after one, two and three treatment failures from the STAR*D trial (Rush et al., 2006), rTMS monotherapy (Carpenter et al., 2012) and rTMS combined with psychotherapy (Donse et al., 2018). Note the relative increase in response and remission rates for rTMS, especially for patients that have had two or three prior treatment failures, which is the typical population rTMS treatment focusses on (TRD).*

As can be seen in figure 3, a treatment resistant population respond to rTMS alone (monotherapy) similarly to an anti-depressant medication (monotherapy) in populations receiving medication or psychotherapy as a first line treatment and, when combined with psychotherapy, rTMS patients respond similarly to a combined medication/psychotherapy approach. These findings suggest a considerable promise for this hard to treat population following many prior failed attempts at depressive symptom reduction.

STIMULATION LOCATION

The dorsolateral prefrontal cortex (DLPFC) has been the primary area of interest for stimulation (see Figure 4 on page 22). The motivation behind choosing this brain area stems from various imaging studies that indicated depression is associated with regional brain dysfunction in, among other regions, the DLPFC (Cummings 1993). Other researchers have not only proposed an 'underactivated' L-DLPFC, but suggested an imbalance between frontal regions. For example, the 'frontal asymmetry hypothesis' of depression states that in depression there is an imbalance in left vs. right frontal brain activation (Henriques and Davidson, 1990). Although a recent meta-analyses failed to demonstrate a difference in frontal alpha asymmetry between depressed and non-depressed groups on the group level (van der Vinne et al., 2017), the DLPFC is still the most commonly used stimulation location in rTMS protocols for depression. In addition, of all brain regions known to be related to the pathophysiology of depression (e.g., prefrontal, cingulate, orbitofrontal and parietal cortical regions) the DLPFC is regarded as most accessible for treatment with rTMS (Wassermann and Lisanby, 2001). On the basis of such previous theories and findings, the supposedly 'activating'/ HF-rTMS protocols are applied over the left DLPFC and supposedly 'inhibiting'/LF-rTMS protocols are applied over the right DLPFC. The choice of the stimulation frequency is thus closely linked to the stimulation location.

Historically, most studies localizing the DLPFC have been performed by means of the '5cm rule'. The hand area of the primary motor cor-

Figure 4: *Image of the location of the (left) Dorsolateral Prefrontal Cortex in the brain (Figure used with permission from Research Institute Brainclinics ©)*

tex (M1) (which elicits a contralateral motor response of the thumb when stimulated), is taken as the detectable reference point. From there, the coil is moved 5 cm anteriorly. Positioning the coil at that location during treatment is assumed to target the DLPFC. It can be argued that this literal "rule of thumb" does not take individual differences into account and is used less frequently these days. Recently, the Beam-F3 / F4 method has been proposed as a new method (Beam et al., 2009) which does take individual differences in skull size into account and is based on the 10-20 EEG location F3 or F4. Free software to easily apply this method can be found at: http://www.clinicalresearcher.org/software.htm. This method has been shown to lead to an adequate determination, with a minimal discrepancy, compared to MRI neuro - navigated location determination (Mir-Moghtadaei et al., 2015).

For MRI neuronavigation, a technique in which an individual MRI

scan is used to determine the exact location of the DLPFC, a single study has found an advantage regarding clinical effectiveness in the treatment of TRD compared to the 5 cm rule (Fitzgerald et al., 2009). However, results from the multi-center OPT-TMS trial in which an individual adjustment could be made based on an individual MRI scan, showed no stronger clinical effect of this MRI-based adaptation (Johnson et al., 2013) and there are no studies that compared the effectiveness of MRI-navigated rTMS with the Beam-F3 / F4 method.
Despite the fact that the majority of the studies target the DLPFC, some authors have argued that it has never been experimentally proven that the DLPFC is the most effective target for rTMS treatment of depression. In addition, the pathophysiology of depression is certainly not limited to the DLPFC (Drevets et al., 2008). Therefore, several other stimulation targets have also been investigated in the treatment of depression. Clinical efficacy in TRD has also been reported for stimulation of the Dorsomedial Prefrontal cortex or mediofrontal cortex using a double-cone coil (a figure-of-eight coil where the cones are presented in an angle of 90 degrees of one another) (Downar et al., 2014; Kreuzer et al., 2015), the right orbitofrontal cortex using a double cone coil (Feffer et al., 2018) and (albeit limited) for the right parietal cortex (Schutter, 2009). Although these findings need to be replicated in larger studies, they are encouraging regarding searching for other cortical targets in the treatment of depression with rTMS outside the DLPFC.

STIMULATION INTENSITY, TRAINS, SESSIONS AND SAFETY

For rTMS to be effective, the magnetic field has to induce currents in the neurons of the cortex. The intensity of the magnetic field that induces this current is referred to as the stimulation intensity. This is usually expressed as a percentage of the motor threshold (MT). The MT is usually determined prior to each session by applying the TMS coil over the 'thumb' area of the motor cortex. Single pulses are applied with stepwise variation of the output intensity of the device. The minimal output intensity which yields a motor response (moving of the thumb) in at least half of the applied trials is determined to be the MT. So if the intensity of a TMS protocol is 100% MT, then it is

the same as the output intensity of the device which was determined to be MT. All other intensity values are reported as a percentage of this MT, e.g. if the MT is at an output intensity of the device of 60%, then an intensity of 110% MT means that the output intensity is 66%. Although this determination of stimulation intensity may seem arbitrary, it takes individual differences in motor cortex excitability (and presumably therefore differences in excitability of other brain regions) into account. This contributes to a safer administration of TMS pulses to an individual. In depression protocols reported to date, the lowest stimulation intensity used was 80% MT (George et al., 1995) and the maximal intensity used was 120% MT (O'Reardon et al., 2007; Rumi et al., 2005). The majority of the depression protocols to date use stimulation intensities of 110%-120% MT.
In most rTMS protocols the stimulation is delivered in pulse trains (see Figure 2). That is, pulses are delivered in trains and are separated by certain time intervals: the inter train interval (ITI). This is done for two reasons. First, the effect of TMS pulses is cumulative in the brain (Rossi and Rossini, 2004; Ridding and Rothwell, 2007; Hallett 2007), and this summation causes an increase of the likelihood of the induction of a seizure. This possible side effect of rTMS - the occurrence of an epileptic seizure – is minimized when adhering to the published safety guidelines (Wassermann 1998; Rossi et al., 2009) that have been compiled based on reported incidents. These guidelines concern stimulation parameters (such as frequency of stimulation (Hz), intensity of stimulation (as a percentage of the 'motor threshold' (MT), and duration of a stimulation train), as well as other risk factors which should be taken into account (such as sleep deprivation, acute withdrawal of benzodiazepines etc.). Since the publication safety guidelines (Rossi et al., 2009; Wassermann 1998), only a few incidents of epileptic seizures have been reported. Currently, the risk of a seizure due to rTMS is worldwide 0.007% of treatments or research sessions. In the US, under clinical use, the risk is estimated at 0.003% of treatments (or 0,1% of patients). This means that the risk of a seizure is comparable or lower than with antidepressants (George et al., 2013).

Secondly, the repetitive release of strong electrical pulses causes heating of the electronics of the TMS device. The ITI between trains allows the device to partially cool down. For safety reasons for the

subject and protection of the device, all devices are manufactured to automatically turn off as soon as a certain heat-limit has been reached. Newer TMS devices are designed with better cooling systems (e.g. air or fluid cooled coils), which reduce the likelihood of overheating. However, the overheating of the device is still possible when multiple sessions are performed within a short period, or if a highly demanding (e.g. high rate of pulse delivery) protocol is performed. Train durations in HF-rTMS protocols are usually between 2 and 10 seconds with an ITI between 20-60 seconds. In LF-rTMS protocols often continuous stimulation is used.

In studies performed thus far, the number of sessions applied has been highly variable, ranging from 5 sessions (Miniussi et al., 2005; Manes et al., 2001) to up to or greater than 30 sessions (Fitzgerald et al., 2006; O'Reardon et al., 2007). Based on more recent studies, a general trend towards a greater number of sessions (>10) is associated with continuing improvement in depression scores. Schutter (2009) suggested that similar to antidepressant medication, rTMS treatment may involve a delayed therapeutic onset. Investigation of the number of sessions optimally required is important for gaining information about the temporal course of the antidepressant effect.

Over the years of rTMS research efficacy has increased in line with increased dosage, which can be expressed as number of pulses applied during a treatment course. Initially, George and colleagues (1995), applied a total of only 5000 pulses. This was doubled in 1996 by Pasqual-Leone and colleagues, escalating to 10 000 pulses. By 2007, O'Reardon and colleagues had increased to 90 000 pulses, which continued to be well tolerated (4.5% dropout), while efficacy was still rising.

In this section it was discussed how manipulations of intensity, frequency and total number of pulses have reportedly affected effectiveness. However, there is room for improvement and studies directly addressing the question of optimal stimulation parameters are urgently required. Furthermore, increasing knowledge about the mechanisms underlying treatment efficacy may result in new protocols with more optimal treatment effects. Thus, the next section presents a discussion of brain mechanisms underlying depression and the possible effects of TMS, which may inform a more rational approach to treatment optimization.

MECHANISMS OF RTMS TREATMENT IN DEPRESSION

Levels of evidence for the treatment of depression via rTMS have accumulated to a point where HF-rTMS on left-DLPFC has received Level A status ('definite efficacy', Lefaucheur et al. 2014). Only HF-rTMS of M1 contralateral to pain side for the treatment of neuropathic pain has also achieved this status. Level B status ('probable efficacy') has been achieved for treatment of depression using LF-rTMS on right-DLPFC and is 'probably' additive to anti-depressant medication. Level B evidence has also been achieved for LF-rTMS of M1 contralateral to pain side; antidepressant effect of HF-rTMS of the left DLPFC in PD patients; LF-rTMS of the contra-lesional motor cortex in chronic motor stroke; and, HF-rTMS of the left DLPFC for negative symptoms of schizophrenia (Lefaucheur et al., 2014). Despite these evidence levels, the neural mechanisms underlying brain modulation via rTMS remain elusive.

DEPRESSION NETWORK

In contrast to earlier beliefs, it is now generally accepted that different brain areas wire together, resulting in a 'network' of brain areas (called 'hubs') that are together involved in different processes or disorders. Thus, there is a 'depression network', with several hubs now identified, especially since the rise of neuromodulation treatments for depression such as rTMS and Deep Brain Stimulation (DBS) (Liston et al., 2014; Fox et al., 2012). These new treatments directly targeted DLPFC (George et al., 2010; O'Reardon et al., 2007), the dorsomedial prefrontal cortex (DMPFC) (Downar and Daskalakis, 2013; Downar et al., 2014) and the subgenual cingulate cortex (sgACC) (Mayberg et al., 2005) and have shown that direct stimulation of these regions is associated with clinical improvement. Recent insights into how these neuromodulation treatments work suggest network connectivity changes within a DLPFC-DMPFC-ACC network to mediate antidepressant response (Liston et al., 2014; Fox et al., 2012), and these changes in connectivity are also possibly implicated in pharmacological treatments. Moreover, new targets are still investigated, such as the orbitofrontal cortex (Frodl et al., 2010; Feffer et al., 2018).
The sgACC and sections of the DMPFC are components of the default

mode network while the DLPFC is partly implicated in the central executive network (CEN). A deficit in switching between the DMN and CEN is well documented in depression (Sridharan et al., 2008; Liston et al., 2014) and is considered to be one of the main reasons behind cognitive dysfunction in depression. The DLPFC has been described to be hypoactive in depression (Korgaonkar et al., 2012), and an increase in fMRI activity of this structure is associated with treatment response (Koenigs and Grafman, 2009; Fitzgerald et al., 2006). Contrary to the DLPFC, the sgACC has been described to be hyperactive in depression, along with hyperconnectivity with other parts of the DMN observed with PET scans and with fMRI (Mayberg et al., 2005; Liston et al., 2014), and a decrease in activity of the sgACC is associated with antidepressant response (Mayberg et al., 2005; Koenigs and Grafman, 2009). In addition, decreased grey matter volume in MDD has been reported in the left anterior cingulate cortex, DLPFC and DMPFC (Drevets et al., 1997; Grieve et al., 2013). The DMPFC, or dorsal nexus, is a core region to multiple networks, including the DMN, CEN and salience network (SN), with increased fMRI connectivity to all three networks in depression (Sheline et al., 2010). The DMPFC has been observed to be abnormally activated during positive and negative affect processing in MDD, which normalizes after successful treatment (Dunlop et al., 2015; Bermpohl et al., 2009; Mayberg et al., 1999).

As the direct effect of rTMS is limited to cortical surfaces, it is hypothesized that DLPFC-rTMS (and DMPFC-rTMS) might exert its antidepressant effect via trans-synaptic connectivity to deeper regions, such as the sgACC (George et al., 1995; George et al., 1997; Padberg and George, 2009; Fox et al., 2012; Fox et al., 2014). Fox et. al., (2012) demonstrated a negative correlation in BOLD activity between the sgACC and the DLPFC which is hypothesized to be associated to the antidepressant mechanism of rTMS (Fox et al., 2012). The higher the negative correlation in activity between the two areas was (at baseline), the better a patient responded to rTMS (Fox et al., 2012). In addition, DBS in the sgACC which suppresses activity, results in an up regulation of the activity in the DLPFC (Mayberg et al., 2005). Thus, there is an intricate interplay between the sgACC and DLPFC, and this interplay is related to MDD symptoms (improvement). This interplay has also been investigated during antidepressant treatment,

and it was shown that electroencephalogram (EEG) linear-lagged connectivity between the DLPFC and the sgACC changed for responders during treatment, although this was only observed in males (Iseger et al., 2017; Chapter 2). Multiple studies have described a normalization of the existing hyper-connectivity (Mayberg et al., 2005; Liston et al., 2014; Koenigs and Grafman, 2009) after treatment. However, some studies still report differences in ACC or DLPFC connectivity after treatment, compared to healthy controls. Therefore it was hypothesized that connectivity tends to normalize or resemble the connectivity pattern of healthy controls, but could still be incomplete (Liston et al., 2014).

These hubs in depression are linked to the limbic system, such as the amygdala and hippocampus but there are divergent findings as to whether volume reductions associated with depression are present in these structures (Grieve et al., 2013). When investigating white matter trajectories, significant white matter fractional anisotropy deficits were found in regions associated with the limbic system, but only for the melancholic subtype of depression (Korgaonkar et al., 2010). Moreover, it is believed that this depression network is connected to the heart. This is not surprising because MDD has been strongly associated with a higher risk for cardiovascular disease (Glassman, 2007; (Lett et al., 2004); Penninx et al., 2001). Several studies have shown that depression increases the risk for cardiovascular illness from two to fivefold (Horrobin & Bennett, 1999). Moreover, autonomic regulation is already disturbed in depressed patients without heart disease, manifested in an overall higher HR, and lower heart HRV in comparison to healthy controls (Ehrenthal et al., 2010; Udupa et al., 2007; Licht et al., 2008), and is more pronounced in patients with severe MDD (Stein et al., 2000).

The connection to the heart is mediated by the vagus nerve, the 10th cranial nerve. The vagus nerve is part of the parasympathetic nervous system and, besides regulating heart rate, is also involved in other autonomic functioning such as blood pressure, inflammation and gastrointestinal function (Cheung et al., 2019). Of these, gastrointestinal and heart function have been studied most extensively with regards to depression. Heart rate consistently decreases when the vagus nerve is stimulated (Lang and Levy, 1989; Buschman et al., 2006), consistent with the parasympathetic action of the vagus nerve. Strong

positive correlations were found between cardiac vagal control and the BOLD signal intensity in the sgACC of healthy controls but not in depressed subjects (Lane et al., 2013). Moreover, RR intervals (intervals between ventricular depolarizations) were positively related with VMPFC activity in healthy participants during rest (Ziegler et al., 2009) and patients with ACC damage all showed abnormalities in cardiovascular arousal during cognitive tasks, indicating a blunted autonomic arousal response during stressful situations (Critchley et al., 2003). Few studies have been conducted investigating the effect of neuromodulation on the VMPFC in relation to heart rate, however one older study on monkeys showed electrical stimulation in the pregenual ACC associated with cardiac slowing (Dua and Maclean, 1964). The same associations with heart rate control have been found for the DLPFC. A recent meta-analysis demonstrated that both tDCS and rTMS aimed at the DLPFC resulted in reduced heart rate, although rTMS was more effective than tDCS (Makovac et al., 2016). These effects were observed both after left and right hemispheric stimulation, although some studies do observe larger heart rate reductions after right DLPFC stimulation.

In summary, the core of the depression network involves the DLPFC and the sgACC, and these regions are interconnected with the limbic system and the heart-brain axis, providing new insights into the pathophysiology of MDD that can be utilized to further optimize treatments such as rTMS, as is explained in more detail below under target engagement regarding Neuro-Cardiac-Guided TMS (NCG TMS).

NEW DEVELOPMENTS

As a new and dynamic field, rTMS is the topic of considerable research and innovative developments are numerous. These developments are of a diverse nature, including technological progress in equipment and software, protocol innovations and optimizations, and advances in the understanding of long-term effects. Some examples are the investigation of theta burst stimulation applicability (the delivery of bursts of 50 Hz pulses usually at a rate of 5 Hz) and new equipment such as the H-coil for deeper brain stimulation.

In addition to the 'traditional' LF and HF frequency studies, a newly developed theta-burst stimulation (TBS) protocol has been proposed; referred to as 'patterned TMS'. This has been put forward as a technique that could have important implications for the treatment of conditions such as epilepsy, depression and Parkinson's Disease (Paulus 2005). TBS usually involves short bursts of 50 Hz rTMS applied at a rate of 5Hz (hence the name theta burst stimulation). In fact there are two frequencies within one train of stimuli; the intra burst frequency of 50 Hz (e.g. 3 pulses at a rate of 50 Hz) and the frequency of delivery of the number of bursts within one second which is at a rate of 5 per second (5 Hz). TBS can be applied as either a continuous (cTBS), or intermittent (iTBS) train (Huang et al., 2005). See Figure 5 for an illustration of both types of TBS protocols.

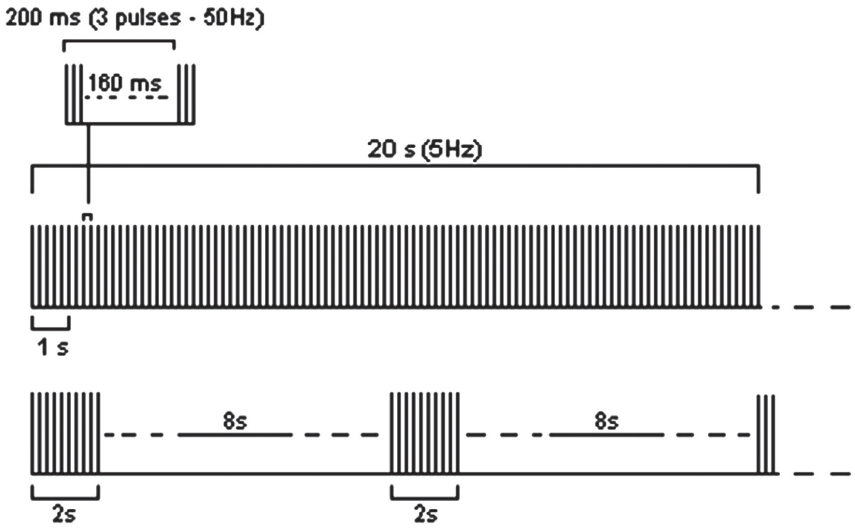


Figure 5: *examples of the two most common TBS protocols: continuous TBS (first trace) and intermittent TBS (second trace). Figure taken and adapted from Rossi et al. (2009).*

Previously, the rapid delivery of pulses required in a TBS protocol was not possible due to technical limitation of older stimulators. TBS has therefore only been investigated since 2005. In the years after its introduction, it has been shown that TBS induces changes in cortical excitability that may last longer than with traditional TMS protocols (Huang et al., 2009; Ishikawa et al., 2007). In regard to the observa-

tion of the more sustained effect, Chistyakov and colleagues (2010) suggested that TBS might be more effective than traditional HF and LF-rTMS in the treatment of depression. However, recent work has demonstrated that TBS protocols seem to be equally effective in the treatment of depression, but may be more efficient as the session length is shorter (Bakker et al., 2014).

TECHNICAL PROGRESS

In order to further improve efficacy of rTMS protocols, a new type of coil 'the H-coil' (Brainsway) was developed. The traditionally used coils (figure of eight/circular coils) are thought to penetrate underlying brain tissue only to a depth of 1.5-2cm (Zangen et al., 2005). H-coils, on the other hand, are capable of stimulating deeper brain regions of 4-6 cm (Zangen et al., 2005), albeit others have argued this coil results in more non-focal, but not deeper stimulation (Fadini et al., 2009).
In 2013 the U.S. Food and Drug Administration (FDA) approved the Deep TMS device (using the H-coil) for the treatment of depression in patients who had failed to respond to antidepressant medications in their current episode of depression. The study that resulted in this FDA approval was published in 2015 by Levkovitz and colleagues (Levkovitz et al., 2015) who conducted a double-blind randomized controlled multicenter study evaluating the efficacy and safety of dTMS in MDD. 212 MDD outpatients, aged 22–68 years, who had either failed one to four antidepressant trials or not tolerated at least two antidepressant treatments during the current episode were randomly assigned to active or sham dTMS. Twenty sessions of dTMS (18 Hz over the left prefrontal cortex) were applied during 4 weeks, and then bi-weekly for 12 weeks. Response and remission rates were higher in the dTMS than in the sham group (HDRS - response: 38.4 vs. 21.4%; remission: 32.6 vs. 14.6%). These differences between active and sham treatment were stable during the 12-week maintenance phase.
For the time being, randomized head-to-head comparisons of effectiveness on reduction of depressive symptoms between the H-coil and regular figure-of-eight coils have not been published yet. However, the H-coil is a prime example of further technological advancements which may lead to increased efficacy in time.

OPTIMIZING TREATMENT

A better understanding of the neurophysiological and clinical features of depressed patients who respond to rTMS, together with more clarity on the neurobiological mechanisms of the induced effect of rTMS treatment in depression as described above in depression network, will contribute to the development of more effective forms of rTMS. The identification of patients' characteristics (clinical, physiological or parametric variables) is referred to as personalized medicine. Demographic and clinical features associated with less favorable treatment outcome are older age (Fregni et al., 2006; Brakemeier et al., 2007) and higher therapy resistance (Fregni et al., 2006; Brakemeier et al., 2007; Brakemeier et al., 2008). However, this has not been confirmed by all studies in this area (Fitzgerald et al., 2006). A recent systematic review on predictors for rTMS response concluded that due to methodological variability generalizability of results was limited, but some indications were found for baseline frontal lobe blood flow and presence of some polymorphisms (5-hydroxytryptamine-1a gene, the LL genotype of the serotonin transporter linked polymorphic region (5-HTTLPR) gene, and Val/Val homozygotes of the brain-derived neurotrophic factor (BDNF)) (Silverstein et al., 2015). While EEG measures have been relatively well studied in the prediction of treatment response to antidepressant medication (Cook et al., 1999; Bruder et al., 2008; Spronk et al., 2011), their potential in predicting response to rTMS treatment is generally considered limited but promising, albeit there is a large need for replication (Arns et al., 2012; Krepel et al., 2018). Thus, current predictors are still rather limited and require further replication (in larger samples) before their use in clinical practice can be recommended.

TARGET ENGAGEMENT

Target engagement comprises the use of a direct functional outcome measure as a validation for targeting the correct TMS location, through which it can be demonstrated that the right area is targeted, either directly or trans-synaptically. As said, the motor cortex is identified by thumb movement as a demonstration of primary motor cortex activation, such functional outcome measures are thus far lack-

ing for the prefrontal cortex and DLPFC. One proposed method is by extracting connectivity patterns to frontal areas using the sgACC as a seed region (Fox et al., 2013; Fox et al., 2012), as discussed in 'the depression network'. Other studies hypothesize that the DLPFC could be more accurately targeted with the aid of heart rate, so called Neuro-Cardiac-Guided TMS (NCG-TMS) (Iseger et al., 2017; Chapter 4). As mentioned earlier, the depression network and the brain-heart axis are interconnected and stimulation of the DLPFC was shown to reduce heart rate (Makovac et al., 2016). The parasympathetic effect on the heart is fast and short-lived; stimulation of the vagus nerve usually results in an immediate response of the heart, typically occurring within the cardiac cycle in which the stimulation occurred, with a peak in heart rate deceleration within 5 seconds (Buschman et al., 2006). The return to a normal HR is very quick after the activity of the vagus nerve is reduced (Shaffer et al., 2014). In a recent study, it was shown that stimulating different prefrontal scalp locations led to different effects on heart rate, and that stimulation of the F4 and F3 locations (10-20 system; see figure 6 on page 34) resulted in the most significant heart rate decelerations, compared to central sites overlying the primary motor cortex (Iseger et al., 2017, Chapter 4). Individual variation was also found, however (i.e. for some individuals the most profound heart rate deceleration was found for slightly more posterior sites e.g. FC3, FC4), indicating that the NCG-TMS method could be used to individualize the correct stimulation target, under the assumption that trans-synaptic activation of the sgACC indeed activates the whole DLPFC-sgACC-Vagal nerve pathway that is involved in MDD. However, it has yet to be established how this correlates with treatment outcome and if such targeting methods result in increased clinical efficacy.

To summarize, rTMS has gained much empirical support, both from controlled as well as large open-label studies demonstrating clinical efficacy in the treatment of TRD. Many developments are currently ongoing to further optimize treatments such as new stimulation protocols, new coil designs, predictors for treatment response and methods of target engagement, likely resulting in further improved efficacy for the future.

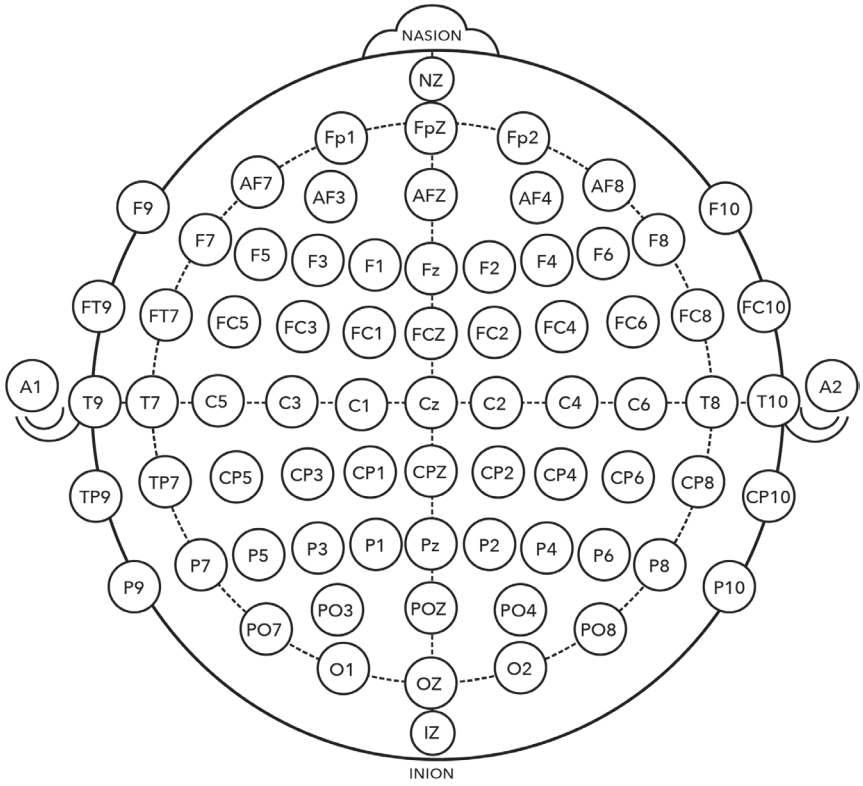


Figure 6: *International system for EEG electrode placement. Adapted from: https://commons.wikimedia.org/wiki/File:International_10-20_system_for_EEG-MCN.svg, under the Creative Commons CC0 1.0 Universal Public Domain Dedication.*

AIMS AND OUTLINE

Within this thesis the depression network in the brain will be further investigated, in order to further unravel the pathophysiology underlying depression and gain more insights into the possibilities to optimize and personalize rTMS treatments.

As described in the **introduction**, rTMS treatment for treatment resistant depression is associated with clinical improvement. However, there is considerable room for improvement of the treatment, since response and remission rates are still between 30-50% (Avery et al., 2006; Garcia-Toro et al., 2001; Mogg et al., 2008; O'Reardon et al., 2007; Padberg et al., 1999; Rossini et al., 2005; George et al., 2010). Improvement of rTMS may lay in the spacing of sessions, the length, stimulation frequency, stimulation intensity, but also localization of the right stimulation target.

The most commonly used rTMS stimulation target in the treatment of depression is the DLPFC, either within the left or the right hemisphere. As mentioned, localization of this target is usually performed by the '5 cm-rule'. With this method, the hand area of the primary motor cortex (M1) (which elicits a contralateral motor response of the thumb when stimulated), is taken as the detectable reference point, and from there, the coil is moved 5 cm anteriorly. This method has been criticized due to the lack of taking individual variation in head size into account. The Beam-F3/F4 method has been proposed as a new method (Beam et al., 2009) which does take individual differences in head size into account and is based on the 10-20 EEG location F3 or F4. This method, as well as 'MRI-neuronavigated rTMS' however, are still not individualized enough since these are based on the 'assumption' that rTMS should be aimed at the 'anatomically defined DLPFC', whereas currently, depression is more commonly seen as a network-disorder. Thus, there is a need for a functional (rather than structural) localization techniques. In line with this, 'target engagement' has gained interest. With target engagement, the right target (or more appropriately, the right node in the network) is identified by including and probing the relevant network that is activated by this node, often by using a functional outcome measure. In its simplest form, the thumb-movement confirming that the primary motor cortex is activated can be seen as target engagement method.

Target engagement has been used to locate the DLPFC as well. Fox and colleagues found that a higher anticorrelation between the sgACC and the DLPFC was associated with better treatment response after rTMS in MDD. Thus, they proposed that the ideal stimulation site would be the location with the highest anticorrelation to the sgACC, and this was measured by fMRI. Though promising, the use of fMRI is not optimal yet in terms of costs and time, but also in terms of reproducibility. One study showed that individual reproducibility for sgACC-DLPFC fMRI connectivity was quite low and could differ a few centimeters between a morning and an afternoon scan (Ning et al., 2018).

Thus, better localization methods are still required. Since fMRI is costly and time consuming, it may be more efficient to use EEG to asses functional connectivity between the sgACC and the DLPFC. Thus, this was investigated within **Chapter 2**, although in this study, DLPFC-sgACC connectivity was not linked to rTMS treatment outcome but to antidepressant medication. Here the iSPOT-D sample was used, that consisted of 1008 MDD patients and 336 controls, where we specifically looked at state and trait aspects of mood and EEG connectivity. Frontal connectivity between some of the major hubs of the depression network, namely the DLPFC, sgACC and DMPFC were investigated, and the predictive value and/or change in terms of response to antidepressant treatment was assessed, as a way to further investigate the depression network. Furthermore, is was assessed whether dysregulated brain connectivity was state – or trait – related. Meaning: do connectivity patterns change over time (due to treatment/ improvement of depressive symptoms), or do they stay the same. For trait-related brain connectivity no change was expected as well as no difference between responders and non-responders to treatment, whereas for state related brain connectivity it was expected that responders to treatment would differentiate from non-responders as treatment progresses. As this chapter indicates, there was a shift in DLPFC-sgACC connectivity within the alpha frequencies, but only for males who responded to treatment. Baseline connectivity was not found to be different between responders and non-responders, thus not predictive of treatment outcome, indicating that EEG does not improve the suggested target engagement method.

Besides target engagement methods using DLPFC-sgACC connectivity, which is only a small part of the depression network, it may be worthwhile to include a larger part of the network. As such, it is known that the depression network in the brain also has connections to bodily functions, as described earlier in **'the depression network'**, meaning that the heart is also an important functionally connected node in the depression network.

MDD has been associated with higher heart rates, lower variability of the heartrate, and a higher risk for heart disease. The exact working mechanism is unclear, but it has been suggested that high heart rate variability protects against multiple disorders. **Chapter 3** shows why solid heart rate variability is important, and what the effect of environmental factors are on heart rate and HRV, such as the influence of season of birth, and how aging and gender influences HRV. Additionally, it is investigated how these relate to personality characteristics and psychological factors such as depression, anxiety and stress.

Besides the association of MDD with heart rate dysregulation, it was also found that several neuromodulation techniques aimed at different nodes of the depression network (DLPFC, sgACC and vagus nerve) *reduced* heart rate. Thus, it seems that a parasympathetic pathway is activated by stimulation of the depression network, indicating this may be used to optimize coil localization for rTMS. Subsequently, neither fMRI nor EEG are required, but only some ECG electrodes. The first proof-of-concept of this approach was investigated within **Chapter 4**. In Chapter 4, knowledge about connectivity within the depression network that extends the DLPFC-sgACC network to include the vagus nerve, is used in order to optimize rTMS treatment, since activation of the vagus nerve will result in heart rate decelerations. In ten healthy subjects, rTMS stimulation was applied at various prefrontal locations and the hypothesis was tested that adequate network activation would be associated with heart-rate deceleration, which was found to be the case and this method could thus be used as a method for target engagement to improve rTMS coil positioning. This target engagement method was called Neuro-Cardiac-Guided TMS or NCG-TMS.

However, since Chapter 4 only describes a pilot study in which 10 healthy subjects were tested, it is essential to replicate the findings in a larger sample, in order to verify whether NCG-TMS is useful as a target engagement method. In addition, a few things need to be addressed further to evaluate the usefulness of this method as a target engagement method and its use in clinical practice:

- Individual test-retest reliability
- Dose-response relationship
- Treatment outcome

The method should replicate in a larger sample, but there must also be adequate individual test-retest reliability and a dose-response relationship. That means that at low doses of stimulation, no effect on heart rate is expected, but as stimulation intensity increases, heart rate changes should be more pronounced. Interestingly, such a dose-response relationship may also tackle determination of the ideal 'prefrontal stimulation threshold' rather than still to rely on the 'motor threshold' for prefrontal stimulation. The intensity at which a first heart rate deceleration is observed may thus be the optimal stimulation intensity for that individual and that prefrontal location. Furthermore, the method has to be validated in a sample of MDD patients and be linked to treatment outcome.

Therefore, in **Chapter 5** a replication study is described in which the results described in Chapter 4 are replicated in a larger healthy control sample as well as in an MDD patient sample. Both cohorts replicated the earlier results. Additionally, in this study, dose response relationships and test-retest reliability were investigated in order to assess the validity of the NCG-TMS method.

Additionally, an independent replication was conducted in a TMS laboratory at Monash University in Melbourne (Australia) which is described in **Chapter 6**. Here NCG-TMS was applied at 3 locations with 10Hz on the left hemisphere, and results indeed replicated the results from Chapter 4. Additionally, this study addressed the potential effect of low frequency stimulation on the cardiac response.

Next to optimization of stimulation location and intensity, the stimulation pattern (or frequency) may be improved. Many studies have investigated different forms of protocols, as described under '**Stimulation intensity, trains, sessions and safety**' and '**progress in protocols**', amongst which iTBS. Unlike 10Hz stimulation, iTBS – or intermittent theta burst stimulation – is a patterned rTMS protocol that mimics endogenous theta rhythms in the brain, thereby improving synaptic long-term potentiation. To this goal, TMS pulses are delivered in twenty sets of ten triplet 50Hz bursts, that are separated by 8s. The ten triplet bursts are applied within 2s at a rate of 5Hz, thus the whole protocol of 600 pulses is delivered within 189 seconds. The advantage of iTBS is that it is less time consuming but has similar or more potent excitatory effects compared to conventional 10Hz stimulation (Blumberger et al., 2018), Since different types of stimulation patterns and frequencies may elicit different cortical and network effects, the effects of iTBS on heartrate were assessed in **Chapter 7**. Chapter 7 presents the results of a treatment resistant MDD patient study sample, patients receiving both sham and active iTBS, while simultaneously cardiac parameters were assessed such as blood pressure and ECG, from which HR and HRV could be derived. It was shown that, compared to sham, active iTBS has significantly larger effects on heart rate, heart rate variability and blood pressure, thereby strengthening the network theory and the potential of NCG-TMS. Additionally, the predictive value of heart rate for treatment response was investigated, since for this MDD patient sample, post-treatment depression score ratings were available. In **Chapter 5**, the potential of iTBS for NCG-TMS (NCG-iTBS) was assessed as well.

Chapter 8 is a review and serves also as a summary of this thesis. In Chapter 8 the heart brain-pathway is sought to be unraveled and a link with depression is being investigated. Furthermore, it elaborates on the effects of neuromodulation on certain hubs in the depression network and the effects on heart rate and heart rate variability. Additionally, an individual participant data meta-analysis is conducted in order to asses laterality differences of the NCG-TMS method.

Finally, **Chapter 9** provides a summary and a discussion.

CHAPTER 2:

EEG CONNECTIVITY BETWEEN THE SUBGENUAL ANTERIOR CINGULATE AND PREFRONTAL CORTICES IN RESPONSE TO ANTIDEPRESSANT MEDICATION

Published as: *Iseger T.A., Korgaonkar M. S., Kenemans J. L., Grieve S. M., Baeken C., Fitzgerald P. B., Arns M., 2017. EEG connectivity between the subgenual anterior cingulate and prefrontal cortices in response to antidepressant medication. European Neuropsychopharmacology. 27(4):301-312. 10.1016/j.euroneuro.2017.02.002*

Author contributions: TAI performed data analyses and wrote the initial draft for the manuscript. MA and TAI conceptualized the study, MA supervised the study, MSK, SMG and MA were involved in data collection. All authors took part in writing the manuscript.

ABSTRACT

Antidepressant medication is the most common treatment for major depressive disorder (MDD), however, the precise working mechanism underlying these treatments remains unclear. Recent neuromodulation treatments demonstrate that direct stimulation of the dorsolateral prefrontal cortex (DLPFC), dorsomedial prefrontal cortex (DMPFC), and subgenual anterior cingulate (sgACC) relate to clinical improvement, suggesting connectivity alterations of the DLPFC-DMPFC-sgACC network to mediate antidepressant response. The international Study to Predict Optimized Treatment in Depression (iSPOT-D) is an international multicentre study that collected EEG data for 1008 MDD patients, randomized to 3 different antidepressant medications (N=447 MDD with complete pre- and post-treatment data and N=336 non-MDD). Treatment response was defined by a decline of >50% on the Hamilton Rating Score for Depression (HRSD17). We investigated whether connectivity in alpha and theta frequencies of the DLPFC-DMPFC-sgACC network changed from pre- to post-treatment between: (i) patients and controls, and (ii) responders (R) and non-responders (NR). Women exhibited higher alpha and theta connectivity compared to males, both pre- and post-treatment. Furthermore, theta, but not alpha, hypo-connectivity was found for MDD patients. A decreased alpha connectivity after treatment was found only for male responders, while non-responders and females exhibited no changes in alpha connectivity. Decreasing alpha connectivity could potentially serve as a treatment emergent biomarker, in males only. Furthermore, it could be useful to a priori stratify by gender for future MDD studies.

INTRODUCTION

Major depressive disorder (MDD) is a chronic mental disease with a remitting and relapsing course. Despite the variety of available treatments, up to 40-50% of patients fail to respond (Kessler and Bromet, 2013). The use of antidepressant medication is a first-line treatment for MDD, in particular the use of selective serotonin reuptake inhibitors (SSRI's) and serotonin-norepinephrine reuptake inhibitor (SNRI's). Despite widespread use, the exact working mechanism behind these treatments is not clear. New treatments such as repetitive Transcranial Magnetic Stimulation (rTMS) and Deep Brain Stimulation (DBS) are emerging (Liston et al., 2014; Fox et al., 2012). These new treatments directly targeted key structures in depression such as the dorsolateral prefrontal cortex (DLPFC) (George et al., 2010; O'Reardon et al., 2007), the dorsomedial prefrontal cortex (DMPFC) (Downar and Daskalakis, 2013; Downar et al., 2014) and the subgenual cingulate cortex (sgACC) (Mayberg et al., 2005) and thereby have shown that direct stimulation of these regions is associated with clinical improvement. Recent insights into how these neuromodulation treatments work suggest network connectivity changes within a DLPFC-DMPFC-ACC network to mediate antidepressant response (Liston et al., 2014; Fox et al., 2012), and are also possibly implicated in pharmacological treatments.

The convergent evidence of involvement of these structures indicates that they are likely to be important hubs in the networks that modulate depression. The sgACC and sections of the DMPFC are components of the default mode network while the DLPFC is partly implicated in the central executive network (CEN). A deficit in switching between the DMN and CEN is well known in depression (Sridharan et al., 2008; Liston et al., 2014) and is considered to be one of the main reasons behind cognitive dysfunction in depression. The DLPFC has been described to be hypoactive in depression (Korgaonkar et al., 2012), and an increase in fMRI activity of this structure

is associated with treatment response (Koenigs and Grafman, 2009; Fitzgerald et al., 2006). Contrary to the DLPFC, the sgACC has been described to be hyperactive in depression, along with hyperconnectivity to other parts of the DMN observed with PET scans and with fMRI (Mayberg et al., 2005; Liston et al., 2014), and a decrease in activity of the sgACC is associated with antidepressant response (Mayberg et al., 2005; Koenigs and Grafman, 2009). The DMPFC, or dorsal nexus, is a core region to multiple networks, including the DMN, CEN and salience network (SN), with increased fMRI connectivity to all three networks in depression (Sheline et al., 2010). The DMPFC has been observed to be abnormally activated during positive and negative affect processing in MDD, which normalizes after successful treatment (Dunlop et al., 2015; Bermpohl et al., 2009; Mayberg et al., 1999). As rTMS is limited to cortical surfaces, it is hypothesized that DLPFC-rTMS (and DMPFC-rTMS) might exert its antidepressant effect via trans-synaptic connectivity to deeper regions, such as the sgACC (George et al., 1995; George et al., 1997; Padberg and George, 2009; Fox et al., 2012; Fox et al., 2014). Serotonergic challenge has been observed to reduce intrinsic functional connectivity in brain regions implicated in mood regulation (Anand et al., 2005; Anand et al., 2007), such as the ventral anterior cingulate cortex (vACC, which includes the sgACC/Cg25 and the rACC/Cg24) and limbic structures such as the amygdala (Drevets et al., 2008; Gudayol-Ferré et al., 2015). However, the full scope of serotonergic and antidepressant action on functional connectivity in the human brain, especially with respect to the DLPFC-DMPFC-ACC network, has not been explored widely.

The international Study to Predict Optimized Treatment in Depression (iSPOT-D) is a multicentre study aimed at finding biomarkers for antidepressant treatment response (Williams et al., 2011). Preferably these biomarkers need to be cost-effective and EEG measurements represent an attractive modality due to the relatively low cost and burden imposed on patients, and informative about underlying brain circuits. To this goal, the study collected EEG data from 1008 MDD patients, randomized to 3 different antidepressant medications, prior to and after 8 weeks of treatment. 336 controls also completed EEG data collection at baseline and at 8 weeks. The aim of this manuscript is to investigate connectivity changes in the DLP-

FC-DMPFC-sgACC network across 8 weeks of treatment, not only for patients and controls, but also comparatively for antidepressant responders and non-responders, and thus to investigate whether these connectivity differences are state, trait or medication related. To this goal, we explored baseline to post-treatment connectivity changes between responders and non-responders to medication in alpha and theta EEG frequencies, as previous studies using the same sample as the current study have found the most relevant differences in alpha and theta (Arns et al., 2015; Arns et al., 2015; Arns et al., 2015). Gender was included as a factor because previous iSPOT-D studies have demonstrated clear qualitative gender differences in topographic distribution of EEG activity and gender-specific predictors of treatment response of alpha asymmetry (Arns et al., 2015) and Event Related Potentials (Arns et al., 2015; Arns et al., 2015; van Dinteren et al., 2015). Quantitative differences could be resolved by using gender as a covariate; however, the clear qualitative differences warrant *a priori* stratification by gender rather than covariation, hence in this study Gender was included as a main factor rather than a covariate. Furthermore, it is well known that MDD is more prevalent in females as compared to males (Gorman 2006; Marténi et al., 2001), further warranting *a priori* stratification by gender.

EXPERIMENTAL PROCEDURES

DESIGN

In the international Study to Predict Optimized Treatment Response in Depression (iSPOT-D), a multi-centre, randomized, prospective open-label trial (Phase-IV clinical trial), 1008 MDD participants were randomized to escitalopram, sertraline or venlafaxine-XR in a 1:1:1 ratio. The design was deliberately chosen to mimic real-world practice—hence no placebo control was included—with the aim of improving the translatability of the findings and ecological validity. The complete study protocol and the consort diagram are available elsewhere (Williams et al., 2011, Arns et al., 2015).

PARTICIPANTS AND TREATMENT

This study included 1008 MDD patients and 336 matched healthy controls. A complete description of the study assessments, inclusion/exclusion criteria, diagnostic procedures and treatment is available elsewhere (Williams et al., 2011; Saveanu et al., 2014). In summary, the primary diagnosis of nonpsychotic MDD was confirmed at baseline visit (before randomization) using the Mini-International Neuropsychiatric Interview (MINI-Plus) (Sheehan et al., 1998), according to DSM-IV criteria, and a score ≥16 on the 17-item Hamilton Rating Scale for Depression (HRSD17). MDD participants were also assessed on the 16-item Quick Inventory of Depressive Symptomatology – Self-Report (QIDS-SR16). All MDD participants were either antidepressant medication-naive or, if previously prescribed an antidepressant medication, had undergone a washout period of at least five half-lives before the baseline visit clinical and EEG assessments. After the baseline visit, MDD participants were randomized to one of three antidepressant medications. After eight weeks of treatment, participants were tested again, while still on treatment, using the HRSD17, QIDS-SR16 and an EEG assessment. This study was approved by the institutional review boards at all of the participating sites and was conducted according to the principles of the Declaration of Helsinki, 2008. After study procedures were fully explained in accordance with the ethical guidelines of the institutional review

boards, participants provided written informed consent. This trial was registered with ClinicalTrials.gov. Registry name: International Study to Predict Optimised Treatment - in Depression. Registration Number: NCT00693849; URL: http://clinicaltrials.gov/ct2/show/NCT00693849.

EEG ACQUISITION

EEG recordings were performed using a standardized methodology and platform (Brain Resource Ltd., Australia). Details of this procedure have been published elsewhere (Williams et al., 2011) as well as details of the reliability and across-site consistency of this EEG procedure (Arns et al., 2015; Paul et al., 2007; Williams et al., 2005). In summary, EEG data were acquired from 26 channels: 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; 10-20 electrode extended international system). EEG data was collected for two minutes with eyes closed (EC). Data were offline referenced to averaged mastoids with a ground at AFz. Horizontal eye movements were recorded with electrodes placed 1.5cm 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. Impedance was <5KOhms for all electrodes. A continuous acquisition system was employed, with a sampling rate of all channels of 500Hz. A low pass filter with an attenuation of 40dB per decade above 100Hz was employed prior to digitization.

EEG ANALYSIS

EEG pre-processing and validation has been described in more detail elsewhere (Arns et al., 2015; Williams et al., 2011; Paul et al., 2007; Williams et al., 2005) . In brief, 1) A high pass filter of 0.3Hz, a low pass filter of 100Hz and notch filters of 50 or 60Hz (depending on the country in which the data were recorded) were applied; 2) Data were

EOG corrected using a regression-based technique similar to Gratton, Coles and Donchin (Gratton et al., 1983), 3) Data were segmented in 4 sec. epochs (50% overlapping), 4) and individual epochs per channel were marked as artefact based on the following criteria: a) EMG detection, b) Pulse and baseline shift detection, c) Crosstalk detection, d) High kurtosis, e) Extreme power level detection, f) Residual eye blink detection and g) Extreme voltage swing detection (Arns et al., 2015; Williams et al., 2011; Paul et al., 2007; Williams et al., 2005). In addition, an electrode-bridging check was carried out (Alschuler et al., 2014), and channels demonstrating bridging were rejected. For eLORETA analysis, rejected channels were replaced using a spherical spline interpolation (only when at least 3 surrounding channels were present, otherwise the data were rejected).

EEG ELORETA ANALYSIS

Based on the scalp-recorded electric potential distribution, the exact low-resolution brain electromagnetic tomography (eLORETA) software (http://www.uzh.ch/keyinst/loreta.htm) was used to compute connectivity values, for phase lags unequal to zero to rule out volume-conduction effects. The method of eLORETA is described in detail in (Pascual-Marqui 2007). eLORETA is an improvement over the original LORETA version and the standardized version sLORETA. sLORETA (Pascual-Marqui 2002) is an improvement over the older LORETA (Pascual-Marqui et al., 1994), and has the ability to localize test point sources with zero localization error in the absence of noise, and is more accurate. eLORETA (Pascual-Marqui 2007) is the newest version of LORETA and is a non-linear imaging method and a solution to the inverse problem, with exact and zero localization errors. In addition, eLORETA offers ways to assess functional brain connectivity between cortically defined regions of interest (ROIs), minimally affected by volume conduction and low spatial resolution.

ROI EXTRACTION

For the exact ROI coordinates see table 1. The following ROIs were defined:

1. Right and left Dorsolateral Prefrontal Cortex (DLPFC) based on the coordinates published by (Fox et al., 2012; Fitzgerald et al., 2009) with a sphere of 20 mm, restricted to grey matter only (Figure 1C, D).
2. The dorsomedial prefrontal cortex (DMPFC) was defined by a seed coordinate (Salomons et al., 2014), with a sphere of 20 mm (Figure 1B).
3. The subgenual ACC (sgACC) was defined based on the averaged coordinates obtained from a meta-analysis (Fox et al., 2012), that included studies that showed reductions in subgenual activity as a result of antidepressant response (Figure 1A).

Table 1: *ROI coordinates in MNI space*

	X	Y	Z	Sphere (mm)	BA
Left DLPFC	-46	45	38	20	46
Right DLPFC	46	45	38	20	46
DMPFC	0	30	30	20	24
sgACC	6	16	-10	10	25

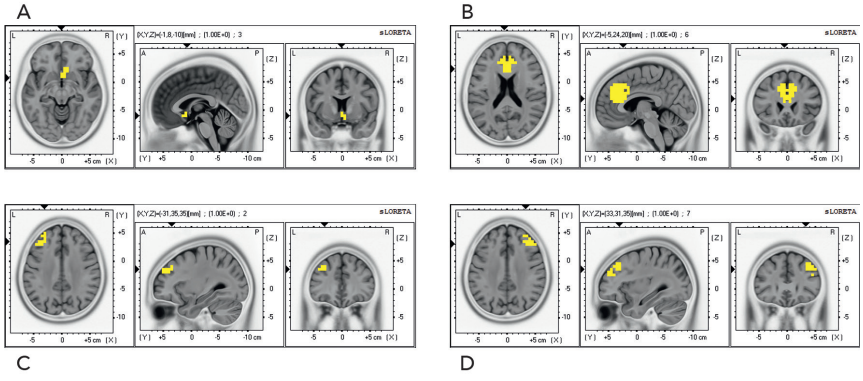


Figure 1: *The four regions of interest (ROI) used in this study; **A**) sgACC; **B**) DMPFC (medial) **C**) DLPFC (left); **D**) DLPFC (right).*

CONNECTIVITY ANALYSIS

Using this region of interest (ROI) approach, linear-lagged connectivity measures were computed. In general, this linear-lagged connectivity represents the linear covariation between fluctuations in activity recorded from distinct neural networks, that were measured in preselected ROI's. These connectivity measures were obtained for theta (4-7.5hz) and alpha (8-13hz) frequencies with LORETA, as previous studies using the same sample as the current study have found the most relevant differences in these frequency bands (Arns et al., 2015; Arns et al., 2015; Arns et al., 2015). It should be noted that the specific frequencies chosen to measure theta differs from the earlier iSPOT-D manuscript on theta in which the 6.5-8.0 Hz theta band was used since that band was specifically used in rACC studies. However, normally the standard theta band of 4-7.5 Hz is used in studies that employ data from iSPOT, thus were also used in the current study. These values were then log-transformed prior to analysis, to meet statistical assumptions of normal distribution.

STATISTICS

Response was defined as a >50% decrease in HRSD17 score from baseline to 8 weeks. Based on the literature, as summarized in the introduction, our primary analysis focused on sgACC-rDLPFC (right); sgACC-lDLPFC (left) and sgACC-DMPFC (medial) connectivity. These combinations are in the analysis referred to as Connectivity-pair. Gender was included as a factor because gender differences have now been reported in several manuscripts using the iSPOT-D sample (Arns et al., 2015; Arns et al., 2015; van Dinteren et al., 2015) .

The primary hypotheses tested in this study are:

1. MDD patients exhibit abnormal baseline connectivity values within these Connectivity-pairs, compared to healthy controls.
2. Baseline connectivity within these Connectivity-pairs in responders, but not non-responders, will normalize over time.
3. Baseline connectivity within these Connectivity-pairs differs between men and women, as a consequence of distinct brain network connectivity, and may lead to different antidepressant medication outcomes.

State effects will show-up as connectivity changes across time for responders but not for non-responders to treatment, medication effects will show-up as changed connectivity for both responders and non-responders, while trait effects would show no time-differences for responders or non-responders, but merely a difference between MDD patients and healthy controls that remains stable across time.

The analysis was divided into multiple parts. First, we examined baseline differences between MDD patients and healthy controls (HC). A repeated measures design was conducted, with baseline Connectivity-pair as within-subject factor. Gender and Group (HC or MDD) were included as between-subject factors in the MDD-HC analysis. When a significant interaction between Group or Gender and Connectivity-pair was observed, analysis was run separately for Group or Gender, or per Connectivity-pair using univariate models.

Secondly, we focused on differences over time between responders (R), non-responders (NR) and healthy controls. Repeated measures ANOVA with Connectivity-pair and Time (pre and post-treatment) were used as within-subject factors. Gender and Group (R, NR or HC) were used as between-subject factors. In a separate analysis we added medication as additional between-subjects factor and evaluated whether medication type (escitalopram, sertraline or venlafaxine-XR) led to significant differences with respect to connectivity changes. *F* ratios were evaluated using degrees of freedom computed using the Greenhouse–Geisser **ε** correction where appropriate to counteract heterogeneity of variance matrices associated with repeated measures. When Gender or Connectivity-pair had significant interactions with Group, Time or combinations of those, analyses were run separately per Gender, Group, Time or Connectivity-pair. When post-treatment connectivity values were analyzed separately from baseline values, the latter were included as a covariate to correct for baseline differences in connectivity values. Furthermore, Group means were used to calculate the effect-size (Cohen's *d*), where needed. The Group means and standard deviations were directly adapted from SPSS. Correlation analysis with these difference scores was conducted to confirm whether a change in connectivity was associated with symptom severity or improvement. In addition,

in case of finding significant differences between R and NR, discriminant analysis was performed and a Receiver Operator Characteristic (ROC) curve was plotted to investigate how well these measures could be used to predict treatment outcome. A ROC curve is a graph displaying the true positive rate vs. the false positive rate for R and NR status.

RESULTS

Of 336 controls, 279 subjects (157 females, 122 males) had useable baseline EEG data and were included in the baseline analysis. 222 of them also had complete week 8 data, and were included in part two of the analysis. Of the 1008 MDD patients, 807 patients had useable baseline EEG data and were used in the baseline analysis (434 females, 373 males). 655 patients (378 females, 277 males) had completed the study according to the protocol. Of these, there were 447 subjects with useable resting EEG and HRSD17 data at baseline and post-treatment (244 females, 203 males) and were used for the second part of the EEG analysis. The log-transformed data were normal distributed. Within the MDD sample, there was a significant age difference between responders and non-responders ($F(1, 637)=7.88$, $p<0.005$). Therefore, age was entered as a covariate in all analyses.

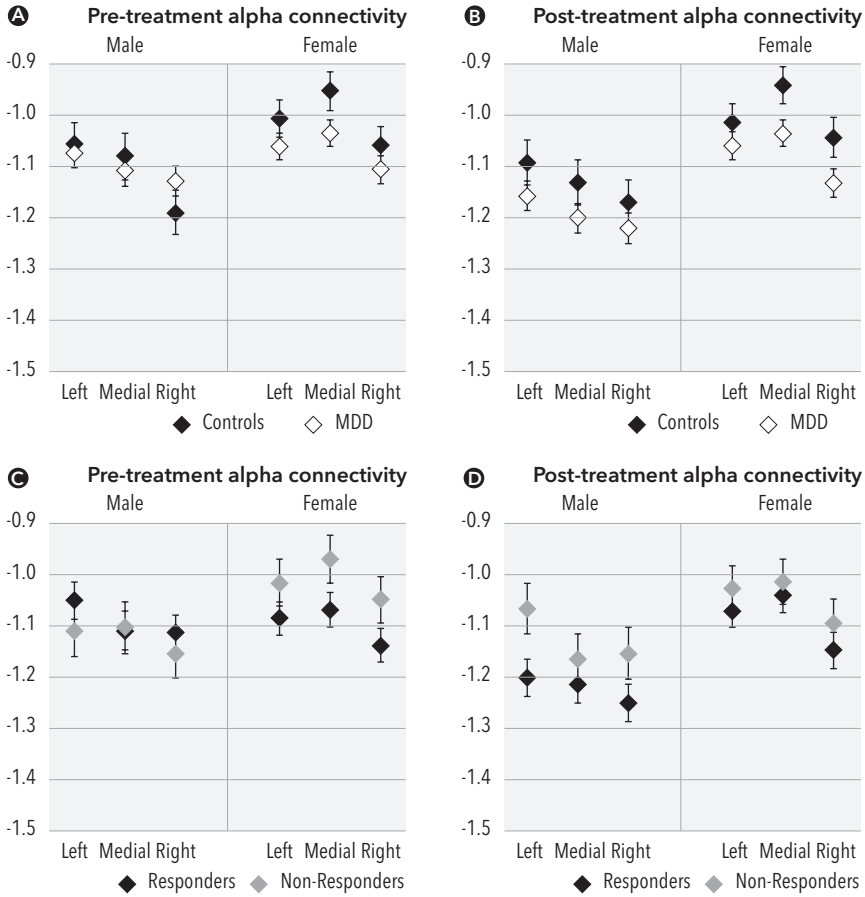
Table 2: *(page 53) Sample characteristics*

	Intention to treat (N)	Per protocol (N)	Gender	N	Response type	N	Treatment	N	Age	HDRS pre	HDRS post	MDD duration	Number of episodes
MDD	1008	655	Male	277	Responders	172	Escitalopram	61	37.16 (11.60)	21.85 (3.92)	4.66 (3.05)	14.03 (11.48)	3.95 (1.51)
							Sertraline	63	38.29 (12.58)	21.57 (4.28)	6.19 (3.08)	13.63 (11.98)	3.98 (1.42)
							Venlafaxine-XR	48	38.95 (12.65)	21.31 (3.66)	5.83 (3.27)	12.02 (11.18)	3.85 (1.58)
					Non-responders	105	Escitalopram	37	43.56 (11.30)	21.62 (3.49)	16.08 (4.19)	16.25 (12.21)	4.25 (1.38)
							Sertraline	32	43.04 (13.14)	22.28 (4.42)	16.75 (4.89)	16.90 (14.43)	3.74 (1.59)
							Venlafaxine-XR	36	38.36 (12.30)	22.17 (4.27)	15.31 (3.85)	16.91 (12.01)	4.09 (1.22)
			Female	378	Responders	243	Escitalopram	70	38.44 (12.58)	21.63 (3.90)	5.10 (3.10)	14.38 (13.00)	3.96 (1.44)
							Sertraline	92	35.79 (11.42)	22.27 (4.06)	5.87 (3.29)	14.54 (12.43)	4.20 (1.30)
							Venlafaxine-XR	81	37.13 (12.73)	20.72 (3.55)	5.80 (3.14)	15.78 (12.14)	4.14 (1.09)
					Non-responders	134	Escitalopram	49	38.04 (13.65)	21.90 (4.60)	15.94 (4.87)	15.73 (12.70)	4.10 (1.31)
							Sertraline	46	40.45 (12.65)	21.61 (4.12)	15.85 (4.35)	13.49 (11.66)	3.75 (1.51)
							Venlafaxine-XR	39	40.73 (14.06)	22.77 (4.17)	17.44 (4.07)	17.84 (14.76)	3.89 (1.49)
HC	336		Male	145					37.16 (12.77)	0.95 (0.14)	0.95 (0.12)		
			Female	191					36.87 (13.35)	1.30 (0.12)	1.15 (0.11)		

ALPHA

MDD-HC (BASELINE)

Women had higher baseline alpha connectivity in all three connectivity pairs, compared to men, indicated by a main effect of Gender (F(1, 1081)=6.96, p<0.008, d=-0.156) (figure 2A,B). There was no effect of Group, nor interactions involving Group, suggesting there were no baseline differences between MDD patients and controls (figure 2A,2B).



R-NR-HC (BASELINE-WEEK 8)

For alpha connectivity, a main effect of Gender was observed (F(1, 662)=11.69, p<0.001, d=0.266), and of Connectivity-pair F(2, 661)=3.63, p<0.027). Women exhibited in general higher connectivity with the

sgACC then men, and the sgACC has higher connectivity with the DMPFC, than with the left or right DLPFC (figure 2C,D).
A significant Time*Group*Gender ($F(2, 662)=3.35$, $p<0.036$) interaction, Connectivity-pair*Gender ($F(2, 661)=9.95$, $p<0.001$) and a Connectivity-pair*Time*Group ($F(4, 1324)=2.78$, $p<0.026$) interaction were found. Separate analysis per gender revealed a significant Group*Time effect ($F(2, 292)=4.11$, $p<0.017$) for males only, not for females. This was driven by a decreasing connectivity over time only for responders ($F(1, 130)=19.44$, $p<0.001$, $d=0.369$), while non-responders and controls remained stable (figure 3). Subsequent analysis within the male group revealed that the group differences were negligible on baseline, but significantly different after 8 weeks of treatment, when covaried for baseline connectivity ($F(2, 291)=4.4$, $p<0.013$). Pooled across connectivity pairs, responders differed from

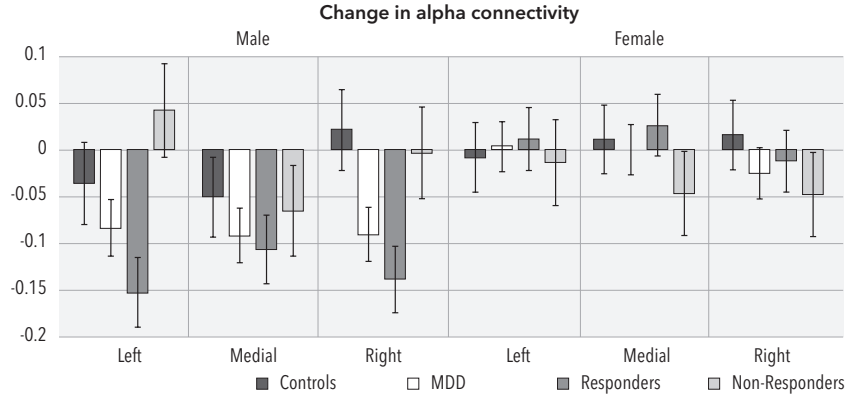


Figure 3: *(above) Alpha connectivity changes over time (post minus pre-treatment) for sgACC-lDLPFC (left), sgACC-DMPFC (medial) and sgACC-rDLPFC (right), separated for males and females, MDD patients and controls, and responders and non-responders. Error bars represent the standard error of the mean (SEM). *The results shown are based on the age-covaried analysis, including only subjects used in the second part of the analysis. Significant changes over time are observed only in male responders.*

Figure 2: *(facing page)* ***A)*** *Pre-and* ***B)*** *post treatment alpha connectivity levels for sgACC-lDLPFC (left), sgACC-DMPFC (medial) and sgACC-rDLPFC (right) connectivity, separated for males and females, between controls and MDD patients.* ***C)*** *Pre-and* ***D)*** *post-treatment alpha connectivity levels for sgACC-lDLPFC (left), sgACC-DMPFC (medial) and sgACC-rDLPFC (right) connectivity, separated for males and females, between responders and non-responders. Error bars represent the standard error of the mean (SEM). *The results shown are based on the age-covaried analysis, including only subjects used in the second part of the analysis.*

non-responders (p<0.013, d=-0.363) and healthy controls (p<0.016, d=-0.327). Non-responders did not differ from healthy controls (p<0.797). These results will be further explored in the discriminant analysis. A positive correlation between HRSD17 difference scores and connectivity difference scores (r=0.189, p<0.007) was found within the male MDD group, but not separately for responders or non-responders, confirming only the results from the ANOVA.
As reported above, there was also a significant Connectivity-pair*-Time*Group and Connectivity-pair*Gender interaction. Separate analyses for each Connectivity-pair investigating the Time*Group effect only revealed a main effect of Group for sgACC-DMPFC connectivity (F(2, 665)=3.75, p<0.024), but no Time*Group effects.
Analyzing the Time*Connectivity-pair interaction for each Group revealed a main effect of Time (F(1, 287)=7.18, p<0.008) in the responders group, but no Time*Connectivity-pair interaction. It should be noted that the apparent difference between males and females with respect to the pattern of results as visible in figure 2, in where a larger post-treatment difference is seen within male responders for sgACC-lDLPFC and sgACC-rDLPFC connectivity compared to sgACC-DMPFC connectivity, could not be confirmed statistically. With respect to medication-type effects, no apparent interactions nor main effects were found.

THETA

MDD-HC (BASELINE)

A baseline gender difference (F(1, 1082)=11.42, p<0.001, d=0.205) was found for theta connectivity, which was higher in woman (figure 4). A main effect of Group was found (F(1, 1082)=5.14, p<0.024; d=0.157), discriminating MDD patients from healthy controls, but a Group*-Connectivity-pair interaction (F(2, 1080)=6.37, p<0.002) was observed as well, indicating that the group difference varied across Connectivity-Pairs. Subsequent analysis showed that the group difference was only significant for sgACC-lDLPFC (p<0.003) and sgACC-DMPFC (p<0.010). In general, MDD patients had lower connectivity then controls (figure 4). A Connectivity-pair*Gender interaction (F(2, 1080)=3.23, p<0.040) was found, but Gender remained signifi-

cantly different in all Connectivity-pairs: sgACC-lDLPFC (p<0.004), sgACC-DMPFC (p<0.001) and sgACC-rDLPFC (p<0.001).

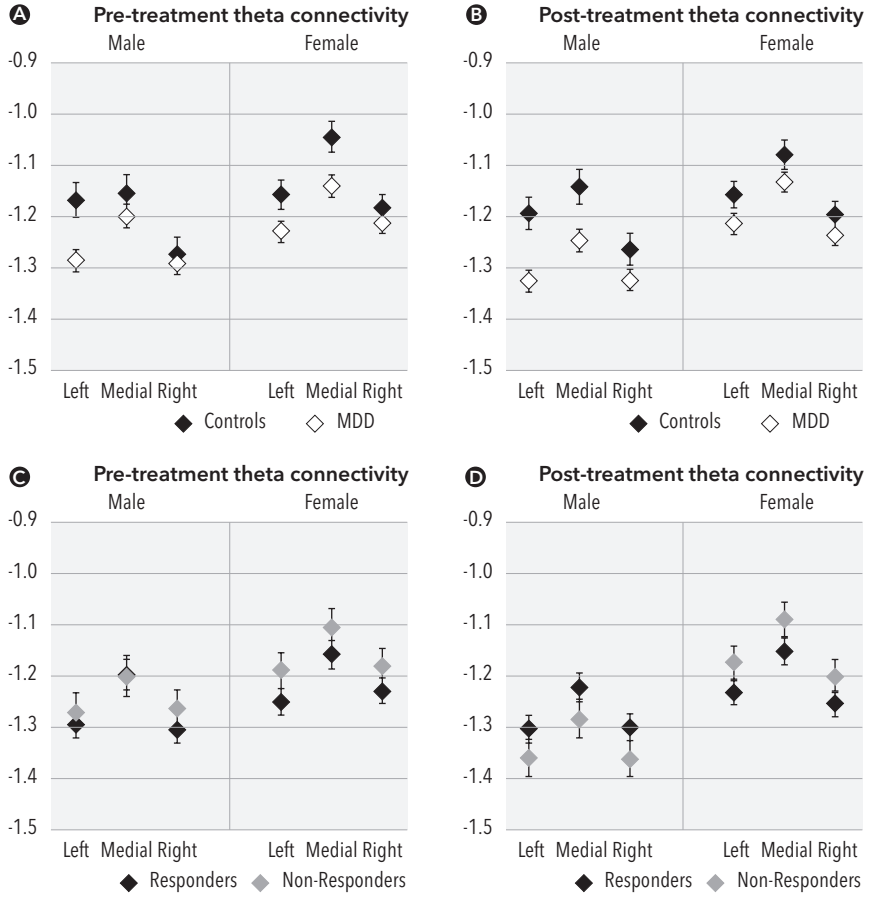


Figure 4: ***A)*** *Pre-and* ***B)*** *post-treatment theta connectivity levels for sgACC-lDLPFC (left), sgACC-DMPFC (medial) and sgACC-rDLPFC (right) connectivity, separated for males and females, between controls and MDD patients.* ***C)*** *Pre-and* ***D)*** *post-treatment theta connectivity levels for sgACC-lDLPFC (left), sgACC-DMPFC (medial) and sgACC-rDLPFC (righ) connectivity, separated for males and females, between responders and non-responders. Error bars represent the standard error of the mean (SEM). *The results shown are based on the age-covaried analysis, including only subjects used in the second part of the analysis.*

Woman exhibited higher connectivity values then men ($F(1, 662)=18.64$, $p<0.000$, $d=0.341$). Furthermore, a main effect of Group was found ($F(2, 662)=6.068$, $p<0.002$), which was driven by controls being different from responders ($p<0.001$) and non-responders ($p<0.027$), thus only emphasizing the difference between patients and controls. A main effect of Connectivity-pair was observed ($F(2,662)=15.93$, $p<0.001$). Higher connectivity is observed for sgACC-DMPFC, in both male and female subjects, when compared to sgACC-lDLPFC or sgACC-rDLPFC connectivity, similar to findings for alpha (figure 5).

Furthermore, Connectivity-pair interacted with Group ($F(4, 1324)=3.23$, $p<0.012$). Subsequent analysis per Connectivity-pair indicated a significant Group difference for sgACC-lDLPFC ($F(2, 662)=9.761$, $p<0.000$), and for sgACC-DMPFC ($F(2, 662)=5.10$, $p<0.006$) but both were due to a difference between patients and controls (respectively sgACC-lDLPFC and sgACC-DMPFC connectivity: R-HC ($p<0.001$; $p<0.001$); NR-HC ($p<0.006$; $p<0.027$)), and not between R-NR ($p<0.337$; $p<0.553$). No apparent time effects were found. Additional analysis of the differential effects across treatment-types did not reveal interaction with treatment-type nor any main effects.

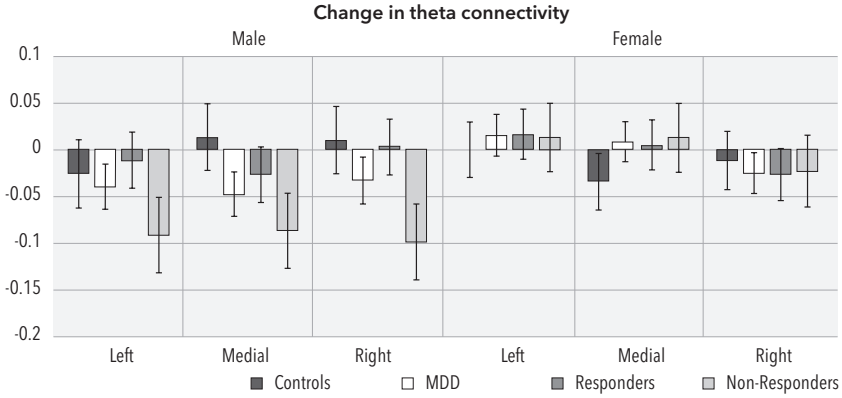


Figure 5: *Theta connectivity changes over time (post minus pre-treatment) for sgACC-lDLPFC (left), sgACC-DMPFC (medial) and sgACC-rDLPFC(right), separated for males and females, MDD patients and controls, and responders and non-responders. Error bars represent the standard error of the mean (SEM). *The results shown are based on the age-covaried analysis, including only subjects used in the second part of the analysis.*

DISCRIMINANT ANALYSIS

A discriminant analysis was performed using alpha sgACC-lDLPFC, sgACC-rDLPFC and sgACC-DMPFC connectivity difference scores. The grouping variable was responder status. The model resulted in a significant Wilks' Lambda (p< 0.004, Wilks' Lambda=0.936, Chi-square(3)=13.204). Figure 6 shows the specificity (18%) and sensitivity (91%) for responders (dotted line) and non-responders (striped line), with an area under the curve of 0.664. 65,5% of the subjects could be classified correctly. Running the same analysis including the baseline characteristics of age, MDD severity and anxiety severity resulted in a significant Wilks' Lambda (p<0.011, Wilk's Lambda=0.897, Chi-Square(8)=19.934), with an area under the curve of 0.698, showing a slight improvement of the model.

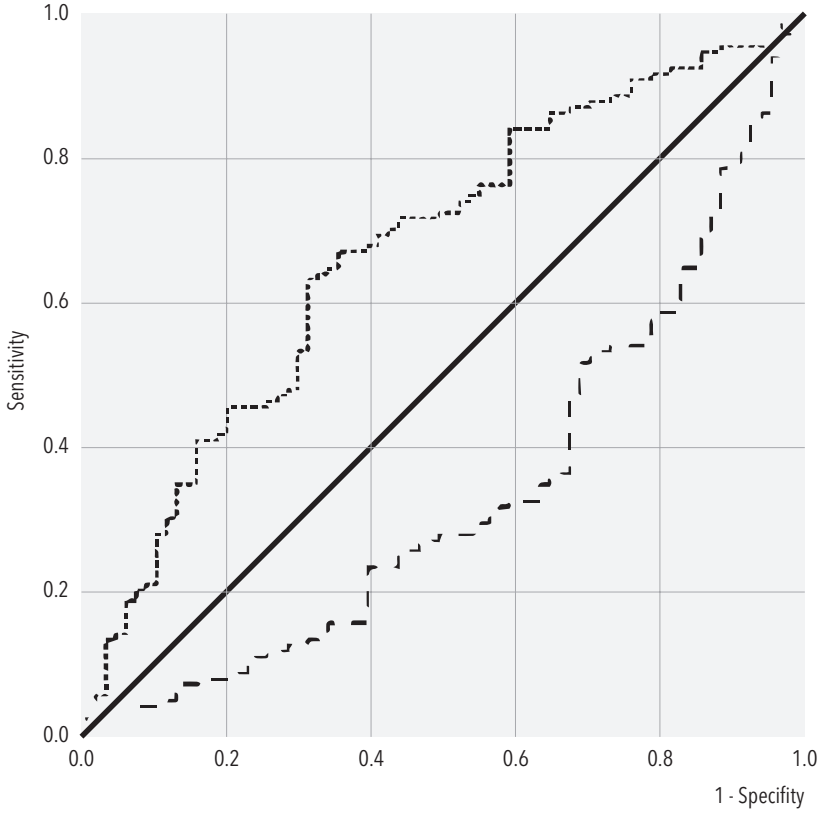


Figure 6: *Receiver Operator Curve (ROC) for the results of a discriminant analysis on response, with an area under the curve of 0.664. The ROC shows the specificity (18%) and sensitivity (91%) for responders (dotted line) and non-responders (striped line).*

DISCUSSION

We explored the sgACC-DLPFC-DMPFC network in relation to depression and treatment response and found a significant treatment-related change over time for male responders in the alpha band for connectivity of the sgACC with the DLPFC and DMPFC, whereas non-responders and control subjects remained stable over time. Based on these results, there was not enough evidence to determine whether decreasing alpha connectivity could be state-related. However, the decreasing alpha connectivity in male responders, suggests that, for males only, decreasing alpha might serve as a treatment emergent biomarker.

Males classified as responders based on the HRSD17 change, displayed a significant decrease in alpha connectivity, with a significant post-treatment difference, while non-responders and control subjects exhibited stable connectivity patterns over the 8-week time course. Interestingly, male responders thus become less like healthy controls for alpha connectivity, which is not in line with previous literature on treatment response in MDD patients, in which multiple studies have described a normalization of the existing hyper-connectivity (Mayberg et al., 2005; Liston et al., 2014; Koenigs and Grafman, 2009). In some studies differences in ACC or DLPFC connectivity after treatment have been reported, therefore it was hypothesized that connectivity tends to normalize or resemble the connectivity pattern of healthy controls, but could still be incomplete (Liston et al., 2014). However, this is also not the case within this sample, where the opposite is seen in male responders: they become less like healthy controls. This could be due to the type of treatment (antidepressant medication rather than neuromodulation), the use of EEG recording rather than fMRI, or due to gender differences not picked up in previous studies that were not statistically powered to tests such gender differences. Decreasing alpha connectivity might serve as a treatment emergent biomarker, as supported by the discriminant analysis and ROC curve, and needs to be investigated in a gender controlled design, on earlier time points within 8 weeks of treatment to evaluate the potential as treatment emergent biomarker for a more clinically relevant usage, for example, similar to the work on treatment emergent biomarkers at 5-7 days such as EEG Cordance (Leuchter et al., 1994) and ATR (Leuchter et al., 2009; Leuchter et al., 2009).

Secondly, strong gender differences in connectivity patterns were found in MDD patients as well as in healthy controls. In general, we observed higher connectivity in females for both alpha and theta activity. This is in line with previous research on network connectivity where especially the frontal parts exhibits more (inter-hemispheric) connections in females, while males had more (intra-hemispheric) connections throughout the whole brain (Ingalhalikar et al., 2014). The higher functional connectivity in females could be due to the frontal locations of our regions of interest e.g. the DLPFC and DMPFC. To rule out that these gender differences would be driven by weight or length, we included these factors as covariate in a post-hoc analysis, which did not change the results. Gender differences have not been widely explored in depression research, particularly for treatment response. Clinically however, depression is found to be more common in female patients (Kessler and Bromet, 2013). The cause for this is not clear, but it could be due to differences in emotional processing between men and women (Gorman 2006).

Thirdly, we found theta hypoconnectivity in MDD patients, both in male and females, in contrast to healthy controls, while previous research has described hyperconnectivity of the sgACC with the CEN (which includes right and left DLPFC) (Mayberg et al., 2005; Liston et al., 2014). However, it is worth noting that these studies performed analysis using fMRI connectivity measurements and it is not yet understood how these relate to EEG measurements of connectivity. While both methods are usually based on the covariation in fluctuations of the signal, the source of the signal differs. fMRI connectivity more often relates to slower fluctuations in blood oxygenation responses (<1Hz), whereas in our analysis we looked at faster oscillations in the theta and alpha bands (3.5-13Hz). EEG reflects the cortical electrical activity of the brain produced by waxing and waning postsynaptic potentials, and the waveforms produced can be classified according to frequency. In contrast, fMRI is usually based on the Blood Oxygen Level Dependent (BOLD) signal, in where the amount of oxygen in the blood is a reflection of activity in an area, and is a result of a long chain of neural and hemodynamic processes (Sato et al., 2010). When there is high covariation between two regions, one

could assume that there is a functional connection between the two regions. It should be noted that our analysis addressed only non-zero phase-lag connectivity so that contamination by volume conduction effects can be ruled out (Pascual-Marqui et al., 2011).

Limitations of this study are in the design, which was not placebo-controlled, while the placebo-response rates are about 31-45%, compared to 50% responses to antidepressants (Peciña et al., 2015) Signal-to-noise ratio is lower in EEG compared to fMRI, however, due to the large sample size available in this study provides improved sensitivity to these effects. EEG is marked by a high temporal but a low spatial resolution. The EEG picks up post-synaptic potentials from cortical layers but is hardly or not at all sensitive to post-synaptic potentials from deeper structures such as the hippocampus and amygdala. The sgACC is a relatively small structure lying deeper in the brain, and while it is still valid to use as an EEG target in LORETA analysis, the measured EEG signal might not be an exact reflection of this precise region of interest and may also reflect activity derived from adjacent areas due to the relatively low spatial resolution of EEG at this depth.

In conclusion, we found strong evidence that alpha connectivity decreases in male responders in response to antidepressant medication, while non-responders and healthy controls remained stable, suggesting that decreased alpha might serve as a treatment emergent biomarker, but, more research is needed to evaluate connectivity on multiple time points within these 8 weeks of treatment. Furthermore, we found gender differences in the DLPFC-sgACC-DMPFC network in a large sample of MDD patients and healthy controls, similar to gender differences reported in other analysis of the iSPOT-D study (Arns et al., 2015; Arns et al., 2015; van Dinteren et al., 2015). These data suggest that future EEG and imaging studies in MDD could benefit by a priori stratifying their analysis by gender (rather than co-vary for gender) to rule out such gender differences that could results in spurious findings or mask real effects.

CHAPTER 3:

HEART RATE VARIABILITY RELATED TO SEASON OF BIRTH: A REPLICATION STUDY

Published as: *Iseger, T.A., Vollebregt, M.A., Krepel, N., Arns., M. 2019a. Heart rate variability related to season of birth: A Replication Study. Psychophysiology. e13419. Epub ahead of print. 10.1111/psyp.13419*

Author contributions: TAI and MAV performed data-analyses, TAI, MAV and MA conceptualized the study, MA supervised the study and TAI wrote the initial draft for the manuscript. All authors took part in writing the manuscript.

ABSTRACT

Low Heart Rate Variability (HRV) has strongly been associated with an increased risk for cardiovascular disease. Cardiovascular disease being the number one cause of global deaths, factors that influence its development are relevant to understand. Season of birth has been suggested to be one of the factors influencing the development of HRV. The current study was set up to replicate the finding that men born in winter have higher HRV later in life compared to those born in other seasons. To this end, we studied a sample of 1871 healthy participants from the Brain Resource International Database (BRID) during rest and during task. Furthermore, sex and age differences and associations with personality traits and psychiatric symptoms were explored. We replicated the earlier finding that men born in winter have a lower ratio of Low Frequency (LF) power to High Frequency (HF) power during rest compared to summer and fall, and, although less pronounced, higher HF compared to summer. A difference between summer and winter for LF/HF in men was internally replicated using data recorded during task. Additionally, for both sexes, LF/HF ratio increased with age and LF and HF both decreased. In general, LF/HF was lower in women, but heart rate was higher. In men, low HRV was associated with depression and the personality trait openness.

Concluding, results from a large multicentre dataset covering the entire lifespan demonstrate that HRV changes with age in both sexes and confirm that season of birth influences HRV later in life in men.

INTRODUCTION

Season of birth (SOB) has been associated with many psychiatric disorders such as schizophrenia, Major Depression Disorder (MDD) (Torrey et al., 1996), Autism Spectrum Disorder (ASD) and Attention-Deficit Hyperactivity Disorder (ADHD), as well as with physical disorders such as epilepsy and cardiovascular disease (Reffelmann et al., 2011; Nonaka and Imaizumi, 2000). Cardiovascular disease being the number one cause of global deaths, factors that influence its development are relevant to understand. So far, being born in winter seems to be cardio-protective (Sohn 2016), whereas men born in spring had lower blood pressure (Banegas et al., 2000). Also the variability in heart rate has been related to SOB (Huang et al., 2015). Heart rate variability (HRV) is the fluctuation around the mean heart rate and is generally regarded as an index for autonomic functioning or sympathovagal balance, representing the flexible shift between sympathetic and parasympathetic activity (Thayer and Lane, 2000). It is often reflected by the ratio of low frequency to high frequency power (LF/HF) in frequency domain analyses of the electrocardiogram. Low HRV has strongly been associated with an elevated risk for heart related problems (Thayer et al., 2010; Hillebrand et al., 2013). Moreover, dysregulated HRV has been related to the prevalence of psychiatric disorders such as MDD (Kemp et al., 2010), ADHD (Griffiths et al., 2017), ASD (Wang et al., 2015), psychosis (Alvares et al., 2016) and anxiety (Chalmers et al., 2014), and even is related to personality (Huang et al., 2013; Shepherd et al., 2015). Thus, HRV seems to contribute to the development of emotional and physical wellbeing, which in turn could be influenced by SOB. A relation between HRV and SOB implies the influence of environmental factors associated with seasonality on HRV during the prenatal and postnatal period. These factors may include climatic factors such as sunlight, humidity, temperature, but also nutrition, exercise (being outdoors), and prenatal stress. Although a relationship between HRV and SOB has been suggested by one study, this study was rather

small and conducted at a research site with a specific (Taiwanese) climate (Huang et al., 2015). In a sample of 382 school children (age 6-10 years) SOB was related to both sympathetic and parasympathetic activity. They found that 1) boys born in winter have higher high frequency (HF) power than boys born in other seasons; 2) boys born in winter have a lower LF/HF power ratio than boys born in other seasons; 3) temperature of SOB and age were predictive factors for HF among boys; 4) humidity during SOB was predictive of normalized low frequency power (LF%) and LF/HF power among girls.
The aim of the current study was to conceptually replicate the findings of Huang et al. (2015). We extended their findings to a lifespan perspective as well as using a multicentric approach to rule out effects driven by specific local and/or cultural aspects rather than season. To this end, an existing dataset of 1871 healthy participants between the ages of 6 to 87 years was used. Additionally, this study investigated age and sex differences in HRV and correlations between personality, depression and anxiety scores and HRV.

METHODS

PARTICIPANTS

Data of participants were extracted from the Brain Resource International Database (BRID). This database contains data from multiple laboratories (New York, Rhode Island, Nijmegen, Sydney, and Adelaide) that have been acquired using standardized data acquisition techniques (identical amplifiers, standardization of other hardware, audio calibration, paradigm details, software acquisition, and task instructions). Inter-lab reliability and test-retest reliability measures are high and have been reported elsewhere (Williams et al., 2005; Clark et al., 2006; Paul et al., 2007). The database consists of healthy participants between 6 to 87 years old. Database exclusion criteria included a personal or family history of mental illness, brain injury, neurological disorder, a serious medical condition, drug/alcohol addiction, first-degree relative with bipolar disorder, schizophrenia, or genetic disorder. Data from participants that were regarded not to follow task instructions were rejected from analyses.

After selecting data from participants for whom electrocardiogram (ECG) data were available, using data from sites that contributed at least N=20 due to our interest in seasonality that geographically varies, the sample consisted of 1871 participants (956 men; mean age=28.31±19.92). Participants were required to refrain from caffeine and smoking (2h), and alcohol (6h) prior to testing. All participants provided written informed consent. Since only an already existing dataset was used, no Institutional Review Board approval was obtained. Demographic characteristics can be found in Table 1 (page 71).

PSYCHOLOGICAL DATA ACQUISITION

Preceding the ECG measurement, participants completed the NEO Five-Factor Inventory (NEO-FFI). The NEO-FFI was used was used to examine 'the Big Five' personality traits. The NEO-FFI is a 60-item, self-report instrument that measures five personality traits, being Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. These domains have shown good internal consistency (Cronbach's alpha range 0.87-0.92) (Renner et al., 2013). To measure anxiety and stress, the Depression, Anxiety and Stress Scale (DASS) (Henry and Crawford, 2005) was used.

EXPERIMENTAL DESIGN

ECG was recorded during an electroencephalography (EEG) test battery. Of this test battery, ECG recordings during Eyes Open at rest (EO) and Auditory Oddball (ODB) were used for HRV analysis. During EO (2 minutes), the participant was instructed to sit relaxed and fixate on the red dot displayed at the screen for 2 minutes, in essence similar to the 5-minute recording in Huang et al. (2015) where participants sat quietly awake. During ODB (6 minutes), the participant was presented with high-pitched and low-pitched tones and instructed to press a button when hearing high-pitched tones.

PHYSIOLOGICAL DATA ACQUISITION

Two ECG electrode channels were combined to create the ECG data series, with one positioned on the inside of the non-dominant wrist, directly above the radial pulse (called ECG) and the other at Erbs point (located two-thirds distal from midline on the clavicle; called Erbs). Both of these channels were referenced to a separate common reference channel at C7 (the 7th cervical vertebra's most pronounced transverse process). The Erbs point and C7 recording sites are both positioned directly above the bone to serve as relatively muscle-free data recordings. The data were sampled at 500 Hz and a low pass filter of 100 Hz was applied prior to digitization (Griffiths et al., 2017).

DATA PROCESSING

Data processing was performed according to (Griffiths et al., 2017): Data reduction was performed using software from Brain Resource Ltd, and the standard methods for the Brain Resource International Database (Kozlowska et al., 2015). R waves (i.e. the main spikes observed in the graphical deflections observed in an ECG) were detected in the ECG and converted to a RR tachograph, which is a graph of the numerical value of the RR-interval (i.e., the interval between two R peaks) and time. The combined ECG and Erbs data (ECG - 5*Erbs) underwent a 5-15 Hz Tukey bandpass filter. Data cleaning was performed using semi-automated methods to identify and remove any tacho-series in which R waves were not reliably detected or scored, series that where ectopic, were arrhythmic beats were present, or where the voltage was too extreme (low or high) to be scored. Beats were considered missing when the inter-beat interval exceeded 1.2 times the moving average (using a forgetting factor of 0.4), and an ectopic beat was classified as an interval of less than 0.8 of the moving average. Intervals around these beats were also removed from the tacho-series.

Table 1 (facing page): *Comparison of the HRV parameters across a sample born in 4 seasons. Note that the descriptives represent the resting condition, task data is not shown. Age (years); Mean RR interval in milliseconds (RR; msec); High frequency power in square milliseconds, ln transformed (HF; ln(ms2)); Low frequency power in square milliseconds, ln transformed (LF; ln(ms2)); LF/HF ratio (ln(ratio)); Very low frequency power in square milliseconds, ln transformed (VLF; ln(ms2)); Normalized LF power (LF%.nu)). * For RR and LF% the sample was smaller.*

Men	Spring N=257 Mean (SD)	Summer N=224 Mean (SD)	Fall N=231 Mean (SD)	Winter N=244 Mean (SD)	F Test (ANOVA)	P-value	Comparison
Age. y	25.49 (17.83)	26.81 (19.81)	26.37 (19.28)	25.62 (19.63)	0.253	0.859	-
RR. msec * (N=904)	841.595 (140.725)	833.592 (132.684)	843.330 (143.182)	826.018 (139.163)	0.747	0.524	
HF. ln(ms2)	6.383 (1.460)	6.101 (1.413)	6.203 (1.495)	6.395 (1.528)	2.222	0.084	Spring-Winter: p=0.931 Summer-Winter: p=0.032 Fall-Winter: p=0.168
LF. ln(ms2)	6.559 (1.165)	6.418 (1.272)	6.398 (1.271)	6.395 (1.287)	0.988	0.398	
LF/HF[ln(ratio)	0.176 (1.057)	0.317 (1.067)	0.195 (1.057)	<0.001 (1.074)	3.548	0.014	Spring-Winter: p=0.066 Summer-Winter: p=0.001 Fall-Winter: p=0.047
VLF. ln(ms2)	5.951 (1.270)	5.803 (1.196)	5.893 (1.272)	5.839 (1.125)	0.681	0.564	
LF%. nu * (N=904)	9.746 (11.026)	9.881 (14.407)	10.290 (13.289)	9.449 (9.964)	0.183	0.908	
Women	**Spring N=217 Mean (SD)**	**Summer N=220 Mean (SD)**	**Fall N=241 Mean (SD)**	**Winter N=237 Mean (SD)**	**F Test (ANOVA)**	**P-value**	**Comparison**
Age. y	29.43 (19.54)	31.94 (21.06)	31.06 (20.79)	30.29 (20.52)	0.601	0.615	
RR. msec * (N=866)	809.534 (132.657)	825.032 (132.001)	828.269 (119.491)	825.660 (136.921)	0.905	0.438	
HF. ln(ms2)	6.198 (1.311)	6.116 (1.443)	6.178 (1.384)	6.181 (1.366)	0.148	0.931	
LF. ln(ms2)	6.109 (1.251)	6.170 (1.256)	6.100 (1.174)	6.132 (1.184)	0.149	0.931	
LF/HF[ln(ratio)]	-0.887 (0.949)	0.054 (1.042)	-0.078 (0.910)	-0.0484 (1.000)	0.986	0.399	
VLF. ln(ms2)	5.703 (1.182)	5.637 (1.196)	5.695 (1.065)	5.743 (1.155)	0.328	0.805	
LF%. nu * (N=866)	7.487 (8.979)	7.819 (10.732)	7.194 (7.683)	7.836 (14.007)	0.181	0.909	

The RR interval signal was interpolated by a cubic spine interpolation algorithm. Mean RR interval, absolute very low frequency power (VLF: 0.0033 – 0.04 Hz), low frequency power (LF: 0.04 – 0.15 Hz) and high frequency power (HF: 0.15 – 0.4 Hz) were calculated, as well as the ratio of low frequency power to high frequency power (LF/HF). Frequency domain measures were measured in power (ms2), by use of a Welch Periodogram. Furthermore, normalized low frequency power was calculated (LF%.nu). The natural logarithm (ln) of VLF, LF, HF and LF/HF was taken, consistent with the analyses of Huang et al. (2015).

CLIMATIC DATA

The dataset was divided into 4 seasons: spring (March to May), summer (June to August), fall (September to November), and winter (December to February) for the northern hemisphere, identical to Huang et al. (2015) and spring (September to November), summer (December to February), fall (March to May), and winter (June to August) for the southern hemisphere.
For the different sites, dewpoint, temperature, and solar irradiance (SI) were calculated per month using "meteonorm 7.2.2" (http://www.meteonorm.com/en/downloads). Per site, "interpolated city" was used as location, interpolating data from different weather stations. Data were based on the month averages over the years 1991-2010 using "standard" Meteonorm output. In addition, the difference between two month-averages was calculated (SI change). Dewpoint was used as an indicator of humidity (studied in Huang et al.).

STATISTICAL ANALYSIS

The dataset was split a priori in men and women. Statistical analyses identical to Huang et al. were performed: HRV metrics among the 4 seasons were compared with univariate analyses, in order to test the following hypotheses one-tailed (i.e. a significance threshold of $p<.1$):
1) Boys born in winter had lower LF/HF than when born in other seasons and, 2) Boys born in winter had higher HF power than when born

in other seasons. These analyses were conducted separately for rest and task conditions. For significant effects, independent sample t-tests were performed to directly compare seasons. Effect sizes were calculated using Cohen's D. Being an international multicenter study, we verified post-hoc whether location had a significant effect on outcome, using site as an additional factor in univariate analysis.

Additionally, influences of climatic factors, such as temperature, dewpoint, and solar irradiance were tested for using correlation analyses. Because climate metrics were not normally distributed, Spearman correlation was used. For correlations for which we did not expect a significant effect based on Huang et al, Bonferroni correction was applied, setting the significance level at $p<.013$.

Next, age and HRV were correlated and sex differences between HRV measures (MeanRR, LF, HF, and LF/HF, VLF) were tested using univariate analyses with sex as fixed factor and the different HRV metrics as dependent variables. For this analysis, men and women were combined. Since VLF power is unreliable in the shorter EO recordings, because any frequencies under 0.0085Hz will not be reliably scored, VLF power was only investigated during the longer Oddball recordings.

Finally, correlation analysis was performed between HRV measures: LF/HF, VLF, LF, HF, MeanRR, and the Big Five Personality traits (neuroticism, extraversion, openness, agreeableness, and conscientiousness), and also between the HRV measures and DASS scores for depression, anxiety and stress, both for HRV measures at rest and at task. For these analyses, Bonferroni correction was applied, setting the significance level at $p<.006$.

RESULTS

REPLICATION

Data were divided based on sex. No significant age differences were observed between seasons (men: $F(3, 1003)=.25$; $p=0.859$; women: $F(3, 990)=0.60$; $p=0.615$). Similar to Huang et al, a comparison of HRV parameters in men revealed significant differences for HF and LF/HF

among the 4 seasons, during the resting condition. A significant main effect of SOB was found for LF/HF ratio (F(3, 952)=3.54, p=.014) and for HF (F(3, 952)=2.22, p=.084) in men. Direct comparison between seasons revealed that LF/HF was lower for birth in winter, consistent with the findings from Huang et al. (winter-spring: t(499)=-1.84, p=.066; winter-summer: t(466)=-3.20, p=.001, d=.296; winter-fall: t(473)=-1.99, p=.047, d=.183; figure 1). Like in Huang et al, this effect was lacking in women. Furthermore, higher HF power in men born in winter was also replicated (winter-spring: t(499)=-.087, p=.931; winter-summer: t(466)= -2.16, p=.032, d=-.200; winter-fall: t(473)=-1.38, p=.168; Table 1 and Figure 1). Post hoc analysis showed no indications for interaction effects with location, for both LF/HF and HF power.

During task condition, there was an association of SOB with LF/HF ratio (F(3, 944)=2.16, p=.092) in men but not women. Direct comparison between seasons revealed that this effect was mainly driven by a difference in LF/HF between those born in summer and those born in winter (winter-spring: t(488)=-1.30, p=.194; winter-summer: t(458)=-2.51, p=.013, d=.234; winter-fall: t(470)=-1.64, p=.102; Figure 1).

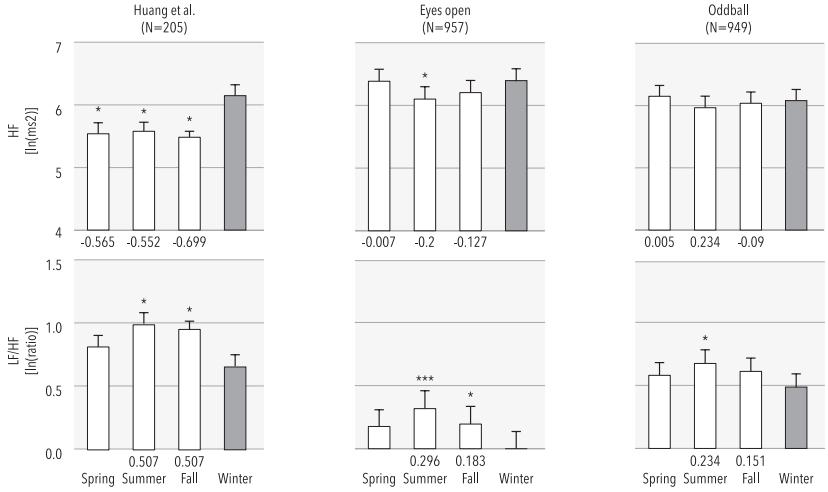


Figure 1: *Heart rate variability plotted for different seasons of birth. The top row depicts variation in high frequency (HF [ln(ms2)]) while the bottom row illustrates variation in low frequency relative to high frequency (LF/HF [ln(ratio)]). In the left column, the original results from Huang et al (2015) are depicted with permission. The middle and right column depict results from the current study for the eyes open resting state and oddball performance conditions respectively. Significance between each season (white) in relation to winter (grey) are indicated (* p < .05, *** p < .001). Cohens d' effect sizes are provided in the columns for the significant comparisons found in Huang et al, also in relation to winter.*

MEDIATING FACTORS

As expected, temperature was significantly different between seasons ($F(3, 3660)=1617.87$, $p<.001$), as was dewpoint ($F(3, 3660)=727.55$, $p<.001$), solar irradiance (SI) ($F(3, 3660)=2340.10$, $p<.001$), and SI change ($F(3, 3660)=3701.54$, $p<.001$). A small significant correlation between dewpoint and LF/HF was found in men ($r(955)=.081$, $p=.012$), but not in women. In women, meanRR shows a significant, yet small correlation to SI ($r(865)=-.085$, $p=.012$).

Table 2: *Climatic data of the 4 seasons during SOB of participants.*

	Spring	Summer	Fall	Winter	P-value
Temperature (°C)	15.72 (3.62)	21.73 (1.54)	16.67 (3.48)	10.47 (3.32)	<.001
Dewpoint	7.21 (3.01)	11.62 (3.31)	9.49 (2.77)	5.05 (3.44)	<.001
Solar Intensity (SI)	158.33 (34.50)	203.45 (29.71)	116.31 (39.84)	75.70 (20.94)	<.001
Change in Solar Intensity	29.70 (11.72)	-17.33 (17.80)	-32.07 (14.11)	22.66 (11.19)	<.001

SEX DIFFERENCES

Because there was a significant age difference between sexes (men: mean=25.83, SD=19.09, women: mean=30.34, SD=20.51, $F(1, 1999)=25.82$, $p<.001$), sex analyses were covaried by age. Univariate analyses revealed that there was a significant difference of sex on most HRV metrics, most pronounced in LF ($F(1, 1868)=12.72$, $p<.001$), LF/HF ($F(1, 1868)=32.38$, $p<.001$), meanRR ($F(1, 1767)=17.96$, $p<.001$) during rest. No sex differences were found for HF.

During task this was similar for LF ($F(1, 1846)=48.40$; $p<.001$), LF/HF ($F(1, 1846)=49.27$; $p<.001$), VLF ($F(1, 1846)=17.48$, $p<.001$) and meanRR ($F(1, 1656)=17.92$, $p<.001$). Men had higher RR interval lengths than women and more LF, resulting in higher LF/HF ratios compared to women.

HRV ACROSS THE LIFESPAN

Since the currently used sample consists of participants across the whole lifespan, we investigated the influence of age on HRV metrics. Using Spearman correlations, strong correlations with age were found for both men and women (see Figure 2). MeanRR increased (men: r(903)=.441, p<.001, women: r(856)=.459, p<.001), especially until 20 years, reflecting a heart rate decrease with age. Conversely, LF (r(955)=-.281, p<.001) and HF (r(955))=-.553, p<.001) power decreased with age (Figure 2 and table 3). There was a positive relationship between LF/HF and age (r(955)=.423, p<.001) in men, as well as in women (LF: r(914)=-.488, p<.001; HF: r(914)=-.513, p<.001; LF/HF: r(914)=.117, p<.001). These results where replicated during task (results not shown), and in addition, a significant correlation with VLF was also obtained (men: r(955)=-.314, p<.001; women: r(914)=-.408, p<.001).

Table 3: *HRV across the lifespan. Presented are the correlations between MeanRR in milliseconds (ms), low and high frequency power in LN(ms2), with age, separate for males and females.*

	Age	
	Males	Females
Mean RR (ms)	r (903) =.441, p<.001	r (865) =.459, p<.001
Low Frequency (LF [LN(ms^2)]	r (955)=-.281, p<.001	r (914) =-.488, p<.001
High Frequency (HF [LN(ms^2)]	r (955)=-.553, p<.001	r (914) =-.513, p<.001

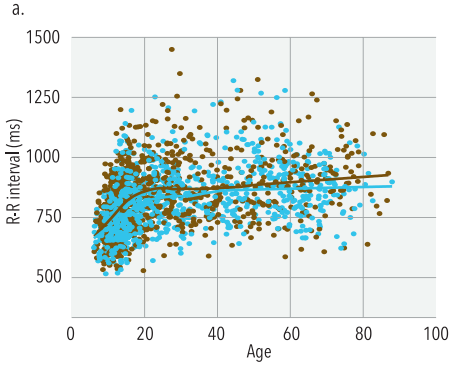


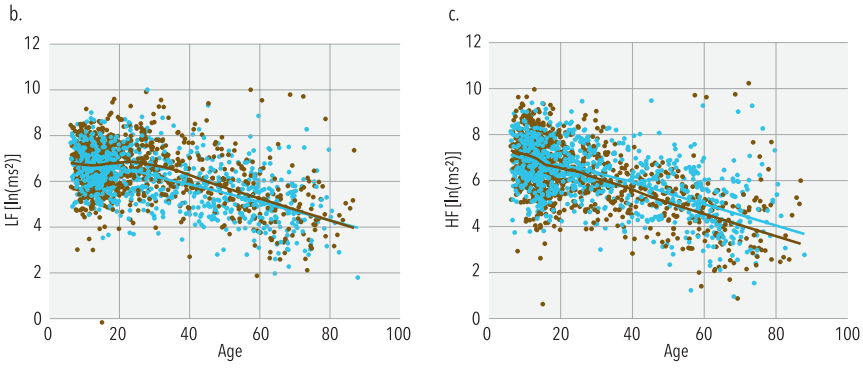
Figure 2: *HRV across the lifespan, separated for men (brown dots and line) and women (blue dots and line). Depicted HRV metrics are (a) meanRR interval, (b) LF power and (c) HF power.*

DEPRESSION, ANXIETY AND STRESS

Although the current sample consists of healthy participants, variation within the healthy range can be observed in the Depression-Anxiety-Stress scales (DASS). There was no interaction between SOB, DASS scores, and HRV. However, HRV measures did correlate to DASS scores. Since In men, age was significantly correlated to depression ($r(1004)=.086$, $p=.007$), stress ($r(1004)=.148$, $p<.001$) and anxiety ($r(1004)=.071$, $p=.025$), analyses were covaried by age. In women, age only correlated to stress levels ($r(990)=.112$, $p<.001$). To correct for multiple testing, a strict p-value of 0.006 was set.

In men, LF/HF correlated to depression severity ($r(680)=.141$, $p<.001$), as well as to stress ($r(680)=.111$, $p=.001$), indicating lower HRV when depression and stress scores are higher. However, this was not found during task.

In women, there were significant correlations found between meanRR length and depression ($r(860)=.120$, $p<.001$) and meanRR and stress ($r(862)=.113$, $p=.001$) during rest. This was replicated during task (depression: $r(808)=.102$, $p=.004$; stress: $r(810)=.114$, $p=.001$), but when covarying for age, these effects where no longer visible. When controlling for age, a significant correlation was found between stress and LF, ($r(669)=.110$, $p=.004$), showing that sympathetic activity is increased when stress levels are high.



Although personality is often assumed to be stable across time, correlations with age were found in both men and women. Neuroticism and extraversion decreased with age (neuroticism: $r(1716)=-.193$, $p<.001$; extraversion: $r(1716)=-.214$, $p<.001$), whereas openness, agreeableness, and conscientiousness increased with age (openness: $r(1716)=.188$, $p<.001$; agreeableness: $r(1716)=.202$, $p<.001$; conscientiousness: $r(1716)=.358$, $p<.001$). For this reason, personality-HRV analyses was covaried by age. Doing so, openness was positively correlated with LF ($r(680)=.173$, $p<.001$) and LF/HF ($r(680)=.167$, $p<.001$) in men, which was replicated during task (LF: $r(680)=.157$, $p<.001$ and LF/HF: $r(680)=.140$, $p<.001$). In women, openness was positively correlated to meanRR, both at rest ($r(699)=.196$, $p<.001$) and during task ($r(699)=.211$, $p<.001$), but not to LF or LF/HF.

Neuroticism in men was significantly related to lower meanRR ($r(699)=-.109$, $p=.004$) at rest, but not during task ($p=.009$, Bonferroni corrected threshold was set at $p=.006$). However, during task, neuroticism was related to VLF power ($r(699)=-.115$, $p=.003$) and extraversion was significantly related to meanRR during task ($r(699)=.116$, $p=.002$), but not Bonferroni corrected significant at rest ($r(699)=.101$, $p=.008$).

DISCUSSION

The aim of the current study was to conceptually replicate the findings of Huang et al. (2015). The replication was conceptual (Schmidt, 2009) because we studied different and wider geographic areas, and a wider -lifelong- age range. Additionally, this study investigated age and sex differences in HRV and correlations between personality, depression, and anxiety scores with HRV. We replicated that men born in winter had lower LF/HF. They also expressed higher HF power, indicating parasympathetic activity, although this effect was less pronounced. For LF/HF, this finding was internally replicated using HRV data during task performance. Furthermore, we found that HRV was different between men and women, and HRV changed with aging. Finally, we found a correlation of LF/HF ratio with depression and stress scores, as well as with the personality trait openness.

Regarding mediating factors, a small association of LF/HF with dewpoint was found in men, whereas Huang et al (2015) found an association with humidity in girls. Although dewpoint and humidity are closely related, they are not the same. Note that Huang et. al., conducted their study in Taiwan, characterized by a subtropical climate, and only tested participants between April and October.

In rest, we found only LF/HF and HF to be significantly different between SOBs. This makes sense since it has previously been suggested that parasympathetic activity is the main driver of LF/HF ratio in rest, also reflected in HF (Uijtdehaage and Thayer, 2000; Billman, 2013) . We used a rest and task condition in order to validate the findings, both requiring the participant to remain stationary, distinguished by cognitive demands. Although LF/HF was found to be significantly different during both rest and task conditions, HF only differed significantly during the rest condition. It is not fully understood why. Task demands may have led to decreased parasympathetic activation, resulting in diminished HF power.

There are several hypotheses for why HRV would be higher when born in winter. First of all, vitamin D has been associated with HRV (Canpolat et al., 2015; Mann et al., 2013). The sex difference may arise from hormonal differences. Previous findings suggest that higher levels of testosterone were associated with higher levels of HRV, while there was no association between estrogen and HRV (Wranicz et al., 2004) and that the level of testosterone is also driven by vitamin D levels (Nimptsch et al., 2012). Another explanation could lie in the levels of serotonin and dopamine metabolites, of which seasonal variation has been reported (Losonczy et al., 1984; Brewerton et al., 2018). That is, serotonin and dopamine metabolite levels are higher in winter. Sex differences have been reported, but a theory as to how these came across has not been suggested (Brewerton et al., 2018). Furthermore, it has yet to be established how seasonality in these metabolites relate to HRV. To explore the influence of estrogen and testosterone, it may be useful to include information about the menstrual cycle for women, as was previously shown to have an effect (Bai et al., 2009), although this will not be informative about hormone levels during birth.
We found that women expressed, in general, lower RR intervals,

while having lower LF/HF's, compared to men. This was similar to a previous meta-analysis (Koenig and Thayer, 2016). However, another study showed that LF/HF was higher in women (De Meersman and Stein, 2007). However, sex differences in HRV seem to be age and measure dependent, and age is an important modulator of HRV. It was shown that sex differences decrease with age, starting from 30 years old, and disappear around the age of 50, dependent on what measure for HRV is used (Umetani et al., 1998). This may be attributed to the level of oestrogen (Liu et al., 2003). Since our sample consisted of participants across the whole lifespan, we correlated HRV metrics with age. Age was found to have a substantial impact on HRV, where meanRR interval increased with age, while VLF, LF and HF power decreased with age, in line with previous work showing a negative correlation between age and HF (Abhishekh et al., 2013) and decreasing heart rates (increased meanRR) (Umetani et al., 1998). No indications of the proposed disappearance of sex differences by the age of 50 were observed in the current sample, but this may be due to the fact that we -in contrast to (Umetani et al., 1998)- have investigated the frequency domain HRV rather than the time domain. Our correlations with age were reversed from the results presented by Huang et al. (2015), which is probably a consequence of their small age range. In order to see if age would have any influence on the primary analyses, we replicated the main analysis with age as covariate, in order to control for age-related HRV differences. Age did not seem to be a modulator of the effect of SOB on HRV, since the results were similar to the earlier obtained results.

Previous research suggests that both depression (Disanto et al., 2012; Torrey et al., 1996) and HRV (Kristal-Boneh et al., 2000) show seasonal variation. As mentioned before, dopamine and serotonin metabolite levels also show such variation, and serotonin levels are associated with depression and anxiety (Deakin 1998). In the current study, LF/HF was positively correlated with depression and stress in men. Previous studies pointed to HRV as a marker for depression (Kemp et al., 2010; Brunoni et al., 2013; Ehrenthal et al., 2010; Udupa et al., 2007; Licht et al., 2008). The latter has been found in the current study in men, but a seasonal effect was not detected. It is important to note that although the sample consisted of healthy participants,

without diagnosed depression, anxiety or stress, we did find results similar to those in previous studies, albeit only for men. For women, only stress was associated with higher LF, which reflects, as can be expected, higher sympathetic tone.

Few studies have examined the relationship between HRV and personality and findings have been inconsistent. Furthermore, different studies have used different personality questionnaires/ factors. In the current study, both in men and women, although for different HRV metrics, positive associations were found for openness with LF, LF/HF ratio and meanRR intervals. An association with openness has been reported a few other studies, in where LF and LF/HF was shown to be positively associated with levels of openness (Čukić and Bates, 2014), and HF negatively, which is believed to be the result of activation of reward/motivation, emotion and arousal pathways in the brain. It has previously been shown that lower HRV was associated with greater difficulties in emotion regulation, but this was measured with RMSSD, which is an HRV variable in the time-domain (Williams et al., 2015). This may suggest that men born in winter have decreased emotion regulation compared to those born in other seasons, but we were not able to test this with the current data.

When interpreting the meaning of the current results, it is important to note that LF/HF's might not truly reflect the balance between sympathetic and parasympathetic activity - HRV is the fluctuation around the mean heart rate, largely under influence of respiration, but also affected by blood pressure and arterial fluctuations, and is often used as an index for autonomic functioning (Thayer and Lane, 2000). LF has been associated with sympathetic activity and HF with parasympathetic activity. Consequently, the LF/HF reflects the interaction between the two (McCraty and Shaffer, 2015). HF has been related to respiratory sinus arrhythmia (RSA) and is a measure of the natural variation occurring in the HR during a breathing cycle (Porges 1995). However, during slow respiration, vagal activity can easily generate oscillations that cross over into the LF band, for example during slow paced breathing. Thus, some research suggests that LF is not merely a reflection of sympathetic activity (Billman 2007; Reyes del Paso et al., 2013) and that LF/HF does not adequately

reflect the balance between sympathetic and parasympathetic activity. Still, during task, one would not expect slow-paced breathing, and the results of ODB replicated the resting state EO data, thus (para)sympathetic balance would be the most sensible explanation within this study. In addition, the standard recording length for HRV is 5 minutes, whereas we had recordings of 120 seconds for the rest condition. Recent studies however suggest that these shorter timeframes are still reliable, although it has been suggested that VLF data requires a minimum of 270 seconds (Shaffer and Ginsberg, 2017). Nevertheless, one study showed shorter VLF recordings still highly correlate with 5-minute recordings, thus the shorter VLF recordings may give a good indication (Baek et al., 2015).

One limitation of the study is that to correct for ectopic heartbeats and artifacts, phases of beat-to-beat intervals were cut out instead of using an interpolation method, possibly having unwanted influences on the frequency spectra. Since we received the HRV metrics from an existing database, we had no influence on this method. Using such a correction method may have had influences on frequency spectra. However, since Huang et al. (2015) did use an interpolation method and the current study does replicate these earlier data; it can be assumed that in this case there was no major influence. Other limitations are that there are many factors influencing HRV, such as BMI, sleep deprivation and menstrual cycle, but also acute effects of having a meal or drink, and these are not controlled for (Bai et al., 2009; Monnard and Grasser, 2017; Takase et al., 2004). Furthermore, recent developments point to using non-linear measures such as entropy to analyze HRV (Voss et al., 2009; Young and Benton, 2015) . Since this was a replication study, we did not use such methods, but it would certainly be of interest to do so in the future.

The currently used sample was a worldwide multicenter study and tested people across the lifespan throughout the whole year, resulting in a high generalizability of the current results. Future studies could focus on unravelling the underlying reason of a relationship between season of birth and HRV, thereby possibly unravelling the aetiology of sex-specific problems associated with certain HRV patterns. Furthermore, the newly found relationships between HRV and

depressive symptoms and personality traits should be replicated and investigated further.

In summary, we replicated the findings of Huang et. al (2015), while extending the study to a larger age range. Men born in winter had lower LF/HF compared to other seasons. This difference was not found in women. Sex differences were widely observed in HRV metrics, as well as age differences. Women expressed higher heart rates, but also higher HRV, than men. LF/HF increased with age. RR increased with age, and LF, VLF and HF power decreased. Furthermore, LF/HF was related to depressive symptoms and personality trait openness in men. Concluding, with this study we have replicated the earlier study by Huang et al, extending the findings to the full age lifespan, a wide geographic distribution, HRV measurements throughout the year, and additional measures that could be of interest to better understand the relationship between HRV and seasonality.

CHAPTER 4:

NEURO-CARDIAC-GUIDED TMS (NCG-TMS): PROBING DLPFC-SGACC-VAGUS NERVE CONNECTIVITY USING HEART RATE – FIRST RESULTS.

Published as: *Iseger T.A., Padberg F., Kenemans J. L., Gevirtz R., Arns M., 2017. Neuro-Cardiac-Guided TMS (NCG-TMS): Probing DLPFC-sgACC-vagus nerve connectivity using heart rate - First results. Brain Stimulation. 10(5):1006-1008. 10.1016/j.brs.2017.05.002.*

Author contributions: MA and TAI conceptualized the study, TAI collected data, performed data-analyses and wrote the initial draft for the manuscript, MA supervised the study. All authors took part in setting up the study and writing the manuscript.

ABSTRACT

Background

Given that many studies suggest a role of DLPFC-sgACC connectivity in depression and prior research demonstrating that neuromodulation of either of these nodes modulates parasympathetic activity and results in a heart rate deceleration, a new method is proposed to individualize localization of the DLPFC. This can, among others, be useful for rTMS treatment of depression.

Methods

Ten healthy subjects received three trains of 10Hz rTMS randomly over 7 target regions (10-20 system). Results: Overall, F3 and F4 expressed the largest heart rate deceleration, in line with studies suggesting these are the best 10-20 sites to target the DLPFC. On the individual level, 20-40% subjects expressed the largest heart rate deceleration at FC3 or FC4, indicating individual differences as to the 'optimal site for stimulation'.

Conclusions

These results show that the NCG-TMS method is valid to localize the entry into the DLPFC-sgACC network.

INTRODUCTION

Autonomic regulation is disturbed in patients with major depressive disorder (MDD), indicated by a higher heart rate (HR) and lower heart rate variability (HRV). Moreover, the heart is functionally connected via the vagus nerve (VN) to other brain structures that are dysregulated in depression, such as the subgenual anterior cingulate cortex (sgACC) (Mayberg et al., 2005), and the dorsolateral prefrontal cortex (DLPFC), suggesting dysregulated network function in MDD (Fox et al., 2012; Shoemaker and Goswami, 2015; Thayer and Lane, 2000). In line with this network dysregulation hypothesis of MDD, optimal transcranial magnetic stimulation (TMS) sites are currently thought to be those that show functional connectivity to the sgACC such as the DLPFC (Fox et al., 2012).

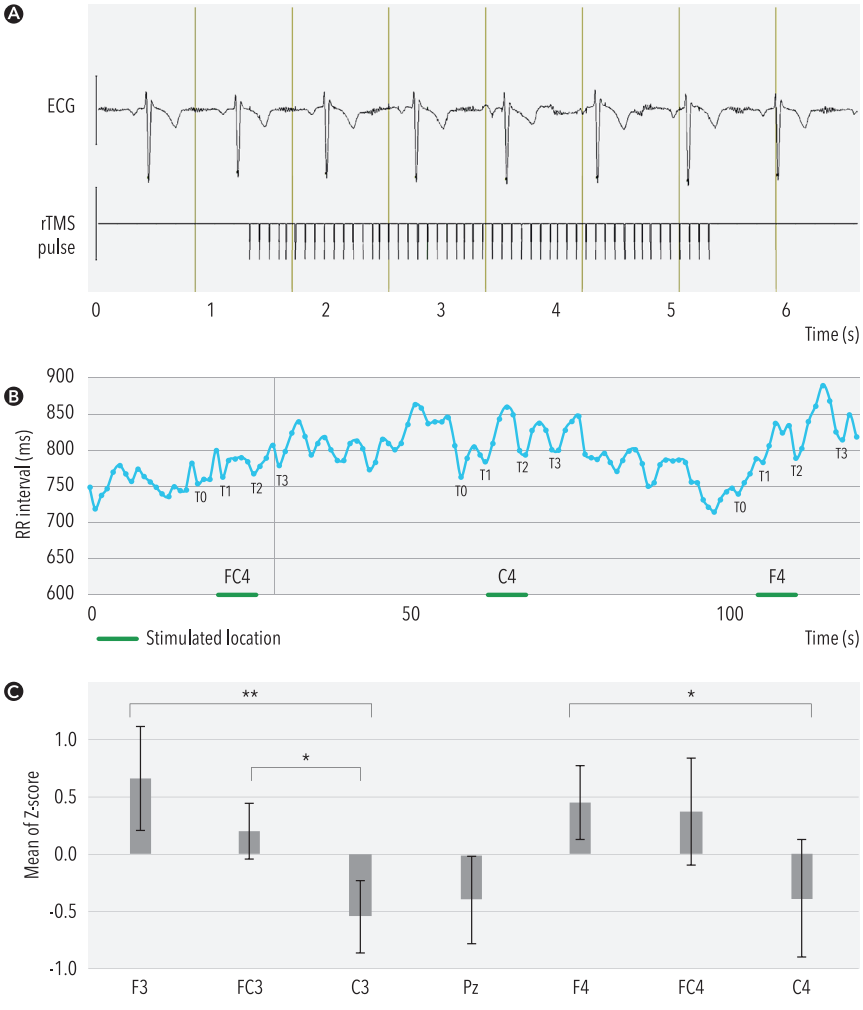
Current DLPFC localization methods for TMS are the 5cm rule, or BEAM-F3 method (Rusjan et al., 2010). These are valid on the group-level, but do only limitedly take individual variation into account (Fox et al., 2013). Functional and structural neuro-navigation methods do, but are expensive, time-consuming and navigate based on blood-oxygen-level-dependent (BOLD) signal or structural targets e.g. Brodmann areas and do not take knowledge about functional connectivity into account. Here, we propose a new functional neuronavigation method for localizing the frontal area representation of DLPFC-sgACC connectivity using HR, called: Neuro-Cardiac-Guided TMS (NCG-TMS).

Multiple studies now indicate that stimulation of the (sg)ACC, as well as transcranial direct current stimulation (tDCS) and TMS at the DLPFC, lead to HR decreases (Rossi et al., 2016; Makovac et al., 2016), indicative of parasympathetic activation. Similarly, stimulating the VN, directly activates the parasympathetic system.
Thus, we hypothesized that this influence on parasympathetic activity could be used as a functional outcome measure reflecting ade-

quate targeting of the DLPFC-sgACC network, similar to the motor evoked potential (MEP) as functional key measure for primary motor cortex stimulation. In a pilot-study, we set out to validate this notion by stimulating various 10-20 sites using repetitive (r)TMS and co-registering the stimulation pulses with the electrocardiogram (ECG). Here we report that on the group-level, in line with earlier work (Mir-Moghtadaei et al., 2015), F3 and F4 demonstrate the largest HR-suppression, the control sites (C3/C4/Pz) show no HR-suppression and FC4 and FC3 show an intermediate suppression. Furthermore, we report individual differences in the site that results in maximum HR-suppression.

MATERIAL AND METHODS

We recruited ten healthy volunteers. All subjects underwent 5 sec. trains of 10Hz rTMS (100% MT) to 7 different locations: left (F3/FC3/C3), right (F4/FC4/C4), and midline (Pz), with 30-second intervals. Each stimulation site was stimulated three times in a randomized order (same order for all subjects). An ECG electrode was attached on both wrists and one ground electrode was placed on one upper wrist. Recordings were obtained using an 'r-wave trigger' device (neuroConn, Ilmenau, Germany). During the stimulation protocol the subject was asked to sit relaxed and breath steadily. The data were imported in Brain Vision Analyzer where automatic R-peak detection was used to mark the R-peaks in the ECG (fig.1A, facing page). The ECG was then converted into R-R intervals (fig.1B). The peaks and troughs within this R-R signal reflect respiration-induced heart-rate modulation. By limiting the subsequent analysis to R-R values at the troughs, the effect of respiration was effectively removed, and the room to detect HR deceleration was maximized. Pre-stimulation troughs were labelled as T0. The first 3 troughs during and/or after stimulation were labelled as T1, T2, T3. The 3 trials per location were averaged and transformed into Z-scores (computed as (T1-T0)/sd(T0), where sd(t0) is the standard deviation of T0 across the 3 repeated stimulations for that location; same for T2 and T3). The normalization using sd(T0) was performed to reduce variance in effects of TMS due to individual differences and to the different timing for different



D

F3	FC3	C3	F4	FC4	C4
8/10	2/10	0/10	6/10	4/10	0/10
80%	20%	0%	60%	40%	0%

Figure 1: ***A)*** *Example of concurrent recording of ECG and TMS pulses;* ***B)*** *Example of ECG converted into R-R intervals. Note that in (A) only a single TMS period is shown, but in B three. The peaks and troughs of the respiratory waves were scored;* ***C)*** *Whole group mean z-scores of T1, T2 and T3. F3 and F4 express the largest HR deceleration. Error bars in standard error of the mean (SEM);* ***D)*** *percentage of subjects showing the largest HR deceleration per specific site, demonstrating inter-individual variability.*

locations. The Z-scores of T1-T3 were subsequently averaged. On group level, paired t-tests were performed for these average z-scores. On individual level, the best location was determined by the largest Z-score.

RESULTS

Ten subjects were included (23-61 years of age; 40% male). One subject was excluded from group analysis due to one extreme Z-score (>3 times the SD across subjects for that location (F4)). Between locations SD(T0)s did not differ. As can be seen in figure 1C (previous page), at the group-level, paired t-tests indicated a significant HR change between F3-C3 ($p=0.009$), FC3-C3 ($p=0.032$), and F4-C4 ($p=0.036$). The largest HR-deceleration was observed for both F3 and F4, followed by FC3 and FC4. Opposite effects were seen for C3, C4 and Pz. Furthermore, inter-individual variability was observed, where for some subjects the largest HR-decrease was found for FC3 (20%) or FC4 (40%) instead of F3 or F4, also see figure 1D on the previous page.

DISCUSSION

This pilot-study shows first preliminary evidence that HR can be used as functional outcome measure to identify specific frontal regions that likely reflect DLPFC-sgACC-VN network activation. On the group-level, we found a site-specific HR-deceleration for F3 and F4, as hypothesized. As can be seen in fig.1C, the effects showed site-specificity with largest effects for F3/F4, followed by FC3/4 and none or reversed effects for control sites overlaying the motor (C3/4) and parietal cortex (Pz), in line with previous results (Makovac et al., 2016; Foerster et al., 1997). Furthermore, the results show a perfect mirror image for left vs. right hemisphere. At individual level, for 20% of subjects HR decelerated stronger after stimulation at FC3 and for 40% at FC4 confirming our expectation of inter-individual variability.

These findings indicate that – in line with the notion put forward by Fox and colleagues (Fox et al., 2012) – this method of Neuro-Cardiac-Guided TMS could potentially be used as a functional outcome measure to localize an individualized stimulation location for rTMS treatment in MDD. Furthermore, such a method could eventually be used in similar ways as the motor threshold for the primary motor cortex and could assist in establishing individual stimulation thresholds for DLPFC-sgACC stimulation, investigate angular sensitivity and investigate in more detail neuroplasticity effects, that are now all modelled on the motor system. Further studies need to validate these results in larger groups and patients and establish the association with treatment response (i.e. do MDD responders exhibit associated HR decreases during stimulation, suggestive of 'accurate targeting'?). With this individualized approach relying on a functional outcome measure, TMS targeting could become more consistent and possibly, could enhance response to DLPFC-TMS treatment.

CHAPTER 5:

NEURO-CARDIAC-GUIDED TMS (NCG-TMS): A REPLICATION AND EXTENSION STUDY

Submitted as: *Iseger, T.A., Padberg, F., Kenemans, J.L., van Dijk, H., Arns, M. 2019b. Neuro-cardiac-guided TMS: a validation study (Under review for publication in Neuroimage).*

Author contributions: MA, TAI, FP and JLK conceptualized the study, TAI collected data, performed data-analyses and wrote the initial draft for the manuscript, MA supervised the study. All authors took part in setting up the study and writing the manuscript.

ABSTRACT

Background

In a recent pilot study, Neuro-Cardiac-Guided TMS using 10 Hz trains (NCG-TMS) was studied for its potential to identify the correct cortical target for rTMS treatment in Major Depressive Disorder (MDD). This method is founded on the interplay between the depression network and the heart-brain connection. It was found that stimulation at F3/4 (10-20 system EEG locations) led to significant heart rate slowing, relative to C3/4.

Objective

In the current study, we aimed to replicate these results in a larger sample, and additionally investigate dose-response effects.

Methods

To this end, two types of cohorts were used: 50 healthy controls, and a patient sample of 33 MDD patients. Both cohorts received an NCG-TMS assessment. Additionally, the healthy control cohort received a second session in which dose-response relationships, test-retest reliability, and the effect of deep TMS were studied. Additionally, for 30 healthy controls, the effects of iTBS stimulation (NCG-iTBS) were assessed.

Results

The current study resulted in a successful replication of the pilot study in both cohorts, showing heart rate decelerations for F3/4, which significantly differed from C3/4. Furthermore, this method shows high internal consistency, as well as indications for a dose-response relationship, albeit only for absolute machine output, and not for %MT. Finally, it was shown that deep TMS had similar effects on heart rate, compared to the figure-of-eight coil. NCG-iTBS requires further study for safety and site-specificity.

Conclusions

These results indicate that we were able to transsynaptically stimulate the autonomic nervous system, and confirm that the NCG-TMS method is adept in detecting the correct target to engage the heart-brain network, potentially involved in MDD.

INTRODUCTION

Major Depressive Disorder (MDD) is a chronic, heterogeneous psychiatric disorder often with a remitting and relapsing or chronic course, affecting 4.7 percent of the global population at any given time (Whiteford et al., 2013), thereby indicating the widespread burden in today's society in terms of both psychological and economic costs (Kessler et al., 2012). Despite the variety of available treatments (e.g. medications and psychotherapy), up to 30-40% of patients fail to enter remission (Kessler and Bromet, 2013). For MDD, the use of antidepressant medication is a first-line treatment, (Anderson et al., 2008), but neuromodulation treatments such as repetitive Transcranial Magnetic Stimulation (rTMS), transcranial Direct Current Stimulation (tDCS), Deep Brain Stimulation (DBS) and Vagal Nerve Stimulation (VNS) also show promising clinical benefit in MDD (Donse et al., 2017; Brunoni et al., 2017; Brunoni et al., 2017; Mayberg et al., 2005; Schlaepfer et al., 2013; Rush et al., 2000). With these treatments, brain structures that are affected in depression such as the dorsolateral prefrontal cortex (DLPFC), the dorsomedial prefrontal cortex (DMPFC), the subgenual anterior cingulate cortex (sgACC) and vagus nerve (VN) are being stimulated. This was shown to be associated with symptom improvement in MDD (Downar et al., 2014; Downar and Daskalakis, 2013; Mayberg et al., 2005). The underlying mechanisms of these neuromodulation treatments, suggest altered network connectivity between the DLPFC, (sg)ACC and VN, which may be mediating clinical response (Liston et al., 2014; Fox et al., 2012). The VN, part of the parasympathetic branch of the autonomic nervous system, influences bodily functions such as respiration and heart rate (HR) and stimulation of the VN consistently leads to HR decelerations (Buschman et al., 2006). Interestingly, several studies have also reported HR deceleration after stimulation of the DLPFC using rTMS and tDCS (Makovac et al., 2016), indicating that there is a connection between the DLPFC and the heart. Furthermore, it has been observed that HR in MDD is

often dysregulated, expressed in overall higher HR and lower heart rate variability (HRV) (Licht et al., 2008; Koenig et al., 2016; Castaldo et al., 2015). In addition, both these measures have been reported to normalize after neuromodulation treatment (Kemp et al., 2010).

Currently, there are several methods for localizing DLPFC for TMS. First, there are structural methods like the 5cm rule or BEAM-F3 method (Rusjan et al., 2010). Although these are valid on the group-level they are limited in their capability to take individual variation into account (Fox et al., 2013). Structural neuro-navigation methods using MRI do recognize individual variation, but these methods are costlier and more time-consuming. Moreover, these methods navigate based on blood-oxygen-level-dependent (BOLD) signal or structural targets (e.g. Brodmann areas) and do not consider knowledge about functional connectivity. Secondly, neuro-navigation methods using functional connectivity have been proposed. For example, one method employs functional connectivity between the DLPFC and the sgACC, where the sgACC is used as a seed region to identify the appropriate prefrontal area that exhibits best functional connectivity to the sgACC (Fox et al., 2012). However, there is considerable inter-individual variation in functional connectivity patterns when assessed on different occasions (Ning et al., 2018). To overcome the described problem of lack of individualization, as well as expenses, we recently proposed and tested another method for identifying the right cortical target for TMS. In a pilot study we showed the potential benefit of using the interplay between the depression network and the heart-brain axis, called Neuro-Cardiac-Guided TMS (NCG-TMS) (Iseger et al., 2017, Chapter 4). We stimulated several cortical brain regions with 10Hz TMS trains (n=10). Relative to C3/C4, stimulation at F3/F4 led to significant HR deceleration, but there was individual variation; for some subjects FC3/FC4 led to the most pronounced HR decelerations, showing the potential to use HR as a functional outcome measure reflecting adequate targeting of the depression network.
In order to investigate whether this method of NCG-TMS is valid as a target engagement method, the following aspects need to be tested: 1) replication of the pilot study in a larger sample, 2) establish individual test-retest reliability, 3) establishing a dose-response relation-

ship, i.e. do higher TMS intensities lead to larger effects on HR, and 4) treatment outcome, i.e. does improved localization lead to better treatment response?

The current study was set up in order to address the first three points. Two cohorts were used in order to replicate the pilot study; a controlled study in 50 healthy controls, and an open-label patient sample of 33 MDD patients who received regular rTMS, where as part of their treatment a standard NCG-TMS assessment was undertaken. Both cohorts received an NCG-TMS assessment in order to replicate the pilot study. The healthy control group received a second session, where dose-response relationships and test-retest reliability were assessed. In addition, a dTMS H1-coil was used in order to study the effects of different coil designs (Levkovitz et al., 2015). It was hypothesized that on the group level, F3/F4 and FC3/FC4 would lead to HR decelerations, similar to Iseger et al., (2017); Chapter 4, and that most subjects would show maximum HR decelerations for these locations. Additionally, the usability of NCG using iTBS stimulation (NCG-iTBS) was assessed.

MATERIAL AND METHODS

STUDY DESIGN AND PARTICIPANTS

COHORT 1 (HEALTHY CONTROL GROUP)

In our previous study, stimulating the left hemisphere, location F3 was found to be the best location to cause a decrease in HR (total of three locations, n=10, $d_{(F3\ vs\ C3)}$ = 1.01), and in the right hemisphere the F4 location ($d_{(F4\ vs\ C4)}$ = 0.66). A power calculation (GPower 3.1.9.2) was performed based on a 1-tailed t-test using two dependent means, with alpha at 0.05 and power at 0.90. For an effect size of 0.66 the required sample size was 22 (for each hemisphere), hence a sample size of 25 subjects per hemisphere (assuming a 10% drop-out rate) was chosen, resulting in a total study sample of n=50. This study was

approved by the local Institutional Review Board (IRB, Utrecht University, Netherlands; NL63092.041.17) and registered on ClinicalTrial.gov (ID: NCT03652597).

Thus, 50 healthy subjects were collected. Due to preliminary data indicating more profound effects of iTBS, it was decided after consultation with the IRB, to change the protocol to iTBS instead of 10Hz rTMS. Therefore, the healthy control sample consisted of a sample of 30 subjects using 10 Hz, and 30 subjects using iTBS stimulation. Ten subjects were tested both with 10Hz and iTBS. All participants provided written informed consent. Details and results of the NCG-iTBS sample can be found in the supplementary material, and key results will be summarized in the manuscript.

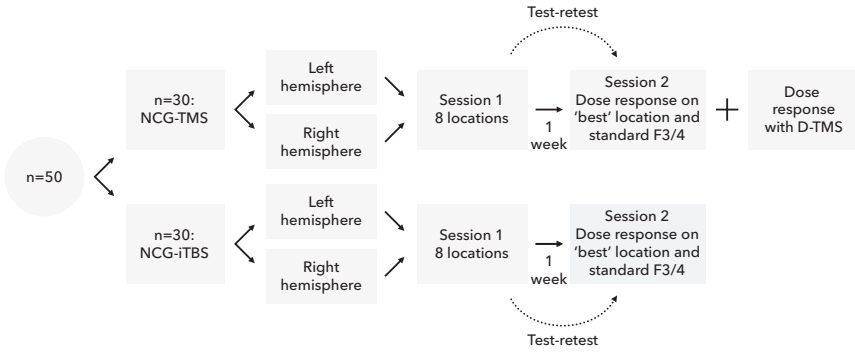


Figure 1: *Flow chart of the NCG-TMS study (n=50 adapted to n=30, see methods).*

COHORT 2 (MDD PATIENT GROUP)

Thirty-three patients with a diagnosis of MDD, who received rTMS for treatment of MDD, underwent an NCG-TMS assessment as part of their rTMS treatment. This assessment was undertaken either at session 10 of treatment, or after unsuccessfully completing treatment (non-response). After the NCG-TMS assessment, treatment was continued as usual, or optionally continued experimentally at the NCG-TMS location. All patients provided written informed consent.

PROCEDURES

COHORT 1 (HEALTHY CONTROL GROUP)

Participants were randomized to NCG-TMS over the left or right hemisphere. For NCG-TMS, single 10Hz trains of 5sec. each were applied to 8 different cortical 10-10 scalp locations on the left: F3, FC3, F1, F5, FC5, C3, FP1, AF3; or right hemisphere: F4, FC4, F2, F6, FC6, C4, FP2, AF4 with a Magstim Super Rapid^2 and a 70mm figure-of-eight coil (The Magstim Company Ltd., Whitland, UK). Every location was stimulated 3 times in random order across all sites (inter-train-interval between two locations: 30sec). A custom EEG cap without electrodes (ANT Neuro) was used to locate the 10-10 system locations. Motor threshold (MT) was determined prior to stimulation. Stimulation at all sites was applied at 100% of the MT. During stimulation, the participant was sitting in a relaxed upward position, was instructed to breath normally and to avoid talking, since this could influence HR. The participant was asked to refrain from drugs and alcohol for 24hrs as well as from caffeine and smoking for 2hrs preceding the sessions. In session 2, the subject received 10Hz trains on 2 different locations: the standard F3/F4 location, and their individual best NCG-TMS location which was obtained from session 1. After again determining the MT, the locations were stimulated at 70, 80, 90, 100 and 110% MT, 3 times at every intensity.

Subsequently, the same protocol was performed with deep TMS (dTMS). Using a H1 coil (Brainsway Ltd, BRIN, Tel Aviv, Israel), after determining again the MT, the standard location was stimulated again at the 5 different stimulation intensities with 3 trains of 10Hz TMS, in order to investigate whether deep TMS had similar effects on HR.

COHORT 2 (MDD PATIENT GROUP)

NCG-TMS was applied at 10Hz trains of 5sec. to 7 different cortical 10-10 system locations on the right: F4, FC4, F2, F6, FC6, FP2, AF4 with either a Deymed DuoMAG XT-100 (Deymed Diagnostic s.r.o., Hornov, Czech Republic) or Magstim Super Rapid^2 (The Magstim Company Ltd., Whitland, UK), both with a 70mm figure-8 coil. C4 was not used for patients as this site only served as a control location in the controlled study and was believed to be only additional burden for patients. Every location was stimulated 3 times in random order across all sites (intertrain interval between two locations: 30sec.). A custom EEG cap without electrodes (ANT Neuro) was used in order to locate the (individual) 10-10 system locations. MT was determined prior to stimulation. Stimulation was applied at 100% MT. During stimulation, the patient was asked to sit relaxed, breath normally and to avoid talking, since this could influence HR. The patient was asked to refrain from caffeine and smoking for 2hrs preceding the sessions.

PHYSIOLOGICAL DATA ACQUISITION

ECG data were co-registered in real-time with the TMS pulses and collected using the NCG-ENGAGE HR (neuroConn, Ilmenau, Germany). ECG was measured with three electrodes placed diagonally on the chest, with the ground electrode placed in the middle.

DATA PROCESSING

Data was processed similar to Iseger et. al (2017; Chapter 4) but automated by the NCG-ENGAGE HR device. R-peaks within the ECG were scored and the interval between two R-peaks were calculated, creating RR interval data. Since breathing has a significant effect on HR, especially at short timeframes, only the troughs of the RR intervals were used, representing HR maxima. The pre-stimulation trough was labelled T0, and the first 3 troughs after the start of stimulation T1, T2 and T3 (see figure 2, facing page). In case of lower quality recordings where the NCG-TMS device could not label R-peaks correctly,

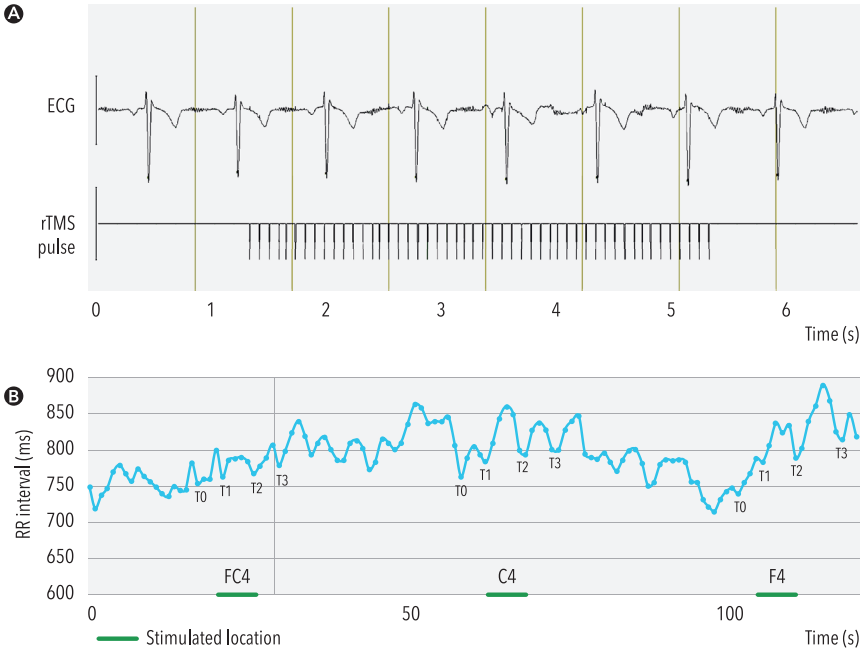


Figure 2: *Example of the NCG-TMS analysis method. ECG is recorded while simultaneously recording TMS stimulation pulses* ***(2A)****. R-peaks from ECG where converted into an RR interval plot* ***(2B)****. The troughs before stimulation where labelled as T0, whereas the 3 troughs after the start of stimulation where labelled as T1, T2, T3.*
Figure adapted from Iseger et. al., 2017, Brain Stimulation.

R-peaks were manually scored when possible, using Brain Vision Analyzer (Brain Products), and further analyzed using Matlab function (The Mathworks), which was similar to the NCG ENGAGE HR.

STATISTICAL ANALYSIS

RR intervals for the three trials per location were averaged and transformed into Z-scores (computed as (T1-T0)/$SD_{(T0)}$, where $SD_{(T0)}$ is the standard deviation of T0 across the three repeated stimulations for that location; same for T2 and T3). The normalization using $SD_{(T0)}$ was performed to reduce variance in effects of TMS due to individual differences and to the different timing for different locations. The

Z-scores of T1-T3 were subsequently averaged. The resulting Z-scores were evaluated on group-level, both for the healthy controls and the MDD patients, in order to replicate Iseger et al (2017; Chapter 4).

Since we collected 30 instead of 50 healthy controls, this resulted in approximately 15 subjects per hemisphere. The power calculation indicated that at least 22 subjects were required per hemisphere, thus left and right hemisphere were combined to obtain enough statistical power. One tailed paired t-tests were used to test the primary hypothesis: Stimulation at F3/4 leads to significantly larger HR decelerations relative to C3/4 (as found in our pilot study (Iseger et al., 2017; Chapter 4)) and secondary: Stimulation at FC3/4 leads to significantly larger HR deceleration relative to C3/4. Cohen's D effect sizes were calculated for the means between locations. All other sites were tested in an exploratory fashion and topographically plotted, but it was expected that on the group level, all would show HR accelerations rather than decelerations. This assumption was made because given the sensation of TMS (uncomfortable, sometimes painful, potentially stimulating surrounding muscles), TMS would in general lead to HR accelerations (sympathetic activation) instead of decelerations, especially in the TMS naïve healthy control group.

Test-retest reliability was tested by correlating RR interval change at the F3/F4 locations from session 1 to session 2 (at 100%MT), and paired t-tests. Additionally, Intraclass Correlation Coefficient (ICC) was obtained by running reliability analysis. Dose-response relationships for HR deceleration were tested by correlating stimulation intensity expressed as a) percentage MT and b) as percentage stimulator output. The likelihood of dTMS to decrease HR were compared to the likelihood of the figure-8 coil to decrease HR, this was compared both at 100%MT and 110%MT.

RESULTS

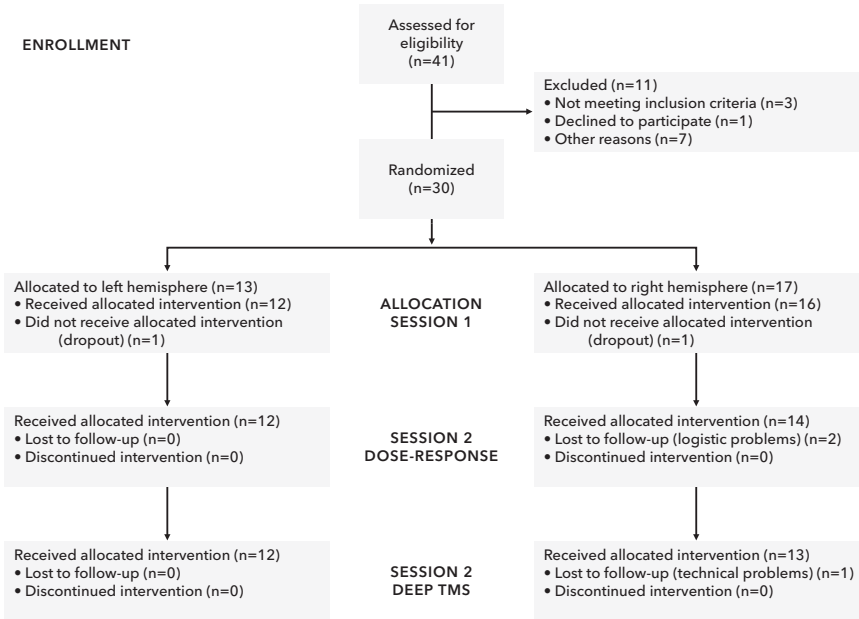


Figure 3: *CONSORT Flow diagram of the NCG-TMS healthy controls study*

Table 1: *Subject characteristics for cohort 1 (healthy controls) and cohort 2 (MDD patients). The separation between left and right hemisphere is only based on cohort 1. Shown are the number of males (N males) as absolute number and percentage of total, age in years (y) (mean (SD)), motor threshold (mean (SD)) for session one and two and deep TMS, head size in centimeters (cm) (mean (SD)) and nasion-inion distance in centimeters (cm) (mean (SD)).*

	Total (cohort 1) (n=28)	Total (cohort 2) (n=33)	P-value	Left hemisphere (n=12)	Right hemisphere (n=16)	P-value
N male	12 (43%)	15 (45%)		4 (33%)	8 (60%)	
Age (y)	31.04 (8.68)	52.01 (12.32)	p<.001	34.83 (8.26)	28.19 (8.09)	p=.042
MT session 1	63.43 (8.73)	68.85 (13.02)		62.33 (11.02)	64.25 (6.82)	
MT session 2	62.31 (9.41)	\		61.83 (11.43)	62.71 (7.71)	
MT deep TMS	53.42 (6.53)	\		53.67 (7.66)	53.21 (5.67)	
Headsize (cm)	56.45 (2.17)	56.37 (1.99)		56.38 (2.64)	56.50 (1.83)	
Nasion-inion (cm)	35.20 (1.58)	34.50 (1.76)		34.92 (1.79)	35.41 (1.42)	

SUBJECT CHARACTERISTICS

For cohort 1, data from 30 healthy control subjects were collected. In total, 28 subjects were included for analyses, of which 12 subjects were allocated to stimulation in the left hemisphere and 16 to stimulation to the right hemisphere (mean age: 31.0, ±6.68, 12 males), see table 1 and figure 2. Two subjects did not complete session 2. No side effects or adverse events were reported.
For cohort 2, data from 33 MDD patients were collected (mean age: 52.01, ±12.32, 15 males), and all were included in the analysis. No side effects or adverse events were reported.

REPLICATION

The primary hypothesis was confirmed, i.e. we found a significantly larger HR deceleration for F3/F4 compared to C3/C4 ($t(27)=2.18$, $p=.038$, $d=.463$) and for FC3/FC4 compared to C3/C4 ($t(27)=1.90$, $p=.069$, $d=.487$), thereby replicating the results from our pilot study (Iseger et al., 2017; Chapter 4). Post-hoc analysis with location as within-subjects factor and hemisphere as between-subjects factor, indicated no differences between hemispheres (F3/F4-C3/C4: $p=.561$; FC3/FC4-C3/C4: $p=.941$). All other locations showed HR accelerations as can be seen in figure 4A (facing page). The spatial distribution can be found in fig. 5A (facing page) (with all data collapsed over one hemisphere for illustrative purposes).

On the individual level, there was an equal number of subjects showing the largest HR deceleration for F3/F4 (18%), as to F1/F2 (fig. 6A, page 106), indicating inter-individual variation for optimal target sites, also in agreement with our pilot results.
Within the patient group, similar trends were observed, showing HR deceleration for F4 and FC4 (fig. 4B) on the group-level. Paired t-tests were not conducted as C4 was not included for this cohort. All other locations showed HR accelerations on the group level (fig. 5B).
On the individual level, most subjects showed the largest HR deceleration for F4 (27%), followed by FC4 (21%) and F2 (18%) (fig. 6B).
As can be seen in figure 4A and 4B, the patterns of response are rather comparable at the group level for controls and MDD patients. While site C3/C4 was not assessed for patients, the effect sizes for the con-

trasts between F3/F4 and FC5/FC6 (d_{CTRL}=0.37 and d_{MDD}=0.39) as well as FC3/FC4 and FC5/FC6 (d_{CTRL}=0.38 and d_{MDD}=0.31) were rather similar for controls and the MDD sample further confirming similarity on the group level.

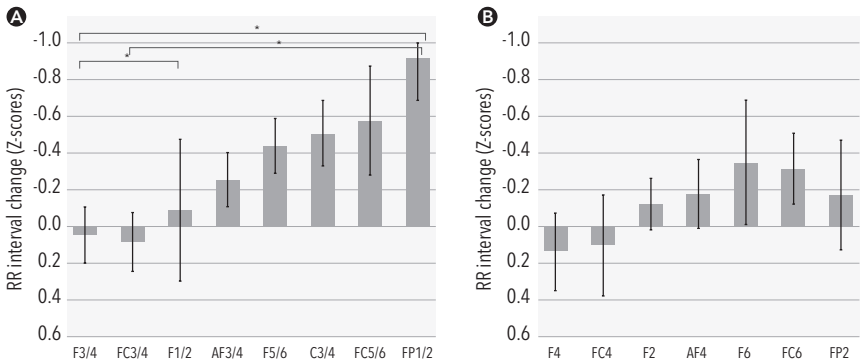


Figure 4: *Group level Z-scores of RR interval changes for the healthy control group (cohort 1)* ***(4A)*** *and the MDD sample (cohort 2)* ***(4B)****. The larger the Z-score, the larger the RR interval change (equaling HR deceleration). Note that the Y-axis is inversed in order to represent HR increases as an upward bar and HR decreases as a downward bar. Only for F3/4 and FC3/4 HR decelerations were observed, whereas all other sites show accelerations.*

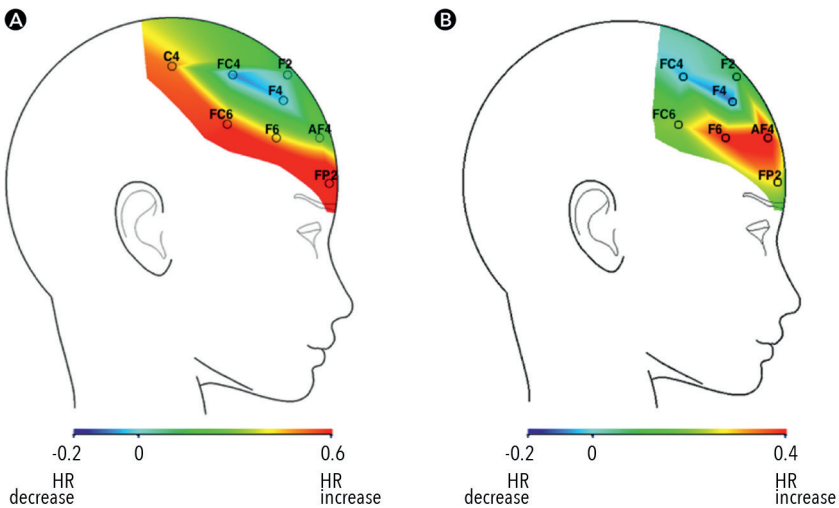


Figure 5: *Group level topographical plots of RR interval changes for the healthy control group (cohort 1)* ***(5A)****, and the MDD sample (cohort 2)* ***(5B)****. The scale represents the inversed z-scores, blue indicates HR deceleration, orange/red indicates HR acceleration, as a result of 10 Hz TMS. Note the similarity in patterns contrasting HR deceleration (F4-FC4) vs. more lateral sites being associated with acceleration.*

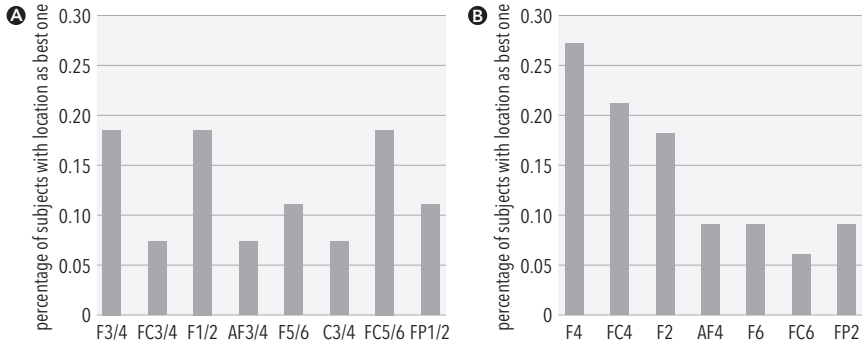


Figure 6: *Percentage of subjects that showed the largest HR decelaration at the respective target site indicated here, for* ***A****, the healthy control group (cohort 1) and,* ***B****, the MDD sample (cohort 2).*

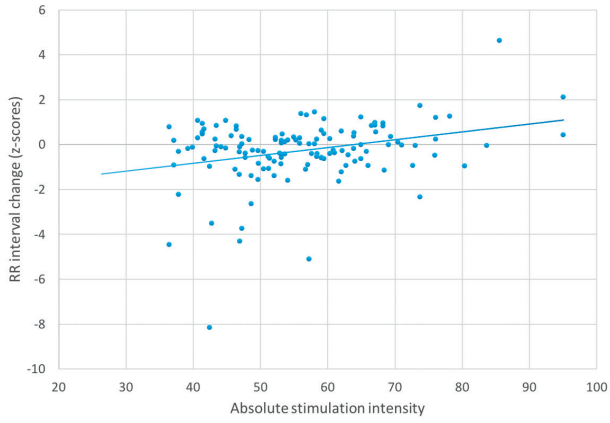


Figure 7: *Correlation plot between RR interval change in z-scores and TMS intensity. The blue dots represent 5 stimulation intensities for each individual subject (n=26, r(129)=.297, p=.001, r²=0.17).*

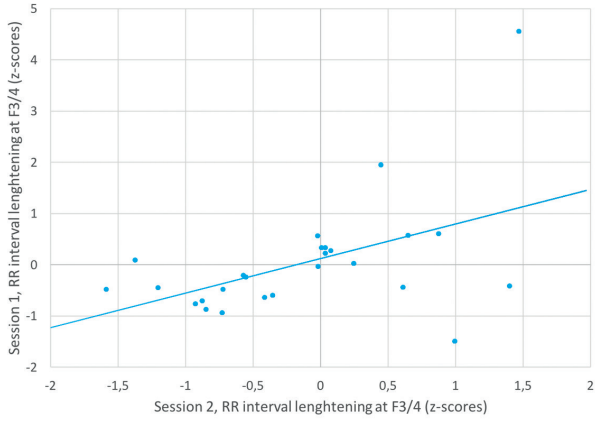


Figure 8: *Correlation plot between RR interval change in session 1 with RR interval change in session 2, during stimulation of the F3/4 region (r=.475, p=.014, r²=0.23).*

TEST-RETEST RELIABILITY

In order to asses test-retest reliability, z-scores from session 1 were correlated with z-scores at session 2. This was tested for F3/F4, since this location was available for every subject and both assessments. A significant correlation of r(25)=.475 (p=.014) was observed, explaining 23% of the variance, thus indicating internal consistency (fig. 8, facing page). A paired sample t-test indicated that there were no significant differences in the amount of HR deceleration (t(25)=.86, p=.399). Additionally, 46.43% of the subjects expressed HR decelerations during session 1, while 42.31% of the subjects expressed HR decelerations during session 2, with an overlap of 73% suggesting sound stability on the individual level. Reliability analysis resulted in an intraclass correlation coefficient (ICC) of .527.

DEEP-TMS

Correlating RR interval lengthening during dTMS with RR interval lengthening at F3/F4 using the figure-8 coil yielded a significant correlation (r(24)=.424, p=.035) at 100%MT. dTMS did not correlate with other locations tested with the figure-8 coil, indicating site-specificity. Additionally, 45.83% of the subjects expressed HR decelerations during dTMS, while 46.43% of the subjects expressed HR decelerations during stimulation at F3/F4 with a figure-8, showing similar effects of both methods on HR. Paired sample t-tests indicated that there were no significant differences in the amount of HR deceleration (t(24)=1.18, p=.248). At 110%MT, similar effects were found.

NCG-ITBS

Detailed methods and results of the NCG-iTBS can be found in the supplementary material. In summary, the results using 1 min. of iTBS stimulation yielded both on the group level, as well on a within-subject comparison in ten subjects (where NCG-TMS and NCG-iTBS were both applied) more lateralized sites that demonstrated the clearest HR deceleration (FC5/6 and F5), also see figure s3. Furthermore, 5/27

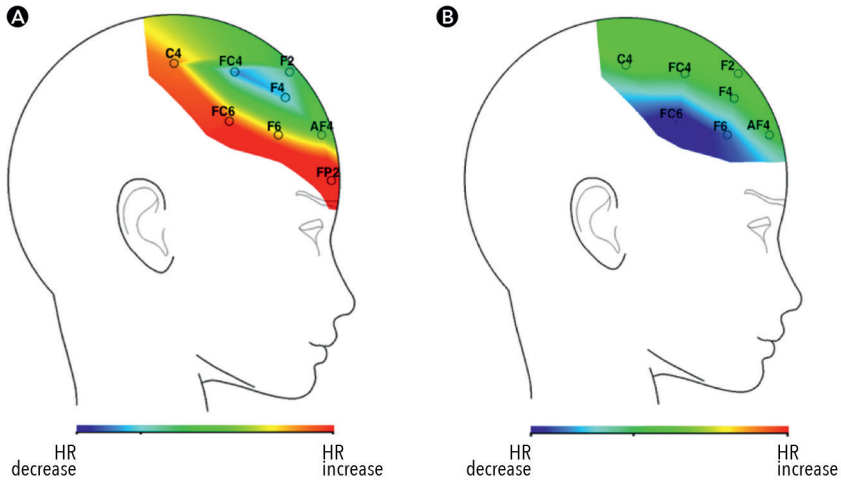


Figure 9: *Group level topographical plots of RR interval changes for the NCG-TMS treatment arm (**9A**), and the NCG-iTBS treatment arm (**9B**). Blue indicates HR deceleration, orange/red indicates HR acceleration.*

subjects (19%) discontinued NCG-iTBS due to adverse events such as painfulness (3), strong emotional reactions (1) and lightheadedness (1), suggesting caution with applying iTBS to various prefrontal locations, whereas no such adverse events were seen for NCG-TMS using 10 Hz trains. These results precluded us from combining the two samples and concluding the NCG-iTBS method requires further study.

DISCUSSION

Here, we present results that replicate our earlier findings, supporting the validity of the NCG-TMS approach based on 10 Hz trains. We show that on the group level, the largest HR decelerations were found at F3/F4 and FC3/FC4 in two independent samples of healthy controls and MDD patients. Furthermore, this method shows sound test-retest reliability, and a dose-response relationship with the percentage machine output, but not with %MT. The results for NCG-iTBS, demonstrated more profound HR decelerations, albeit with a different topography requiring further study and warranting caution due to the adverse events reported.

Similar to Iseger et. al. (2017; Chapter 4), HR decelerations were found for F3/F4 and FC3/FC4 on the group-level in both samples. All of the other tested locations show HR accelerations rather than decelerations. In this study, left and right hemisphere conditions were merged in order to obtain adequate statistical power. Post-hoc analyses yielded no significant differences between hemispheres, although for the left hemisphere a trend was observed towards a more anterior location. This was also found with neuronavigation methods (Fitzgerald et al., 2009) and associated with better treatment response (Herbsman et al., 2009). The finding that HR decelerations were only found for F3/F4 and FC3/FC4 indicates that not only were we able to transsynaptically stimulate the autonomic nervous system, it also confirms that the method of NCG-TMS is an efficient manner for detecting these site-specific rTMS induced HR decelerations. The HR decelerations observed in this study were of medium effect size (d=0.46) and smaller compared to the pilot study, where an average HR deceleration of only 1.85 BPM was found.

The NCG-TMS method is based on three rounds of 10Hz 5sec. trains applied to every location in a random order, with a 30sec. interval. The troughs of the RR interval are used in order to elliminate the effect of respiration and the first trough before and three troughs after the start of stimulation are used to calculate z-scores. The short timeframes that were taken per round, will inevitably result in more variability and lower accuracy. The effect of respiration on HR was corrected for as much as possible, but as a result of taking three troughs after the start of stimulation, timeframes differ and are not standardized. Optimization of the method could lie in the length of timeframes that were taken after start of stimulation, e.g. taking all the troughs in a fixed timeframe; peaks instead of throughs for the RR interval plot; or no troughs at all but a linear regression line over a fixed timeframe. These options were analysed and tested, however no substantial improvements were found (unpublished results). Furthermore, z-scores are used to combine and normalize the data but may not be nescessary or optimal. Test-retest reliability showed that the use of z-scores led to a correlation of r=.475. However, when re-running this analysis without z-scores, a similar significant correlation was obtained of r=.423. Other optimization options may lie in the stimulation protocol, i.e. using longer than 5sec. stimulation at 10Hz, continous 1Hz, or iTBS, in

order to better control for the respiratory effects. Results from NCG-iTBS using a 1 minute iTBS stimulation, indeed confirmed a more pronounced effect and the 1-minute stimulation duration also resolves the respiratory issue. However, this NCG-iTBS method requires further study and replication. The more pronounced heart rate effects may be attributed to the time-period in which heart rate decelerations were analyzed. For example, for iTBS this was a fixed timeframe of 1 minute, while for 10Hz, these were variable timeframes of around 20 seconds, while taking only the troughs of the RR interval. Thus, the methods may not be completely comparable.

Dose response effects were only significant when using absolute values of machine output intensity rather than the percentage of MT. This may be due to differences in cortical structure and excitability between motor and non-motor regions as was also shown for the comparison of motor and phosphene thresholds (Gerwig et al., 2003; Stewart et al., 2001). These data suggest that the stimulation threshold established by motor threshold is not directly applicable to the frontal cortex, and thus advocates for establishing individual frontal excitability thresholds. Thus, NCG-TMS could not only be used to identify the optimal stimulation area, but also to identify the individualised *frontal* stimulation intensity.

Regarding dTMS, it was shown that the use of a H1-coil resulted in a similar likelihood of reducing HR, compared to the figure-8 coil at 100% and 110%MT. HR changes correlated only to F3/F4 and not with other sites, confirming the preferential overlap of dTMS with areas regularly targetted by figure-8-coils. We did not test dTMS at 120% which is a limitation of the study, since that stimulation intensity is most often employed, also resulting in most optimal frontal activation (Levkovitz et al., 2009; Levkovitz et al., 2015), therefore no firm conclusions can be drawn regarding the comparison between dTMS and the figure-8 coil.

A further question that deserves attention, is whether stimulation sites obtained with the NCG-TMS method will eventually result in improved clinical outcomes by immediately targeting the heart-brain network, which needs to be further prospectively tested in larger samples.

Not all subjects show HR decelerations. TMS gives an unpleasant sensation, sometimes painful, which consequently may lead to sympathetic activation and thus HR accelerations, rather than a deceleration. As such, the amount of HR change may not be informative if not compared to other (control) locations. Thus, an important notion deserving further study is that not the location showing a HR deceleration is the most effective location, but the location showing the least HR acceleration, partly depending on the subjective aversiveness of the stimulation itself.

The heart rate deceleration found at FC5/6 and F5/6 with NCG-iTBS was unexpected but raises some interesting hypotheses. For example, using cTBS, Pollatos and colleagues stimulated a region located between FC6-F6-F8, which is located just beneath our stimulated location, and aiming at the insular cortex (Pollatos et al., 2016). The group investigated heart beat evoked potentials and found reduced amplitude of these potentials with cTBS. Another hypothesis is that not the insular cortex is stimulated, but the trigeminal nerve. Stimulation of the trigeminal nerve has been used to treat MDD (Cook et al., 2016) and has previously also been associated with heart rate decelerations (Meuwly et al., 2015). This makes sense, since stimulation on both FC5/6 and F5/6 often leads to muscle activity in the jaw, which can be a result of trigeminal nerve stimulation. Irrespective of which theory may is true, it is a fact that with 10Hz rTMS, stimulation at these regions did not lead to heart rate decelerations, indicating that this may be a frequency specific effect. Since iTBS is designed to mimic specific endogenous theta frequencies in the brain, it is not unlikely that it may have different effects compared to 10Hz rTMS. However, this activation of the mastoid muscles also led to uncomfortable sensations, which were at least for 3 subjects too painful to continue the assessment. Since NCG-iTBS results were inconclusive, it is not recommended for targeting the depression network, before further studies have been conducted.

Finally, besides the use of HR as a measure for target localization, it can be used as real-time validation of coil-to-scalp contact during TMS treatment. HRV may also be informative, as it was shown previously to

also be associated with neuromodulation effects (Udupa et al., 2007). Finally, the focus of this study was the use of NCG-TMS for treatment optimalization in MDD, however this method could possibly be translated to other psychiatric disorders that impact the autonomic nervous system as well.

CONCLUSION

To conclude, this replication of the earlier results shows that stimulating specific prefrontral areas using the NCG-TMS approach based on 10 Hz stimulation trains has an impact on HR, in a site-specific manner. This indicates effects on autonomic function, but also shows that the NCG-TMS method is sufficient in detecting rTMS induced HR changes, and that these changes are relatively stable within subjects. Potentially, NCG-TMS could also be used to determine the ideal stimulation intensity.

SUPPLEMENTARY MATERIAL

The primary aim of this study was to replicate the earlier Iseger et al. (2017; Chapter 4) results, for which all data are presented in the main manuscript. After 30 subjects and based on preliminary data (also see Iseger et. al., 2019c, Chapter 7) it was decided to switch to 1-minute iTBS per site instead of three 10 Hz TMS trains of 5 sec., since the effects of iTBS were more pronounced. However, as will become evident from the results below, iTBS stimulation resulted in a rather different topography, even within-subject, precluding us from combining the data. Given the 10 Hz NCG TMS data replicate the earlier findings and an independent succesful replication has also been conducted (Kaur et. al., 2019, Chapter 6), we here present the iTBS results as preliminary evidence requiring further replication and extension.

METHODS

PROCEDURES NCG-ITBS

Participants were randomized to NCG-iTBS over the left or right hemisphere (fig. 1). For NCG-iTBS, one minute of iTBS was delivered to 7 different cortical 10-10 scalp locations on the left: F3, F1, F5, FC5, C3, AF3; or right hemisphere: F4, F2, F6, FC6, C4, AF4, with a MagVenture MagPro R30 or a Deymed XT-100 both equipped with a 70mm figure-of-eight coil. Between every location a resting period of 1-2 minutes was accommodated to allow the HR to stabilize. A custom EEG cap without electrodes (ANT Neuro) was used to locate the 10-10 system locations. Motor threshold (MT) was determined prior to stimulation. Stimulation at all sites was applied at 100% of the MT. During stimulation, the participant was sitting in a relaxed upward position, was instructed to breath normally and to avoid talking, since this could influence HR. The participant was asked to refrain from drugs and alcohol for 24hrs as well as from caffeine and smoking for 2hrs preceding the sessions. In session 2, the subject received 1 minute of iTBS stimulation on 2 different locations: the standard F3/F4 location, and their individual best NCG-iTBS location which was obtained from session 1. After again determining the MT, these locations were stimulated at 70, 80, 90, 100 and 110% MT for 1 minute per location.

PHYSIOLOGICAL DATA ACQUISITION

ECG data were co-registered in real-time with the TMS pulses and collected using the NCG-ENGAGE HR (neuroConn, Ilmenau, Germany). ECG was measured with three electrodes placed diagonally on the chest, with the ground electrode placed in the middle.

DATA PROCESSING

R-peaks within the ECG were scored and the interval between two R-peaks were calculated, creating RR interval data. In case of lower quality recordings where the NCG-TMS device could not label R-peaks correctly, R-peaks were manually scored when possible, us-

ing Brain Vision Analyzer (Brain Products). The slope of RR intervals across each minute of stimulation was calculated.

STATISTICAL ANALYSIS

One tailed paired t-tests were used to test the primary hypothesis: Stimulation at F3/4 leads to significantly larger HR decelerations relative to C3/4 (as reported in our pilot study (Iseger et al., 2017, Chapter 4), and in the main manuscript for NCG-TMS using 10 Hz trains) and secondary: Stimulation at FC3/4 leads to significantly larger HR deceleration relative to C3/4. Effect sizes were calculated for the means between locations. All other sites were tested in an exploratory fashion and topographically plotted, but it was expected that on the group level, all would show HR accelerations rather than decelerations. This assumption was based on the sensation of TMS (uncomfortable, sometimes painful, potentially stimulating surrounding muscles).

Test-retest reliability was tested by correlating RR interval change at the F3/F4 locations from session 1 to session 2 (at 100%MT), and paired t-tests. Additionally, Intraclass Correlation Coefficient (ICC) was obtained by running reliability analysis. Dose-response relationships for HR deceleration were tested by correlating stimulation intensity expressed as a) percentage MT and b) as percentage absolute stimulator output.

RESULTS

SUBJECT CHARACTERISTICS

Thirty subjects were randomized, and complete data from 27 subjects were collected (3 subjects cancelled their participation), of which 14 were allocated to stimulation in the right and 13 to stimulation in the left hemisphere (mean age: 31.78, ± 10.36, 8 males), see table S1 (facing page) and figure S1 (below). One subject experienced an adverse reaction to all stimulation sites (thus data were excluded from the primary analysis), and 4 subjects experienced adverse reactions to the

exploratory sites (thus were excluded from the exploratory analysis, and additionally from session 2). These subjects were excluded from analyses due to lightheadedness (1), emotional reactions, i.e., crying (1) and painfulness (3). No serious adverse events were reported. For the second session, 2 subjects cancelled the appointment due to scheduling constraints. One subject did complete the second session, but the data were of bad quality, therefore excluded from analysis, leaving 19 subjects for analysis of session 2.

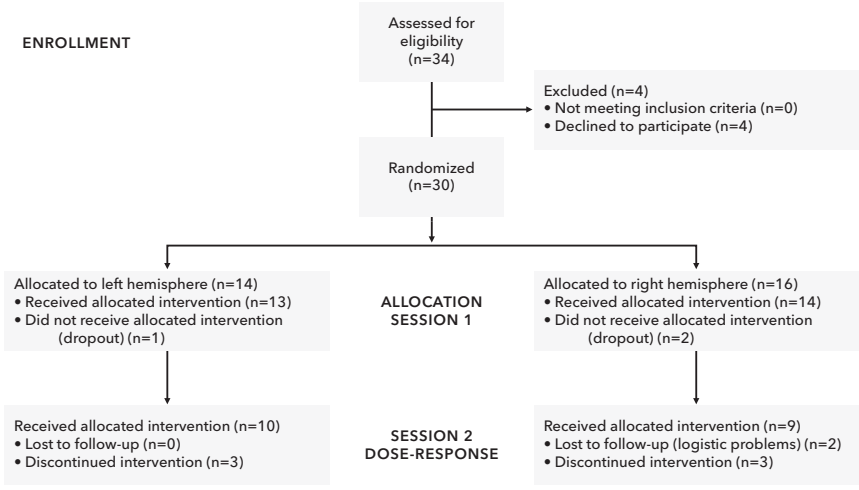


Figure S1: *CONSORT Flow diagram of the NCG-iTBS healthy controls study.*

Table S1: *Subject characteristics for the NCG-iTBS subjects (cohort 1: healthy controls), also separated by right and left hemisphere. Shown are the number of males (N males) as absolute number and percentage of total, age in years (y) (mean (SD)), motor threshold (mean (SD)) for session one and two, head size in centimeters (cm) (mean (SD)) and nasion-inion distance in centimeters (cm) (mean (SD)).*

	Total (n=27)	Left hemisphere (n=13)	Right hemisphere (n=14)
N male	8 (30%)	5 (38%)	3 (21%)
Age (y)	31.78 (10.36)	34.63 (11.07)	28.93 (9.15)
MT session 1	47.70 (6.43)	49.38 (7.59)	46.14 (4.91)
MT session 2 (N=19)	45.00 (6.05)	43.60 (6.80)	46.56 (5.00)
Headsize (cm)	56.16 (1.58)	56.19 (1.56)	56.14 (1.66)
Nasion-inion (cm)	34.87 (1.33)	35.11 (1.45)	34,64 (1.22)

REPLICATION

We found a larger, but not statistically significant HR deceleration for F3/F4 compared to C3/C4 (t(25)=1.65, p=.112, d=.266), and for FC3/FC4 compared to C3/C4 (t(25)=1.62, p=.118, *d*=.218) in 26 subjects (see figure S2, below).

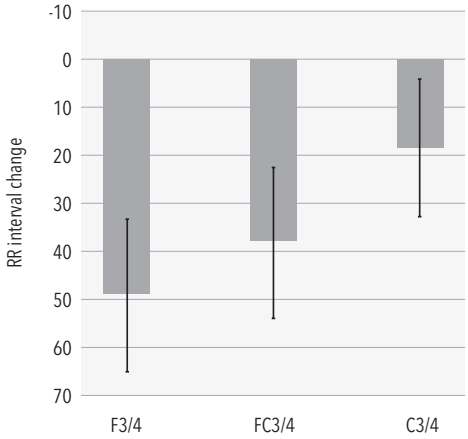


Figure S2: *RR interval changes for the NCG-iTBS group. The larger the bar, the larger the RR interval change (equaling HR deceleration). Note that the Y-axis is inversed in order to represent HR increases as an upward bar and HR decreases as a downward bar.*

When including all other locations, there was a significant effect of location (F(6, 16)=3.84, p=.014), but this was due to unexpected high heart rate decelerations at other locations, namely FC5/6 and F5/6. These locations showed significant differences with every other location (see figure S3 below, and fig 9, on page 108). The FC5/6 location differed significantly from F3/4 (p=.001), FC3/4 (p=.001), C3/4 (p=.001), F1/2 (p=.001), AF3/4 (p=.004) and F5/6 differed significantly from F3/4 (p=.002), FC3/4 (p=.003), C3/4 (p=.001), F1/2 (p<.001), AF3/4 (p=.001).

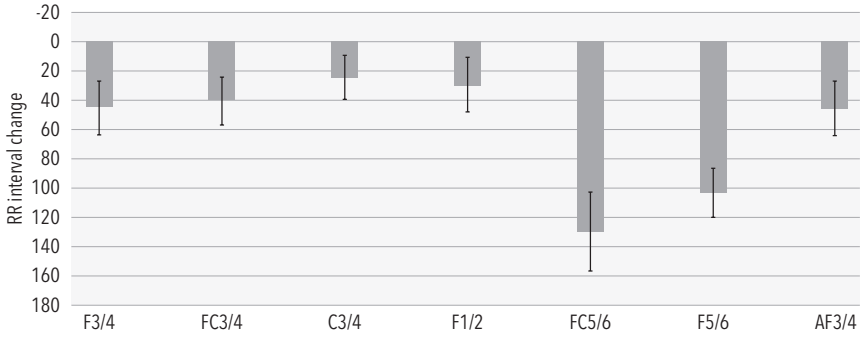


Figure S3: *RR interval changes for the NCG-iTBS group. The larger the bar, the larger the RR interval change (equaling HR deceleration). Note that the Y-axis is inversed in order to represent HR increases as an upward bar and HR decreases as a downward bar.*

On the individual level, most subjects were showing the largest HR deceleration for FC5/FC6 (11) followed by F5/6 (4) and for F3/4 only 2 subjects, indicating inter-individual variation for optimal target sites.

DOSE-RESPONSE RELATIONSHIP

Analyses was corrected for machine type (partial correlation), since a different TMS device was used.

There was no significant effect of %MT on RR interval lengthening during stimulation on the F3/4 location (neither with repeated measures ANOVA nor with correlation analysis ($r(18)=-.004$, $p=.968$)). When using absolute stimulation intensity values rather than %MT, also no significant correlation was observed ($n=19$, $r=.137$, $p=.193$; figure S4A). However, since F3/4 are not the 'best locations' on group level (only for 9% as indicated above), correlation analysis was also performed for the 'individual best location' and in particular for subjects for who FC5/6 was the best location.

For the individual best location, there was no correlation with %MT ($r(18)=.009$, $p=.929$), but there was a significant correlation with absolute stimulation intensity ($r(91)=.206$, $p=.048$, figure S4A. This was probably driven by the individuals with FC5/6 as best location, since when selecting on these individuals, an increased significant correlation was found with absolute stimulation intensity ($r(51)=.374$, $p=.006$, figure S4B).

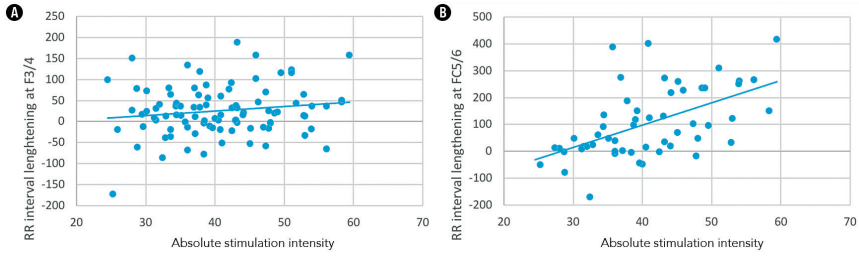


Figure S4: ***A****: Correlation plot between RR interval change and TMS intensity for the F3/4 location. The blue dots represent the 5 stimulation intensities for each individual subject ($n=19$, $r(91)=.137$, $p=.193$, $r^2=.037$). In* ***B****, the correlation plot between RR interval change and TMS intensity for each individual with FC5 or FC6 as best location is depicted. The blue dots represent the 5 stimulation intensities for each individual subject ($n=11$, $r(51)=.374$, $p=.006$, $r^2=.14$).*

TEST-RETEST RELIABILITY

In order to asses test-retest reliability, RR interval lengthening at session 1 was correlated with RR interval lengthening at session 2. This was tested for F3/F4, since this location was available for every subject and both assessments. No significant correlation was observed (r(18)=.257, p=.288). Reliability analysis resulted in an intraclass correlation coefficient (ICC) of .240, indicating no internal consistency. However, since F3/4 are not the 'best locations' on group level, correlation analysis was also performed for only subjects for who FC5/6 was the best location. This resulted in a significant correlation between session 1 and 2 (r(10)=.720, p=.012, figure S5, below) and a high ICC score of .720. However, paired t-tests did indicate differences in the amount of heart rate deceleration that was reached per session (t(10)=3.184, p=.010), showing smaller heart rate decelerations during session 2. This may be explained by the fact that individual MT's were in general lower during session 2 (p=.022). When controlling for MT differences, correlation between session 1 and 2 did not change.

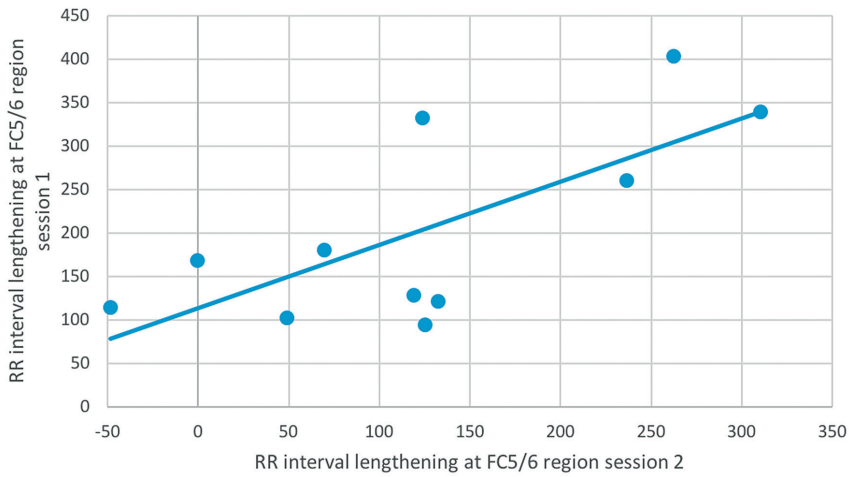


Figure S5: *Correlation plot between RR interval change in session 1 with RR interval change in session 2, during stimulation of the FC5/6 region (r(10)=.720, p=.012, r^2 =.519).*

CHAPTER 6:

NEURO-CARDIAC-GUIDED TMS: TARGETING THE BRAIN-HEART CONNECTION TO PERSONALISE AND OPTIMISE RTMS TREATMENT FOR DEPRESSION.

AN INDEPENDENT REPLICATION

Submitted as: *Kaur, M., Michael, J.A., Hoy, K.E., Fitzgibbon, B.M., Ross, M., Iseger, T.A., Arns, M., Hudaib, A-R., Fitzgerald, P. 2019. Neuro-cardiac-guided TMS: Targeting the brain-heart connection to personalize and optimize rTMS treatment for depression. An independent replication. (Under review for publication in Brain Stimulation).*

Author contributions: MK initialized the study and wrote the first draft of the manuscript. MK, JAM and KH took part in data collection and analysis. TI and MA advised on the study procedures and analyses. FP supervised the study; all authors took part in writing the manuscript.

ABSTRACT

Background

Approaches for determining a functionally meaningful dorsolateral prefrontal cortex (DLPFC) stimulation site is imperative for optimising rTMS response rates for treatment-resistant depression. One approach is neuro-cardiac-guided rTMS (NCG-TMS) in which high frequency rTMS is applied to the DLPFC to determine the site of largest heart rate deceleration. This site indexes a frontal-vagal autonomic pathway that intersects a key pathway believed to underlie rTMS response.

Objective

We aimed to independently replicate previous findings of high-frequency NCG-TMS and extend it to evaluate the use of low-frequency rTMS for NCG-TMS.

Methods

Twenty healthy participants (13 female; aged 38.6±13.9) underwent NCG-TMS on frontal, fronto-central (active) and central (control) sites. Three 5 sec trains of 10 Hz were provided at each left hemisphere site for high-frequency NCG-TMS. For low-frequency NCG-TMS, 60 sec trains of 1 Hz were applied to left and right hemispheres and heart rate and heart rate variability outcome measures were analysed.

Results

For high-frequency NCG-TMS, heart rate deceleration was observed at the left frontal compared with the central site. For low-frequency NCG-TMS, accelerated heart rate was found at the right frontal compared with central sites. No other site differences were observed.

Conclusion

Opposite patterns of heart rate activity were found for low- and high-frequency NCG-TMS. The high-frequency NCG-TMS data replicate previous findings and support further investigations on the clinical utility of NCG-TMS for optimising rTMS site localisation. Further work assessing the value of low-frequency NCG-TMS for rTMS site localisation is warranted.

INTRODUCTION

Two decades of research on the efficacy of repetitive transcranial magnetic stimulation (rTMS) for treatment resistant depression has culminated in the translation of rTMS in clinical practice in multiple countries. Spurred by its FDA approval in 2008, rTMS has provided a safer and cognitively benign alternative to ECT for some treatment resistant depressed patients who have essentially no other treatment options. Response rates for rTMS are reported as between 30% (Berlim et al., 2013) and 50% (Ren et al., 2014), with emerging research suggesting that this rate may be enhanced by profiling brain structure and function prior to treatment (Cash et al., 2019). Indeed, optimization of rTMS protocols is necessary to maximize response rates and reduce burden of non-response on patients and services.

A common approach for improving response rates to rTMS for depression is by advancing site localization methods. To date, relatively rudimentary localization methods are used in the clinic for which the scalp location overlying the dorsolateral prefrontal cortex (DLPFC) is estimated; namely, 5 cm, 6 cm and F3 (10-20 EEG system) methods (Fitzgerald and Daskalakis, 2013). The importance of considering differences in inter-individual brain morphology have been underscored by a spate of imaging studies. Firstly, the 5 cm method which determines treatment site by measuring 5 cm anterior to the motor cortex 'hotspot' (where a consistent response in the contralateral hand is evoked with TMS), was shown to localize a region within a circumscribed DLPFC area in only 32% of participants (Herwig et al., 2001). A subsequent study determined the superiority of the 10-20 EEG system method over the 5 cm method for localization as it resulted in less inter-individual error when both methods were compared to localization of the DLPFC using structural MRI scans (Rusjan et al., 2010). This study additionally showed that the region of least inter-individual variability was actually in-between F3 and F5 sug-

gesting that neither the 5 cm method nor the F3 method were consistently accurately localizing the DLPFC. The clinical applicability of MRI guided neuronavigation of the DLPFC has been additionally demonstrated by our group with better treatment outcomes reported using this method compared with the 5 cm method (Fitzgerald et al., 2009). However, structural MRI guided neuronavigation has not been adopted into clinical practice largely due to the translational limitations (i.e. cost and practicality of imaging).

Another significant limitation of structural MRI guided neuronavigation for rTMS is that it does not target functionally connected brain areas, i.e. it is based on structural rather than functional brain activity. There is increasing support for depression as a 'network disorder' that is characterized by altered neuronal activity within spatially and temporally distinct functional networks (Fox et al., 2012). Specific and connectivity-related abnormalities of the DLPFC and subgenual anterior cingulate cortex (sgACC), respective nodes of the central executive network and the default mode network, are the most reproducible findings in depression (Lane et al., 2013; Fox et al., 2012). Importantly, normalization of DLPFC activity (Gyurak et al., 2016) corresponds with antidepressant medication response and normalization the sgACC activity (Lane et al., 2013) corresponds to response multiple depression treatments (e.g. SSRIs, rTMS, ECT, deep brain stimulation, vagus nerve stimulation). Moreover, rTMS reportedly normalizes sgACC hyperconnectivity and modulates the interplay between the default mode network and the central executive network, both strongly implicated in the pathology of depression (Liston et al., 2014). These findings support the hypothesis that rTMS exerts its therapeutic benefit through the propagation of effects from stimulation of a critical cortical node (i.e. the DLPFC) to deeper subcortical structures in one or more interconnected networks. Compelling evidence for this relationship between DLPFC to sgACC functional connectivity and rTMS treatment response is now emerging. Specifically, Fox and colleagues (2012) showed in a retrospective analysis that the degree of negative (i.e. anti-) correlation of the DLPFC site of stimulation and sgACC functional connectivity predicted response to rTMS for depression, accounting for more than 70% of the variance in efficacy. The utility of DLPFC to sgACC functional connectivity in

predicting clinical efficacy was further demonstrated by a prospective validation study (Weigand et al., 2018) and an independent validation study of these findings (Cash et al., 2019). Critically, this body of work provides a promising avenue for the development of functionally meaningful site localization methods for rTMS treatment of depression that are likely to result in improvements in treatment efficacy.

A potential avenue for identifying a functionally meaningful site based on the connectivity of the DLPFC to sgACC pathway for rTMS treatment is to probe the brain-heart connection. The brain-heart connection in the context of affective regulation has been long recognized by a plethora of neuroimaging and pharmacological studies in humans and animals (Makovac et al., 2017). The top-down modulation of heart rate by the prefrontal cortex involves a frontal-vagal pathway from frontal nodes (including the prefrontal cortex, anterior cingulate and insula) to subcortical nodes within the medial visceromotor network (Thayer and Lane, 2009). Through vagal (i.e. parasympathetic) activation, indexed by heart rate deceleration, the prefrontal cortex is touted to have a parasympathoinhibitory influence over subcortical nodes (Thayer and Lane, 2009). The well-known autonomic nervous system characteristics in depression (i.e. reduced parasympathetic to sympathetic balance and the high incidence of cardiovascular disease) is therefore thought to result from reduced prefrontal cortex parasympathoinhibition (Kidwell and Ellenbroek, 2018). Within the medial visceromotor network, several lines of evidence support the sgACC as the cardinal frontal region for autonomic regulation; it directly and monosynaptically connects frontal nodes to subcortical structures (including the nucleus tractus solitarious of the vagus nerve) (Lane et al., 2013). A meta-analysis has reported that rTMS induces a reduction in heart rate and an increase in heart rate variability (HRV) of moderate effect, particularly when applied to the prefrontal cortex compared with the motor cortex. These findings suggest that rTMS applied to the prefrontal cortex results in stimulation of the frontal vagal network involved in cardiovascular control (Makovac et al., 2017). The effect of stimulating the frontal vagal network with rTMS on heart rate thus provides an avenue for probing DLPFC-sgACC connectivity shown to underlie rTMS clinical efficacy. This method, neuro-cardiac-guided TMS (NCG-TMS), has

recently been developed and is proposed for individualising DLPFC site of stimulation selection for rTMS treatment (Iseger et al., 2017; Chapter 4).

Iseger and colleagues (2017; Chapter 4) published the first insight into NCG-TMS; in this pilot study (n=10), the DLPFC site of greatest heart rate deceleration with rTMS was proposed as the optimal site for rTMS treatment and was shown to vary across individuals. In this study, high-frequency rTMS was applied to left and right 10-20 EEG frontal sites, F3/F4 and FC3/FC4 and, central sites C3/C4 as the control sites. On the left hemisphere, the greatest heart rate deceleration was observed at F3 for 80% of participants, however, in 20% of participants the greatest heart rate deceleration was found at FC3. Similarly, on the right hemisphere, the great heart rate deceleration was shown at F4 for 60% of participants and at FC4 for 40% of participants. These data therefore indicate that for a minority of participants FC3 (and FC4) were more functionally connected to the frontal vagal network than F3 (and F4). As such, for these participants rTMS provided at these sites may result in better treatment response compared with F3 (and F4), presenting a strategy for using NCG-TMS for personalized targeting of rTMS treatment.

The current study aimed to replicate the findings of Iseger et al. (2017; Chapter 4) on high-frequency NCG-TMS applied to the left hemisphere in a larger and independent sample. Secondarily, we aimed to evaluate the applicability of NCG-TMS for low-frequency rTMS treatment, since it is equally efficacious for depression, is better tolerated (Kaur et al., 2019) and potentially safer (Sen et al., 2008) than high-frequency rTMS. In addition, a low-frequency NCG-TMS protocol allows for a longer period of concurrent heart rate recording during rTMS, therefore allowing us to further assess the value of heart-rate variability measures for NCG-TMS as well as testing the direct effects of this rTMS protocol.

MATERIALS AND METHODS

PARTICIPANTS AND PROCEDURE

Twenty healthy participants (13 females) aged between 18-65 years (range=20-62; mean=38.6±13.9) were recruited via flyers, public notice boards and on social media. Exclusion criteria were contraindications to TMS (including but not limited to the presence of metal inside the head excluding dental work, professional drivers, pregnancy or currently breast-feeding and history of seizure), history of neurological or psychiatric disorders and history of or current cardiac abnormalities. Participants were screened for psychopathology using the M.I.N.I International Neuropsychiatric Interview (Nibuya et al., 1996). Prior to testing, participants were asked to refrain from consumption of nicotine (for at least 3 hours), caffeine (for at least 2 hours) and alcohol (for at least 12 hours), respectively and abstinence was confirmed with participants immediately before the testing session.

After the provision of informed consent, participants underwent clinical interview to screen for psychopathology and substance use with a trained researcher (JM) and then, resting motor threshold (RMT) assessment of the left- and right-motor cortices. Thereafter, electrocardiogram (ECG) electrodes were attached and participants were seated, asked to relax, limit any movement and avoid talking during testing. Once ECG trace quality was checked via visual inspection and participants were settled, 2 minutes of resting ECG was recorded. High-frequency and low-frequency rTMS protocols were then administered and ECG was recorded concurrently. The order of frequency type was counter-balanced across participants.

This study was approved by the Alfred Hospital and Monash University Human Research Ethics Committees and written informed consent was provided by all participants, in keeping with the declaration of Helsinki.

NEURO-CARDIAC-GUIDED RTMS DATA ACQUISITION

Stimulation was applied using a Medtronic MagPro stimulator and a 70-mm diameter figure-of-8 coil. Single-pulse TMS was applied to the left- and right-motor cortices to measure the RMT for each hemisphere using electromyography using standard published methods (Fitzgerald et al., 2002). ECG electrodes were attached to the middle of the breast bone (ground electrode), to the right above the right breast and to the left below the left breast. ECG was recorded during stimulation with the nCG-ENGAGE HR (neuroCare, Munich, Germany) and NCG-TMS purposely designed 10-20 EEG caps (without electrodes) were used to guide site of stimulation. Fz was the first site stimulated with each rTMS protocol in order to accustom the participant to the sensation of each type of stimulation and to titrate the intensity up to 100% left or right RMT, which ever was higher. For the high-frequency protocol, three five second trains of 10 Hz at 100% of RMT with a 30 second inter-train interval were applied to 3 locations (excluding Fz) on the left hemisphere, F3, FC3 and C3. Left and right central sites (C3 and C4) were stimulated as control sites for comparison to left and right frontal and fronto-central sites (F3, FC3 and F4, FC4, respectively). For the low-frequency protocol, a single 60 second train of 1 Hz at 100% of RMT with a 30 second inter-train interval was applied at 6 locations (excluding Fz) on the left (F3, FC3 and C3) and right (F4, FC4 and C4) hemispheres.

DATA PROCESSING

Automatic R-peak detection was performed by the nCG-ENGAGE HR. All data were visually inspected to determine correct detection of R peaks and movement artefacts. All data were sampled at a rate of 1000 Hz. The high-frequency data analyses were identical to the study by Iseger et al. (2017; Chapter 4). To control for the effect of respiration on heart-rate, R-R values from the trough at pre-stimulation, trough 1, trough 2 and trough 3 (at each site) were used in analyses. Data were averaged and normalized to z-scores to reduce inter-individual and inter-train effects using the formula: trial 1 – trial 0 / sd trial 0 (repeated for trials 2 and 3). Note, due to the short duration of high-frequency rTMS trains (i.e. 5 secs), heart-rate variability mea-

sures could not be determined. The low-frequency NCG-TMS data were imported into Kubios Premium (ver. 3.0.2) (Tarvainen et al., 2014) for analysis of mean R-R interval and heart-rate variability data during the stimulation period. Data were corrected for ectopic or misplaced beats and movement artefact by interpolation (using the automatic correction feature in Kubios Premium). Data requiring corrections for more than 5% of total R-peaks were excluded. Two heart-rate variability measures known to reflect parasympathetic system activity (Camm et al., 1996) were chosen for analysis; i) the time domain measure, Root Mean Square of the Successive Differences (RMSSD) calculated from the R-R intervals, and ii) the frequency domain measure, high-frequency power log (HF-HRV; 0.15 Hz to 0.4 Hz). HF-HRV was calculated by Fast Fourier Transform based on Welch's periodogram (window width of 300 secs, 50% overlap).

STATISTICAL ANALYSES

For the high-frequency data, SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA) for Windows was used to perform analyses. To replicate the differences in heart-rate change between sites described by Iseger and colleagues (2017; Chapter 4), the high-frequency data were analyzed using paired samples t-tests (one-tailed). Cohen's d effect sizes were generated to assess pair-wise differences in heart-rate change between sites with high-frequency rTMS. The optimal location for individuals was determined by the site of greatest heart-rate deceleration.
Analyses for the low-frequency NCG-TMS data were run with SAS 9.4 (SAS institute, Cary NC). A 3 (site) x 2 (hemisphere) repeated measures analyses of variance (ANOVA) were written to test the hypothesis of site differences for outcomes mean R-R interval, mean RMSSD and mean HF-HRV across left and right hemispheres. The site (F, FC, and C) and hemisphere (left, right) were modelled as within subject variables so each participant was represented by 6 data points or rows. To minimize the correlation between the mean and variance for each outcome measure, natural log transformations were applied. Further, whenever the sphericity assumption was not met, Huynh-Feldt corrected p values were reported. Bonferroni post-hoc pairwise comparisons between sites within site by hemisphere (site*hemisphere) were

adjusted for multiplicity using Bonferroni correction. Additionally, the effect sizes or Cohen's d were calculated for significant score differences within site*hemisphere. The alpha level was set at two-tailed 0.05.

RESULTS

Figure 1: *An example of high-frequency NCG-TMS (left) and low-frequency NCG-TMS (right) data with the ECG trace represented in blue, the TMS triggers represented in red and the R-peak detection by the NCG-TMS module in yellow.*

PARTICIPANT SAMPLE

Following inspection of ECG data (see Figure 1), eighteen participants (11 females; aged 38.7±13.5) with a mean left resting motor threshold (RMT) of 49.7±5.9 were included in the high-frequency NCG-TMS analysis after n=2 were excluded due to technical recording issues. No high-frequency data were lost to incorrect R peak detection. For the low-frequency data, 19 participants (12 females; aged 38.9±14.1) were included in the analysis after n=1 were excluded due to >%5 of R peaks rejected in at least 1 variable. For this sample, the mean RMT for left and right motor cortices were 50.0±5.7 and 49.9±8.9, respectively.

HIGH-FREQUENCY NCG-TMS

As expected, the difference in heart rate reduction (mean z-scores) with high-frequency rTMS between F3 (mean=1.50 ± 3.68) and C3 (mean=-0.36 ± 1.11) was significant and of medium to large effect [t (17) = 2.08, p=.02, d=0.69]. While the difference in heart-rate change between FC3 (mean=0.22 ± 1.60) and C3 was not significant [t (17) = 1.27, p=.22], significance emerged for the difference between F3 and FC3 [t (17) = 1.77, p<.05]. Small to medium effect sizes were found for the pair-wise comparisons between FC3 and C3 (d= 0.42) and, F3 and FC3 (d = 0.45). The pattern of heart-rate change across sites is displayed in Figure 2 and percentage distributions of optimal site at the individual level are shown in Table 1.

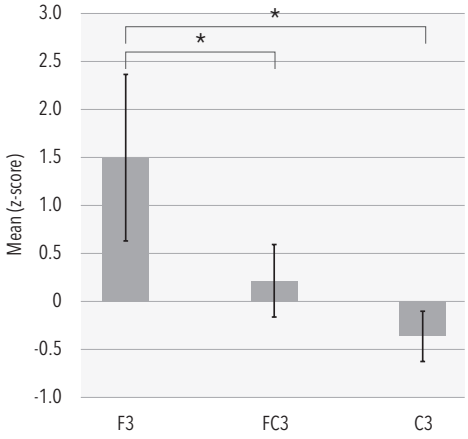


Figure 2: *A bar graph showing the pattern of heart-rate change (mean z-scores and standard error; a positive score means RR interval lengthening / heart rate reduction) across sites of stimulation with F3 and FC3 as test sites and C3 as the control site. Note: * denotes significance at p<.05.*

Table 1: *A table presenting the number (N) and percentage (%) of individuals with the greatest heart-rate deceleration at each site (i.e. the chosen optimal site) with high-frequency NCG-TMS.*

	F3	FC3	C3
N	10/18	5/18	3/18
%	55.6%	27.8%	16.7%

LOW-FREQUENCY NCG-TMS

The raw means and standard deviations for R-R interval, RMSSD and HF-HRV are shown in Table 2. For mean R-R interval, the analyses showed a significant effect of site [F (2, 36) = 4.87, p=0.01]. Post-hoc comparisons revealed a significant mean difference between F4 and C4 (d=-0.67, p=0.02). The hemisphere and site*hemisphere effects were not statistically significant at 0.05 level (Table 3). We noted a significant hemisphere effect on RMSSD scores [F (1, 18) =5.28, p=0.03) but no effect of site (p>.05). For HF-HRV, there were no significant effects of site, hemisphere or their interaction on scores (all p>.05). As noted in Table 4, no post-hoc mean differences within site within site*hemisphere have approached significance for HF-HRV or RMSSD outcomes. Simply stated, for R-R interval, while there was no effect of laterality across sites, there was an effect of site across both the hemispheres, with differences across sites showing a significant heart rate acceleration at F4 compared to C4. For RMSSD, there was a difference in left versus right hemispheres across sites but no effect of site across hemispheres. Figure 3 (facing page) shows that the pattern of R-R interval means (log transformed) across sites is similar for both hemispheres, with accelerated heart rate at frontal compared with central sites. Figure 4 depicts the different RMSSD (log transformed) patterns across hemispheres. The percentage distributions of site with the most accelerated heart rate at the individual level are shown in Table 5 (facing page).

Table 2: *The raw means ± standard deviations for the left (F3, FC3, C3) and right (F4, FC4, C4) for R-R interval, RRMSD and HF-HRV power (log) for low-frequency NCG-TMS.*

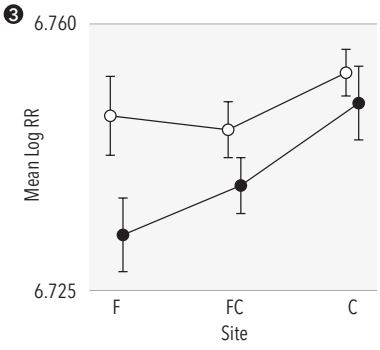
	Left hemisphere			Right hemisphere		
	F3	FC3	C3	F4	FC4	C4
R-R	863.7±131.8	862.4±135.4	868.0±130.4	850.4±131.9	855.0±125.9	864.3±129.8
RMSSD	32.9±19.6	31.3±17.3	34.0±19.0	30.1±16.6	32.1±19.6	30.0±19.0
LOG HF-HRV	5.7±1.3	5.7±1.5	5.7±1.4	5.4±1.5	5.7±1.4	5.5±1.4

Table 3: *The repeated measures ANOVA results for site, hemisphere and site by hemisphere interaction with corresponding F statistics (degrees of freedom, error degrees of freedom) and p values for R-R interval, RRMSD and HF-HRV (all log transformed) for low-frequency NCG-TMS sample (n=18). Note: * denotes p<.05.*

Outcome	Site		Hemisphere		Site * hemisphere	
	F(2, 36)	*p*	*F*(1, 18)	*p*	*F*(2, 36)	*p*
LOG R-R	4.87	0.01*	3.90	0.06	1.20	0.31
LOG RMSSD	0.09	0.91	5.28	0.03*	2.56	0.09
LOG HF-HRV	0.68	0.51	3.97	0.06	2.60	0.09

Table 4: *Mean differences matrix for Bonferroni pairwise comparisons for each outcome (log transformed) for site within site*hemisphere. Note: * denotes p<0.05.*

OUTCOME	HEMISPHERE	SITE	MEAN	F	FC
LOG R-R	Left	F	6.7479	-	-
		FC	6.7461	0.0018	-
		C	6.7537	0.0057	0.0075
	Right	F	6.7324	-	-
		FC	6.7388	0.0064	-
		C	6.7497	0.0173*	0.0109
LOG HF-HRV	Left	F	5.7199	-	-
		FC	5.6645	0.0554	-
		C	5.7389	0.0191	0.0745
	Right	F	5.4049	-	-
		FC	5.6906	0.2857	-
		C	5.4626	0.0577	0.2280
LOG RMSSD	Left	F	3.3191	-	-
		FC	3.2796	0.0395	-
		C	3.3625	0.0434	0.0829
	Right	F	3.2412	-	-
		FC	3.2666	0.0254	-
		C	3.2093	0.0319	0.0573



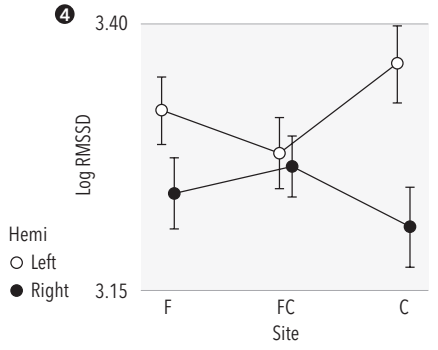


Figure 3 (above, left): *A line graph depicting the pattern of R-R interval means (log transformed) across the frontal (F), fronto-central (FC) and central (C) sites for left (white) and right (black) hemispheres for low-frequency NCG-TMS.*
Figure 4 (above, right): *A line graph depicting the pattern of root mean square of the successive differences (log transformed) across the frontal (F), fronto-central (FC) and central (C) sites for left (white) and right (black) hemispheres for low-frequency NCG-TMS.*

Table 5: *A table presenting the number (N) and percentage (%) of individuals with the greatest accelerated heart-rate (R-R interval) at each site with low-frequency NCG-TMS.*

	F3	FC3	C3	F4	FC4	C4
N	9/19	6/19	4/19	11/19	6/19	2/19
%	47.4%	31.6%	21.1%	58%	31.6%	10.5%

DISCUSSION

The present study forms part of an early literature on NCG-TMS and is the first independent replication study of heart rate deceleration with high-frequency NCG-TMS at F3 compared to C3. In addition, while not significant, a similar pattern of heart rate deceleration was seen at FC3 compared with C3. At the individual level, these data showed marked inter-individual variability in the left hemisphere site of greatest heart rate deceleration. Additionally, the current study is the first to assess low-frequency NCG-TMS for which we showed no effect of laterality but an effect of site with accelerated heart rate at F4 compared to C4. For heart rate variability measures with low-frequency NCG-TMS, there was a laterality effect for RMSSD, however, there were no post-hoc site differences within the site by hemisphere interaction. There was no effect of hemisphere, site or site by hemisphere interaction for HF-HRV. Marked inter-individual variability in site of greatest accelerated heart rate for left and right sites with low-frequency NCG-TMS was noted, with a similar pattern of distribution compared with high-frequency NCG-TMS. These findings replicate the earlier pilot study (Iseger et al., 2017; Chapter 4) supporting that the frontal-vagal pathway may be probed using high-frequency NCG-TMS, with the frontal location of vagal activation varying across individuals. Furthermore, this study highlights the differential physiological effects of low- and high-frequency rTMS.

Our independent replication of frontal patterns of heart rate deceleration with high-frequency rTMS corroborates previous NCG TMS findings (Iseger et al., 2017; Chapter 4) and the meta-analysis of the effect of rTMS on heart rate (Makovac et al., 2017). These data support the theory that rTMS of the prefrontal cortex engages the frontal vagal pathway (Thayer and Lane, 2009) which critically intersects DLPFC to sgACC pathway thought to underpin the rTMS therapeutic mechanism (Fox et al., 2012; Cash et al., 2018; Weigand et al., 2018). In turn, these data provide support for the potential use of high-frequency NCG-TMS for individualized targeting of site for rTMS treatment of depression. On inspection of the optimal site distribution at the individual level, for the majority of participants, the greatest heart rate deceleration was at F3 followed by FC3, in keeping with the NCG-TMS (Iseger et al., 2017; Chapter 4) and the broad-

er rTMS site localization literature (Beam et al., 2009). However, it was unexpected that in a small number of participants (n=3), greatest heart rate deceleration was at C3, the control site. This could be attributed to individual variability in site of greatest connectivity to the sgACC or brain morphology. Another explanation relates to factors that could not be kept constant for stimulation at different sites (at different times) such as anxiety levels or discomfort during stimulation which may have influenced the sympathetic to parasympathetic balance. In this instance, it is plausible that for some individuals, stimulation on F3 or FC3 could have been more uncomfortable than at C3 and, the ensuing increased sympathetic activity could mask potential parasympathetic activity. Future studies should therefore collect data on the subjective experience of rTMS on each site to investigate the potential influence of this on heart rate change. Another point for discussion is that heart rate deceleration at FC3 compared to C3 was not significant, unlike the previous study (Iseger et al., 2017; Chapter 4). Notwithstanding, the effect size we report for this comparison (d=.42) is comparable to a larger replication study by Iseger and colleagues (Iseger et. al., 2019b, Chapter 5) (d=.49) suggesting that the lack of significance may be due to inadequate power.

The difference in heart rate with low-frequency NCG-TMS between F4 and C4 is consistent with high-frequency NCG-TMS findings (Iseger et al., 2017; Chapter 4) indicating that low-frequency rTMS at frontal sites can modulate the frontal vagal network. However, the direction of this difference (i.e. heart rate is accelerated at F4 compared with C4) is opposite to that observed with high-frequency NCG-TMS (i.e. heart rate deceleration at F3/4 compared with C3/4). This difference is moderate to large in effect and the visual heart rate pattern across sites on both hemispheres is similar, lending confidence in the finding. Taken together with the null findings in heart rate variability measures indexing parasympathetic activity, the heart rate acceleration reported here reflects net sympathetic over parasympathetic activity (Thayer and Lane, 2009). Of note, cerebral laterality effects of cortical autonomic control have previously been documented (Barron et al., 1994), including a study showing more pronounced effects of low-frequency rTMS on the right hemisphere (Gulli et al., 2013), in line with the current results. Lastly, the opposite

pattern of heart rate measures with high and low--frequency rTMS is consistent with the broader literature reporting of differential biological effects of these rTMS types (Fitzgerald et al., 2006). While the specific effects of low- and high-frequency are not clear due to the several inconsistencies in the literature, the most replicable finding for low-frequency rTMS is that it produces a decrease in cortical excitability, whereas, more contentiously, high-frequency produces an increase in cortical excitability and potentially, a reduction in cortical inhibition. It is possible that with low-frequency rTMS on the prefrontal cortex, the net effect is a dampening of parasympathoinhibition which then allows an increase in sympathoexcitation. With high-frequency, if the net effect is increased cortical excitability, this could result in parasympathoinhibitory potentiation. It is also worth noting that low-frequency trains were far longer than high-frequency trains (1 min vs 5 sec); the effect of low-frequency rTMS on heart rate was measured by net heart rate change over this period and any short-term heart rate changes with rTMS may have been masked. Further research into low-frequency NCG-TMS is required to understand the mechanism of low-frequency stimulation of frontal vagal networks in order to assess its value in informing NCG TMS guided site localization for treatment of depression.

Notably, NCG-TMS is an emerging field of research and whether heart rate deceleration at frontal sites correlates with frontal-vagal connectivity, as proposed, has not yet been confirmed by mechanistic neurobiological studies. Research with larger samples of depressed participants is required to substantiate the current results and the initial study by Iseger et al (2017, Chapter 4). In order to assess the utility of NCG-TMS as an optimized approach for rTMS site selection, studies investigating rTMS treatment outcome with NCG-TMS are needed. It is also unclear to what extent the sensory experience of rTMS on different frontal and central 10-20 sites influences heart rate. It is plausible that stimulation at subjectively more uncomfortable sites would results in greater sympathetic activity and thus, increased heart rate, a potential limitation that deserves evaluation in future research. Further, the low-frequency NCG TMS data could not be corrected for baseline heart rate (unlike for high-frequency NCG-TMS); due to the dynamic nature of heart rate fluctuations

over the 1-minute length of the heart rate recording, any baseline correction obscured the results. Therefore, heart rate at each site for this condition was compared relative to heart rate at the other sites. Lastly, although a 'practice' site (i.e. Fz) was included in this protocol to accustom participants to the sensation and to up titrate the intensity of rTMS, the stimulation order of sites was not controlled for in this study and any site order effects may have influenced the results.

CONCLUSIONS

The present study extends previous findings on high-frequency NCG-TMS and provides further evidence of this promising approach to locate an optimal site for rTMS based on recent knowledge on the therapeutic rTMS mechanism for depression using an affordable method (i.e. requiring only ECG and rTMS). While it is unclear whether low-frequency NCG-TMS has value in determining optimal site for stimulation, these data substantiate the extant literature showing differential effects of high- and low-frequency rTMS.

CHAPTER 7:

CARDIOVASCULAR DIFFERENCES BETWEEN SHAM AND ACTIVE ITBS RELATED TO TREATMENT RESPONSE IN MDD

In press: *Iseger, T.A., Arns, M., Downar, J., Blumberger, D, Daskalakis, Z., Vila-Rodriguez, F. 2019c. Cardiovascular differences between sham and active iTBS related to treatment response in MDD. Brain Stimulation.*

Author contributions: JD, DB, ZD and FVR conceptualized the study. TAI performed data-analyses and wrote the initial draft for the manuscript. All authors took part in writing the manuscript.

ABSTRACT

Background

Heart rate in MDD is often dysregulated, expressed in overall higher heart rates (HR) and lower heart rate variability (HRV). Interestingly, HR decelerations have been reported after stimulation of the DLPFC using rTMS, suggesting connectivity between the DLPFC and the heart. Recently, a new form of rTMS called theta burst stimulation (TBS) has been developed. One form of TBS, intermittent TBS (iTBS), delivers 600 pulses in just 3 min.

Objective

To determine whether iTBS aimed at the DLPFC acutely affects HR, blood pressure and HRV, and whether these acute cardiac responses are associated with treatment response.

Methods

ECG and blood pressure were analyzed during both sham and active iTBS in 15 MDD patients, over 30 treatment days.

Results

We found a significantly larger HR deceleration for active iTBS, compared to sham, within the first minute of stimulation. Also, a trend towards an association between HR deceleration and treatment response was found, explaining 26% of the variance. Furthermore, several measures of heart rate variability were significantly higher during iTBS stimulation compared to sham, and systolic and diastolic blood pressure were lower during active iTBS.

Conclusion

Active iTBS applied to the DLPFC is able to transsynaptically activate the autonomic nervous system, in particular the parasympathetic branch, similar to what has been found for conventional rTMS methods. Furthermore, the data suggest that the larger the acutely induced heart rate deceleration, the better the clinical response after 30 treatment days of iTBS.

INTRODUCTION

Neuromodulation treatments such as r*epetitive Transcranial Magnetic Stimulation* (rTMS), transcranial Direct Current Stimulation (tDCS) and *Deep Brain Stimulation* (DBS) show promising clinical benefit in Major Depressive Disorder (MDD) (Donse et al., 2017; Brunoni et al., 2017; Brunoni et al., 2017; Mayberg et al., 2005; Schlaepfer et al., 2013), and target key structures that are affected in depression such as the *dorsolateral prefrontal cortex* (DLPFC), the *dorsomedial prefrontal cortex* (DMPFC) *and the subgenual anterior cingulate cortex* (sgACC). Stimulation of these regions is associated with symptom improvement in MDD (Downar et al., 2014; Downar and Daskalakis, 2013; Mayberg et al., 2005), and it was suggested that there is network connectivity between the DLPFC, (sg)ACC and the vagus nerve (VN), mediating clinical response to these treatments (Liston et al., 2014; Fox et al., 2012). The vagus nerve is part of the parasympathetic branch of the autonomic nervous system and influences bodily functions such as *heart rate* (HR) and respiration. Stimulation of the vagus nerve was found to consistently lead to HR deceleration (Buschman et al., 2006). Supporting this network hypothesis, several studies have also reported HR deceleration following stimulation of the DLPFC using rTMS and tDCS (Makovac et al., 2016). Additionally, heart rate in MDD is often dysregulated, expressed in overall higher HR and lower *heart rate variability* (HRV) (Licht et al., 2008; Koenig et al., 2016; Castaldo et al., 2015), which has been reported to be normalized after neuromodulation treatment (Kemp et al., 2010). Recent studies have shown that heart rate decelerations may be used as a functional outcome measure for verifying correct targeting of the depression network, called Neuro-Cardiac-Guided TMS (NCG TMS) (Iseger et al., 2017; Chapter 4), meaning that correct stimulation of the DLPFC results in an acute heart rate deceleration.

Currently, naturalistic remission rates to rTMS are around 37% (Carpenter et al., 2012), and can be higher when combined with psycho-

therapy 56% (Donse et al., 2018). Improvements in TMS protocols that include the spacing of sessions (accelerated TMS), frequency of stimulation or improvement of localization of treatment targets may enhance outcomes. Recently, a new form of rTMS called theta burst stimulation (TBS) has been developed (Huang et al., 2005). Unlike 10Hz stimulation, TBS mimics endogenous theta rhythms, improving synaptic long-term potentiation. One form of TBS, intermittent TBS (iTBS), delivers 600 pulses in 189 seconds, showing similar or more potent excitatory effects compared to conventional 10Hz stimulation. iTBS usually involves 10 triplet bursts of 50 Hz rTMS applied at a rate of 5Hz, for 2 seconds, with an intertrain interval of 8 seconds, for a total of 189 seconds; 200 triplet bursts. A recent study demonstrated equivalent efficacy for iTBS compared to 10Hz TMS in reducing depressive symptoms in patients with depression, leading to both FDA approval in the United States and CE marking in Europe (Blumberger et al., 2018). Due to its rapidity of administration, iTBS may be particularly well-suited to NCG TMS in clinical settings.

In the current study, ECG and blood pressure were recorded during both sham and active iTBS. It was hypothesized that 1) subjects would show heart rate decelerations during active iTBS, but not during sham treatment, similar to conventional 10 Hz TMS, and in line with the results reported by Iseger et. al. (2017; Chapter 4) and 2) MDD patients expressing large HR decelerations during the first treatment day of active iTBS, would be more likely to respond to treatment. Furthermore, we expect general parasympathetic activation via iTBS induced vagal stimulation, resulting in decreased blood pressure and heart rate and increased HRV, compared to sham.

METHODS[1]

STUDY DESIGN AND PARTICIPANTS

15 MDD patients between 20 and 54 years old who had a MINI International Neuropsychiatric Interview¬-confirmed diagnosis of major depressive disorder, as a single or recurrent episode, were included. Patients met inclusion criteria if their current episode showed a 17¬-item Hamilton Rating Scale for Depression (HRSD¬-17) score of at least 18, showed no clinical response to an adequate dose of an antidepressant (based on an antidepressant treatment history form score of more than 3 in the current episode) or were unable to tolerate at least two separate trials of antidepressants of inadequate dose and duration, and they had received a stable antidepressant regimen for at least 4 weeks before treatment, which continued during treatment. Exclusion criteria included substance abuse or dependence in the past 3 months, active suicidal intent, pregnancy, bipolar disorder, any psychotic disorder or current psychotic symptoms, previous rTMS treatment, a lifetime history of non¬response to an adequate course— i.e. a minimum of eight treatments—of electroconvulsive therapy, personality disorder deemed to be the pri¬mary pathology, an unstable medical illness, substantial neurological illness, abnormal serology, or the presence of a cardiac pacemaker, intracranial implant, or metal in the cranium. Participants were also excluded if they were taking more than 2 mg lorazepam (or equivalent) or any anticonvulsant or if more than three adequate antidepressant trials had failed (determined by anti¬depressant treatment history form). Medical-ethical approval was granted by the research ethics board at University of British Columbia and Vancouver Coastal Health Authority. All participants provided written, informed consent.

[1] The data were collected in the context of a larger study (not published yet, Vila-Rodriguez et al., in prep.). The data presented in this thesis form a subset for which ECG and blood pressure have been recorded.
This study was designed to study the effects of spacing between sessions. Thus, there were two treatment arms: one group received 2 sessions of iTBS directly following each other and the other group received iTBS spaced an hour apart. The blanks were filled with sham treatment to ensure equal protocols. This created the following treatment arm for the first group: sham-sham, active-active; and for the second group: sham-active, active-sham.

PROCEDURES

Before treatment, participants had high resolution anatomical MRIs, and during the first treatment day real-time MRI-guided neuronavigation with a Visor neuronavigation system (ANT Neuro, Enschede, Netherlands) was used for coil positioning. The remainder of treatment days used the MRI guided spot without real-time neuronavigation (Beam et al., 2009). The left dorsolateral prefrontal cortex target was located in each participant by reverse co-¬registration from the MNI152 stereotaxic coordinate (x–38, y+44, z+26), which was previously identified as optimal on the basis of clinical outcomes and whole-brain functional connectivity (Fox et al., 2012). iTBS was delivered with a MagPro X100, equipped with a B70 fluid¬ cooled coil and high¬ performance cooler (MagVenture, Farum, Denmark). Each participant's resting motor threshold (RMT) was determined by use of visual observation in accordance with standard clinical practice. iTBS was delivered at 120% RMT, with triplet 50 Hz bursts, repeated at 5 Hz; 2 sec. on and 8 sec. off, for a total duration of 3 min. and 9 sec., resulting in 600 pulses per session. A B70 sham (internally-shielded) coil without magnetic stimulation was positioned over the vertex for the sham stimulation. Subjects were told that the sensation would be different because of the different sites of stimulation.

Subjects were randomized to two treatment arms receiving four treatment sessions in blocks of two sessions one hour apart. 50% of the sessions were sham and the other 50% of the sessions were active. Thus, all subjects received both sham and active stimulation. Treatment comprised 30 treatment days (on weekdays), each with 2 sessions spaced by one hour as described above. An HRSD¬-17 score was determined by trained research staff at baseline, after every five treatments, and 1 week, 4 weeks, and 12 weeks after treatment. Participants missing scheduled sessions due to illness or scheduling conflicts received additional sessions at the end of the treatment course to achieve the intended course length. Participants missing 4 consecutive treatment days were withdrawn.

PHYSIOLOGICAL DATA ACQUISITION

Physiological data were recorded on treatment days 1, 10, 20 and 30 during all four sessions of that day. All physiological recordings were obtained with a Biopac MP 150 system (Biopac Systems Inc., Goleta, CA, USA) comprising modular hardware and "AcqKnowledge" software. The ECG100C Electrocardiogram Amplifier module records electrical activity generated by the heart on one ECG channel using three electrodes placed on the participant's arms and left ankle. To measure continuous blood pressure signal, a non-invasive NIBP100D system (CNAP Monitor 500; CNSystems Medizin technik AG, Graz, Austria) was used.

DATA PROCESSING

ECG data were time locked with the TMS pulses and segmented onto the actual stimulation period (i.e 189 seconds). R waves (i.e. the main spikes observed in the graphical deflections observed in an ECG) were detected in the ECG and converted to a RR tachograph, which is a graph of the numerical value of the RR-interval (i.e., the interval between two R peaks) and time, using Kubios software. Here, medium artefact correction was applied, correcting possible occurrences of ectopic beats or other outliers. From the recording 4 timeframes were derived: the whole 189 seconds, the first 30 seconds, the first 45 seconds and the first 60 seconds, since the heart rate changes might be more pronounced at the start of recording. For each timeframe, the mean RR intervals and the slope of the RR-interval data were calculated. HRV variables were obtained over the whole 189 seconds, since HRV variables are more reliable in recordings of longer duration. Within the frequency domain, absolute very low frequency power (VLF: 0.0033 – 0.04 Hz), low frequency power (LF: 0.04 – 0.15 Hz) and high frequency power (HF: 0.15 – 0.4 Hz) were calculated in ms^2, as well as the ratio of low frequency power to high frequency power (LF/HF) The standard deviation of the NN (RR) intervals (SDNN) and root mean square of the successive differences (RMSSD) were taken within the time-domain. The natural logarithm (LN) was calculated for the HRV variables in order to obtain a normal distribution. Furthermore, mean blood pressure, systolic blood pressure and

diastolic blood pressure were measured over the whole 189 seconds of which also the LN was calculated to meet statistical assumptions of normal distribution. For all variables the two sham sessions were averaged per individual as well as the two active sessions.

STATISTICAL ANALYSIS

In the first part, data were analyzed in a blinded fashion, where stimulation type (sham or real) was not known to the processing analysts (TAI and MA). RR-interval waveforms were visually inspected for RR interval lengthening and the primary outcome measure was defined in this blinded stage. After unblinding, sham and active TMS during treatment day one were compared using repeated measures ANOVA with Stimulation Type (sham or active) and Timeframe as within-subject factors. Following significant effects, paired and one-sample t-tests were performed.

Secondly, after the data was unblinded and the primary outcome measure for measuring RR interval changes was set, further analyses were performed. Sham iTBS slopes were subtracted from active iTBS slopes, creating slope difference scores which were correlated to HRSD difference scores, in order to investigate whether heart rate decelerations during treatment day one could predict treatment outcome.
In addition, RR interval change, mean RR, HRV and BP were analyzed over Treatment Days, using repeated measures ANOVA with both Treatment Day (1, 10, 20, 30) and Stimulation Type as a within-subject factors. In case of a significant interaction, effects of Stimulation Type were analyzed per treatment day.

RESULTS

HRV and blood pressure data were available for 15 MDD patients (5 males) with a mean age of 32.0. One subject withdrew consent treatment before day 5 due to lack of effect, leaving 14 subjects for Treatment day 10, 20 and 30. At baseline mean HRSD score was 21.72 compared to 11.57 at end of treatment (in 14 patients) (Table 1).

Of the 14 subjects that completed Treatment day 10, 20 and 30, 9 subjects had complete ECG data during sham and active iTBS over these Treatment Days. Complete blood pressure data was available for also 9 subjects.

Table 1: *Demographic characteristics of participants. Presented are age in years (y), Hamilton depression score ratings, baseline and post-treatment. Age of depression onset in years (y), and the length of the current episode in months.*

	TOTAL (N=15)	MALES (N=5)	FEMALES (N=10)
AGE (Y)	32.00 (8.90)	29.00 (8.16)	33.50 (9.29)
HRSD BASELINE	21.72 (4.61)	19.40 (1.14)	22.90 (5.28)
HRSD POST TREATMENT (14 subjects)	11.57 (5.77)	14.25 (3.21)	10.50 (6.35)
AGE OF ONSET (Y)	17.80 (8.56)	14.60 (8.792)	19.40 (8.43)
LENGTH CURRENT EPISODE (months)	47.53 (57.48)	29.20 (18.78)	56.70 (68.58)

RR INTERVAL CHANGE: ACTIVE VS. SHAM ITBS ON FIRST TREATMENT DAY.

Analysis was initially performed blinded to treatment condition. For Treatment Day 1, two subjects had no sham ECG recordings, resulting in 13 subjects for sham analyses. By visually inspecting the data, subjects showed pronounced differences between recordings, mainly visible in the increased variation in RR interval length, but also the slope of RR interval lengthening over the first minute. Since parasympathetic responses are fast, the largest changes were expected in the first 30-60 seconds as observed in the blinded data of stimulation (figure 1 on page 148/149).

The slope of RR intervals were analyzed with a repeated measures ANOVA with Timeframe and Stimulation Type as within subject factors. This resulted in a main effect of Stimulation Type ($F(1, 12)=10.0$, $p=.008$), and of Timeframe ($F(3, 10)=11.9$, $p=.001$), and an interaction between Stimulation Type and Timeframe ($F(3, 10)=4.5$, $p=.031$). Paired t-test between sham and active stimulation per Timeframe indicated that the RR change was significantly larger for active iTBS, with large effect sizes, compared to the sham condition: 30s ($t(12)=2.3$, $p=.041$, $d=-0.9$); 45s ($t(12)=-3.5$, $p=.004$, $d=-1.2$) and 60s ($t(12)=-3.6$, $p=.003$, $d=-1.3$).

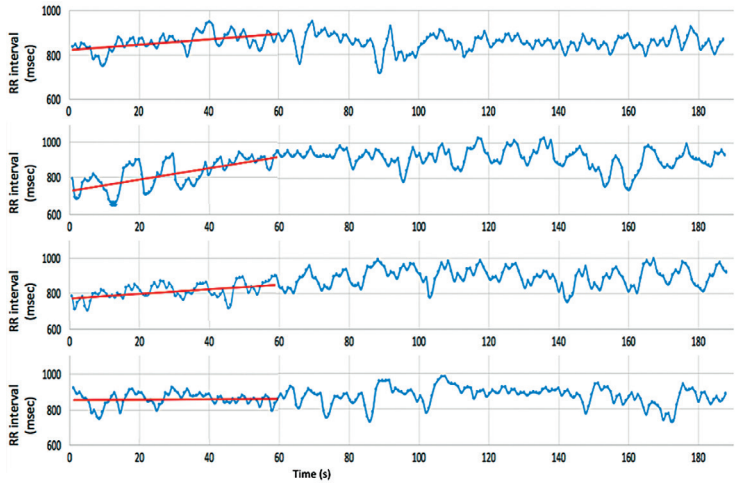


Figure 1: *(page 148/149) Example of RR interval plot of two individuals on the first Treatment Day. On the y-axis RR interval in milliseconds (msec) is shown. The x-axis represents time in seconds (s). The blue line represents the RR intervals. Note especially for the active-iTBS condition (the second and third recording of each block) the clear difference in RR intervals, with an initial HR deceleration (indicated by increasing RR slope, red lines) and more pronounced HRV (indicated by increased variability in RR, or larger amplitudes). Both subjects received the same protocol: Sham-Active-Active-Sham (SAAS). The slope of RR intervals in the first 60 seconds is shown in red.*

For the whole 189s, there is no difference between the two, as expected (see figure 2 below, and table 2, facing page).

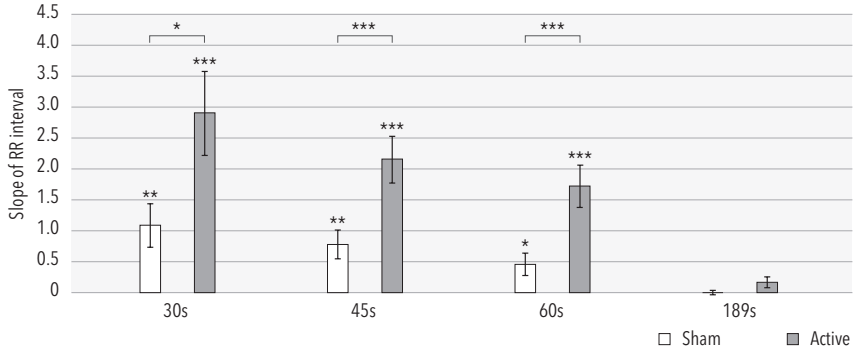
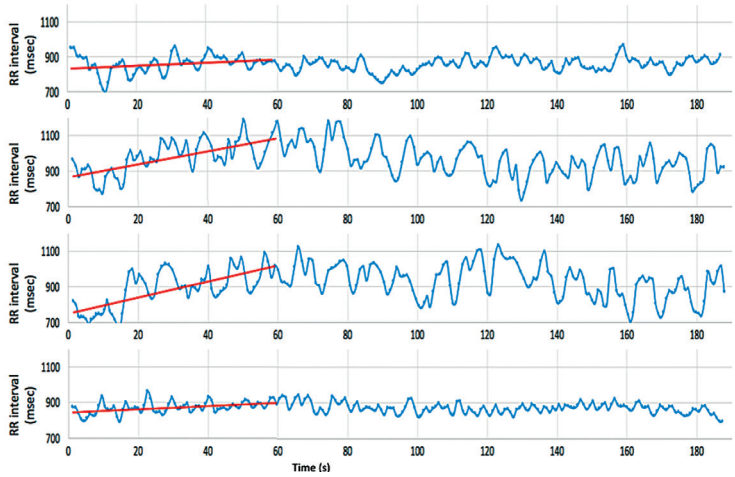


Figure 2: *Slope of RR intervals for different timeframes: first 30, 45, 60 second and total stimulation interval (189 seconds). White bars represent sham stimulation, grey bars active stimulation. *p<.05, **p<.01, ***p<.005*



One-tailed t-tests indicated that slopes significantly deviated from zero, for active iTBS: 30s (t(14)=4.07; p=.001); 45s (t(14)=5.55; p<.001); 60s (t(14)=4.72; p<.001), but also for sham stimulation: 30s (t(12)=3.13; p=.009); 45s (t(12)=3.33; p=.006) and 60s (t(12)=2.55; p=.025). This was not the case for the whole 189s.

Table 2: *Slope of RR intervals for 30, 45, 60 and 189 seconds. Means, SD, t statistics, p-values and effect sizes (Cohens D) are presented.*

	Sham mean (SD)	Active mean (SD)	Statistics
RR slope 30s	1.09 (1.26)	2.90 (2.44)	t(12)=2.28, p=.041, d=-.931
RR slope 45s	0.78 (0.85)	2.15 (1.37)	t(12)=-3.50, p=.004, d=-1.207
RR slope 60s	0.46 (0.65)	1.72 (1.23)	t(12)=-3.64, p=.003, d=-1.275
RR slope 189s	0.01 (0.13)	0.17 (0.32)	t(12)=-1.86, p=.088, d=-.670

PREDICTING TREATMENT RESPONSE

The RR interval slope difference between sham and active iTBS was used to predict treatment outcome at the end of the course. A non-statistically significant trend was obtained between the difference in slope of the first 30 seconds and HRSD difference scores (r(11)=.507, p=.092), explaining 26% of the variance. None of the other variables correlated with HRSD change (figure 3, following page).

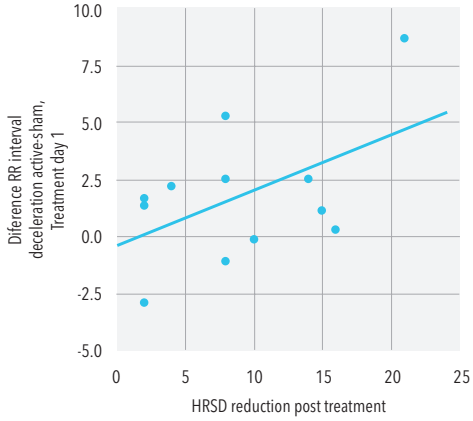


Figure 3: *Correlation between the difference in slope of RR intervals between sham and active iTBS, in the first 30 seconds after start of stimulation on Treatment Day 1, with HRSD reduction post treatment. Each dot represents a single subject. The linear regression line explained 26% of the variance, demonstrating that a larger reduction of HR during the first Treatment Day was associated with a better clinical response to treatment, as hypothesized.*

TREATMENT EFFECTS: ACTIVE VS SHAM ITBS OVER TIME

MEAN RR

Repeated measures ANOVA with Stimulation Type (sham vs. active) as a between subjects factor and Treatment Day (ie. measurements on day 1, 10, 20 and 30 of the treatment) as a within subjects factor resulted in a significant main effect of Stimulation Type ($F(1, 8)=45.87$, $p<.001$, $d=1.062$) but no main effect of Treatment Day. Thus, RR interval lengthening (lower heart rates) occurred consistently during active iTBS stimulation, and this effect persisted across Treatment Days (fig. 4G).

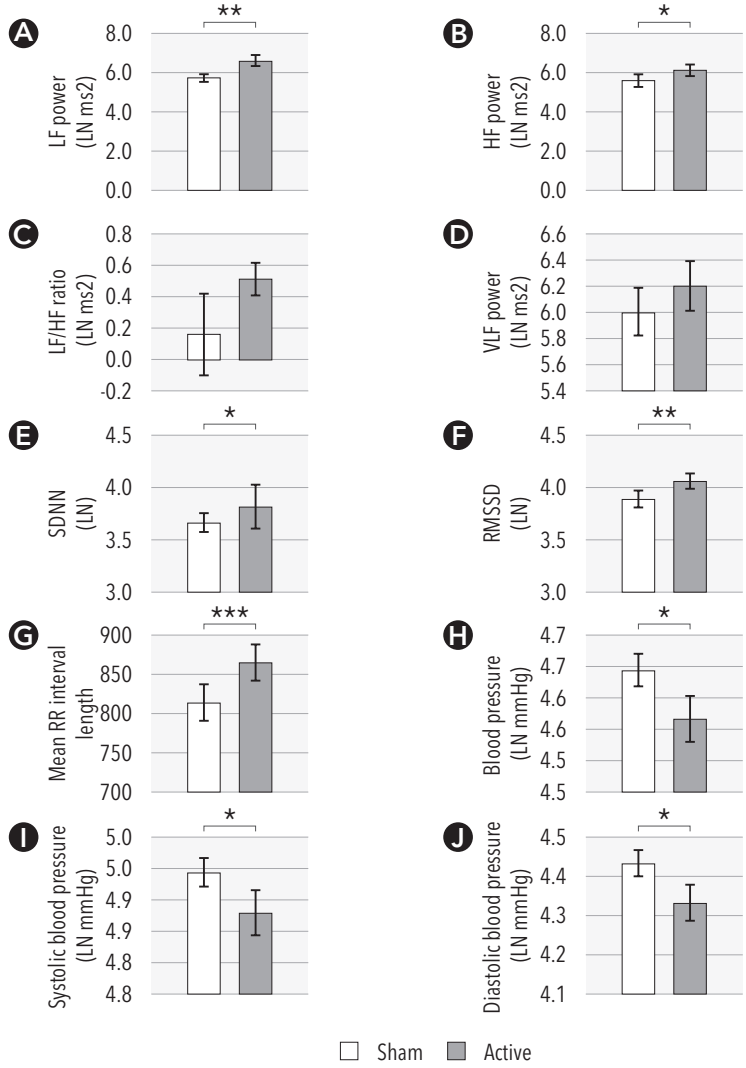
RR INTERVAL CHANGE

Within the first 30 seconds, a main effect was found for Stimulation Type ($F(1, 8)=12.61$, $p=.007$, $d=1.325$), as well as the first 45 seconds ($F(1, 8)=12.81$, $p=.007$, $d=1.303$) and 60 seconds ($F(1, 8)=11.36$, $p=.010$, $d=1.364$), where larger heart rate decelerations were found during active iTBS. For none of the timeframes an interaction with session was found, thus, there were no changes over time.

Figure 4 (right): *Bar graphs showing mean values across al treatment days for sham (white bars) versus active (grey bars) iTBS, for LF (A), HF (B), LF/HF ratio (C), VLF (D), RMSSD (E), SDNN (F), mean RR interval (G), blood pressure (H), systolic blood pressure (I) and diastolic blood pressure (J). All variables except VLF were significantly different between sham and active iTBS over Treatment Days. Error bars represent standard error of the mean.*

HEART RATE VARIABILITY

A main effect of Stimulation Type was found for low frequency power (F(1, 8)=12.48, p=.008, d=-1.156), high frequency power (F(1, 8)=9.23, p=.016, d=-.559), SDNN (F(1, 8)=10.15, p=.013, d=-.805), RMSSD (F(1, 8)=13.45, p=.006, d=-.671), and marginally for the low to high frequency ratio (F(1, 8)=4.33, p=.071, d=-.406). None of the HRV variables changed significantly over Treatment Days and all HRV variables were found to be higher during active stimulation compared to sham stimulation (fig 4A-F).



BLOOD PRESSURE

Since there was no predefined hypothesis of how fast blood pressure changes would appear, a repeated measures ANOVA with both Timeframe and Stimulation Type as within subject factors was used. This showed a main effect of Stimulation Type over Treatment Days ($F(1, 8)=10.93$, $p=.011$, $d=1.079$), showing that blood pressure was significantly lowered during active stimulation. This was not more prominent in one of the different timeframes ($F(3, 6)=.46$, $p=.721$), and also did not change over Treatment Day ($F(3, 6)=.25$, $p=.860$) The difference was not attributable to either systolic or diastolic pressure, both lowered significantly during active stimulation (fig. 4H-J).

DISCUSSION

This study examined the effect of iTBS on cardiovascular parameters such as heart rate, heart rate variability and blood pressure. As hypothesized, active iTBS led to significantly larger heart rate decelerations than sham iTBS. This difference was readily detectable even in the first 30-60 s of the first treatment day.

These findings are relevant for the use of heart rate as a direct outcome measure to verify target engagement for rTMS during treatment, via the methodology we have previously described as Neuro-Cardiac-Guided TMS (NCG-TMS), as described earlier (Iseger et al., 2017; Iseger et. al., 2019b; Chapter 4&5). HR decelerates more during active iTBS, compared to sham stimulation. Although the original NCG TMS method was based on 10Hz TMS, we now demonstrate that iTBS has comparable effects on heart rate. Furthermore, we showed an association between heart rate decelerations and treatment response, which has not been investigated before. A trend towards a significant correlation was found demonstrating that within 30 seconds, the larger the slope of RR intervals for active iTBS compared to sham, the larger the HRSD change. Thus, larger heart rate decelerations were associated with better treatment response. This effect was seen already during the first Treatment Day. Although in this small sample not significant, the explained variance was quite high. It has to be noted that both sham and active iTBS

led to significant heart rate decelerations, when compared to zero, but that these were significantly larger for active iTBS. This suggests that there might be some non-specific habituation effect during iTBS stimulation, expressed as an initial higher HR, subsequently leading to lower heart rates, however active iTBS provides additional HR deceleration with a large effect size (d>1.3) difference, compared to sham stimulation.

Furthermore, blood pressure and most HRV variables were significantly different between sham and active iTBS sessions, but no changes were found over the treatment course. Both systolic and diastolic blood pressure were lower during active stimulation. The effects of non-invasive brain stimulation on blood pressure were reviewed by Sampaio et. al., (2012) (Sampaio et al., 2012). In short, one study found in 6 healthy subjects a trend for active rTMS decreasing blood pressure (Jahanshahi et al., 1997), as well as for tDCS (Knotkova et al., 2012). Another study found only a decreasing effect for the left hemisphere, but not the right hemisphere (Jenkins et al., 2002), just in one session. A recent meta-analysis reported a small effect of rTMS on blood pressure (Makovac et al., 2016), although this study did not differentiate between motor strip and DLPFC stimulation, left or right sided stimulation, and frequency.

Regarding HRV variables, the increases in RMSSD and SDNN indicate higher heart rate variability during active stimulation. LF power is often associated with sympathetic tone, while HF is associated with parasympathetic tone (Thayer and Lane, 2000). In this study, both LF and HF power increased during active stimulation, suggesting that both the sympathetic and parasympathetic pathways increase signaling. However, this is often criticized as LF may not truly reflect sympathetic tone (Billman 2007; Reyes del Paso et al., 2013). HF has been related to respiratory sinus arrhythmia (RSA) and is a measure of the natural variation occurring in the HR during a breathing cycle (Porges 1995), but during slow respiration, vagal activity can generate oscillations that cross over into the LF band. Consequently, LF/HF would not adequately reflect the balance between sympathetic and parasympathetic activity, because LF power represents both sympathetic and parasympathetic activity. Therefore, it may be possible that

in fact only parasympathetic signaling increased as a result of active iTBS stimulation. This is strengthened by the fact that there is no significant difference in VLF power, which is also thought to represent sympathetic activity, although this too remains unclear (Frenneaux 2004). However, the validity of VLF power can be questioned since our recordings where only 189 seconds long, while at least 303 seconds are required in order to have the lower frequencies adequately assessed. In the current data, frequencies between 0.0033 and 0.0053 Hz could not be scored accurately, which may have influenced the results. Nevertheless, one study showed shorter VLF recordings still highly correlate with 5 minute recordings, thus the shorter VLF recordings may give a good indication (Baek et al., 2015).

None of the variables changed over time for the group as a whole, in contrast to the findings of Udupa et. al., (2007) whom reported normalization of HRV variables after rTMS treatment (Udupa et al., 2007). It has to be noted that due to the small sample size, the power to detect such effects may be limited. As a post-hoc analyses, we related the variables to response, showing only a positive correlation for VLF power with HRSD difference scores. VLF power is associated with sympathetic activity and it has recently been shown that a reduction in relative VLF power during REM sleep was associated with improvement in HRSD (Pawlowski, 2018), and at baseline predictive of treatment outcome (Jain et al., 2014), and associated with antidepressant treatment outcome (Jain et al., 2014). However, as mentioned earlier, the validity of our VLF recordings may be questionable, indicating that these results should first be replicated with longer HRV recordings.

A limitation of this preliminary study is the relatively small sample size. Therefore, the association with treatment response, although predicted, requires replication in a larger sample. In addition, for this study, the sham stimulation procedure targeted the vertex rather than the F3 site, leaving open the possibility that different scalp sensations could contribute to the observed differences between active versus sham stimulation. However, this possibility is less likely, given that we have previously shown that even for active stimulation across a variety of different frontal sites, the HR decelerations are seen spe-

cifically with stimulation over dorsolateral regions, and less so over more anterior regions where the sensations of stimulation are more intense (Iseger et al., 2017; Chapter 4).

CONCLUSION

iTBS appears to have an effect on cardiac activity comparable to that of conventional 10 Hz rTMS. There was a pronounced difference between sham and active iTBS for all cardiovascular measures that were used. This suggests that it may be useful to use heart rate as a direct method of verifying correct targeting of the depression network as suggested earlier (Iseger et al., 2017; Chapter 4). Additionally, HRV measurements and blood pressure might be useful too but require longer recordings. The direction of all found effects suggest that parasympathetic signaling increases as a result of active stimulation on the DLPFC.

CHAPTER 8:

A FRONTAL-VAGAL NETWORK THEORY FOR MAJOR DEPRESSIVE DISORDER: IMPLICATIONS FOR OPTIMIZING NEUROMODULATION TECHNIQUES

In press: *Iseger, T.A., van Bueren, N.E.R., Kenemans, J.L., Gevirtz, R., Arns, M. 2019d. A frontal-vagal network theory for Major Depressive Disorder: implications for optimizing neuromodulation techniques. Brain Stimulation.*

Author contributions: MA and TAI conceptualized the study, TAI and NB both equally contributed to writing the first draft of manuscript and the literature search. TAI performed the data analysis. MA supervised the study, JLK, RG and MA all contributed to finalizing the manuscript.

ABSTRACT

Major Depressive Disorder (MDD) is a psychiatric disorder with high comorbidity with cardiovascular disease. Furthermore, a combination of high heart rate (HR) and low heart rate variability (HRV) has been frequently reported in depressed patients. The present review proposes a brain-heart network that overlaps with functional nodes of the depression network.

Moreover, we summarize neuromodulation studies that have targeted key nodes in this depression network, with subsequent impact on heart rate or heart rate variability, such as the dorsolateral prefrontal cortex (DLPFC), subgenual anterior cingulate cortex (sgACC), and the vagus nerve (VN). Based on this interplay, we emphasize the importance of including HR and HRV measurements in human depression studies, in particular those that conduct neuromodulation, in order to obtain a clearer understanding of the pathways that are affected, and we explore the possibilities of using this interplay as a method for target engagement in neuromodulation experiments. Such a target engagement metric opens-up the possibility for individualizing neuromodulation treatments, e.g. Neuro-Cardiac-Guided TMS (NCG-TMS).

INTRODUCTION

Major Depressive Disorder (MDD) is a chronic, heterogeneous psychiatric disorder with a remitting and relapsing course. Regardless a wide range of available treatments (e.g. antidepressants and psychotherapy), up to 40-50% of patients fail to respond (Kessler and Bromet, 2013). Antidepressant medication such as serotonin reuptake inhibitors (SSRI's) and serotonin-norepinephrine reuptake inhibitors (SNRI's) are considered a first-line treatment for MDD (Anderson et al., 2008), but newer neuromodulation treatments such as repetitive Transcranial Magnetic Stimulation (rTMS), transcranial Direct Current Stimulation (tDCS) and Deep Brain Stimulation (DBS) now also show promising clinical benefit in MDD (Donse et al., 2017; Brunoni et al., 2017; Brunoni et al., 2017; Mayberg et al., 2005; Schlaepfer et al., 2013). With these treatments brain structures that are associated with MDD, such as the dorsolateral prefrontal cortex (DLPFC), the dorsomedial prefrontal cortex (DMPFC) and the subgenual cingulate cortex (sgACC), and the vagus nerve (VN), are being stimulated, leading to symptom improvement (Downar et al., 2014; Downar and Daskalakis, 2013; Mayberg et al., 2005; Rush et al., 2000). It has been proposed that network connectivity between the DLPFC and the (sg)ACC may be mediating clinical response (Liston et al., 2014; Fox et al., 2012). The VN, part of the parasympathetic branch of the autonomic nervous system, influences bodily functions such as heart rate (HR) and respiration, and stimulation of the VN consistently leads to HR decelerations (Buschman et al., 2006). Interestingly, several studies have also reported HR decelerations after stimulation of the DLPFC using rTMS and tDCS (Makovac et al., 2016). It is further known that HR in MDD is often dysregulated, expressed in overall higher HR and lower heart rate variability (HRV) (Licht et al., 2008; Koenig et al., 2016; Castaldo et al., 2015), which has been reported to be normalized after neuromodulation treatment, but not after treatment with SSRI's (Kemp et al., 2010). The goal of the current review is to outline the brain-heart

network or frontal-vagal network, and the overlap with the depression network. Next, disturbances in this pathway of MDD patients will be discussed in order to identify a potential target for future neuromodulation interventions and to help refine target selection in neuromodulation treatments.

AN ANATOMICAL FRAMEWORK OF THE BRAIN-HEART CONNECTION

Parasympathetic nerves together with sympathetic nerves at the sinoatrial (SA) node, the atrioventricular (AV) node, and the atria control HR (Shaffer et al., 2014). The sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) are divisions of the autonomic nervous system (ANS) and are both tonically active during rest. Sympathetic activity induces HR acceleration and parasympathetic activity induces HR deceleration (Levy 1984; Levy 1997), via direct nerve fibers descending from the brain, thus suggesting central nervous system (CNS) control over the ANS. The following sections will focus on the efferent anatomical pathways (including both the SNS and the PNS) that are in direct contact with the heart.

AUTONOMIC NERVOUS SYSTEM - SYMPATHETIC PATHWAY

The accelerator nerve originates from three regional ganglia: the superior cervical ganglia, the cervicothoracic ganglia, and the thoracic ganglia. Axons from these three ganglia are connected to the cardiac surface and can influence both atria (Pardini et al., 1989; Kuder and Nowak, 2015). These sympathetic, efferent nerves that project to the SA and AV node in the heart also project to the myocardium (heart muscle) and originate from the intermedio lateral cell column of the spinal cord (IML). Depolarization of the postganglionic sympathetic nerves in the IML results in a release of the neurotransmitters norepinephrine and epinephrine which subsequently results in a depolarization of both nodes of the heart after binding to beta-adrenergic receptors which will lead to an increase in HR (Shaffer et al., 2014). The IML receives information from the rostral ventrolateral medul-

la (RVLM) through preganglionic cholinergic neurons (Thayer and Lane, 2009). The sympathetic pathway is mostly known for its role in stress responses i.e. fight-or-flight. In contrast to the immediate effects of parasympathetic activation on HR, which will be discussed in the next section, sympathetic activation is slow and takes up to five seconds due to unmyelinated efferent fibers from the accelerator nerves. HR is stable after 20-30 seconds if sympathetic activation is persistent (Hainsworth 1995; Shaffer et al., 2014).

AUTONOMIC NERVOUS SYSTEM - PARASYMPATHETIC PATHWAY

The first relay station of the parasympathetic pathway to the heart is the vagus nerve. The vagus nerve originates from the medulla of the brainstem and is directly connected to the heart. This nerve includes axons which converge onto four nuclei of the medulla: the dorsal nucleus of the vagus nerve (DVN), the nucleus ambiguus (AMB), the nucleus of the solitary tract (NTS) and the spinal trigeminal nuclei. Vagal afferents from the heart target cardiac baroreceptors, arterial baroreceptors, and arterial chemoreceptors which eventually terminate in the NTS (Thayer and Lane, 2009). This area integrates information descending from the brain, more specifically, the cerebral cortex and the limbic system.

Parasympathetic efferent nerves from the DVN, one of the two nuclei in the vagus nerve that has been identified to have direct connections with the heart, exit the brainstem just above the cervical nerves (C1)(Crossman and Neary, 2014). The vagus nerve branches into the cervical branches (preganglionic parasympathetic fibers). The superior branches terminate subsequently in the ganglia of the cardiac plexus and enter the heart through the SA node to the AV node and the atrial cardiac muscle (Shaffer et al., 2014). They exert their effects after enhanced activity by means of the release of acetylcholine (ACh). A release of this neurotransmitter leads to the depolarization of both nodes in the heart which consequently lowers HR (Gordan et al., 2015). This effect on the heart is short-lived and depends on the phase of the cardiac cycle at the time the signal is received (Lacey and Lacey, 1978). Stimulation of the vagus nerve therefore usually results in an immediate response of the heart, typically occurring within the

cardiac cycle in which the stimulation occurred and lasting only for about one or two heartbeats after stimulation. Thus, return to a normal HR is very rapid after the activity of the vagus nerve is normalized (Hainsworth 1995; Shaffer et al., 2014) .

Some axons situated in the DVN have their cell bodies near and among the motor neurons of the NA, the second nucleus in the vagus nerve that has been related to a parasympathetic influence on heart function. The axons of these cardiac vagus motor neurons (CVM) terminate in small ganglia and project to the heart through motor fibers (Kiernan and Rajakumar, 2013). Animal studies indicate that 10 percent of the CVM's are situated in the DVN, but that the AMB provides the major cardiac innervations in most mammals (Taylor et al., 1999). However, connections between the DVN and AMB and the heart seem to vary between species. For example, anatomical studies show that more neurons situated in the DVN project to the heart in rats, and least in pigs and dogs (Taylor et al., 1999). It remains unclear whether the AMB or the DVN contain most of the vagal neurons that regulate the human heart. One logical explanation includes the thought that the AMB and DVN together process the incoming stimuli and that the net activity influences the SA node.

What should be noted, is that the vagal efferent pathway from the AMB is myelinated which is not the case regarding the DVN (Porges 2007). As a result, the vagus nerve can act as a neural brake mechanism to change cardiac output by rapidly decreasing HR. It has also been proven that the SA node is under tonic inhibitory control via the vagus nerve in humans and mammals (Levy 1990). This means that during rest in healthy people the tonic parasympathetic activation predominates over sympathetic activation. The vagus nerve, in particular the pathway from the AMB, receives information from the NTS. This fiber bundle is the first relay station that enters the brain following the pathway from the heart and the vagus nerve.

The NTS is a bundle of nerve fibers, including facial, glossopharyngeal and vagus nerve fibers located in the medulla oblongata. Besides efferent signaling to the NA, the NTS also sends excitatory, glutamatergic information to the caudal ventrolateral medulla (CVLM), into

the sympathetic division. Moreover, afferent information is projected to the hypothalamus, nucleus paragigantocellularis (PGi) and the central nucleus of the amygdala (CeA). Unlike the sympathetic pathway, the parasympathetic pathway also contains an afferent connection directly from the SA node in the heart to the NTS which might serve as feedback mechanism. This connection is formed by baroreceptors originating in the heart (Park and Thayer, 2014). The NTS furthermore receives information from the periaqueductal gray (PAG), parabrachial nuclei (PBN), CeA and the *medial prefrontal cortex* (mPFC).

AUTONOMIC NERVOUS SYSTEM - CENTRAL CONTROL

Although the divisions of the sympathetic and parasympathetic nervous system are identified through the thoracolumbar and craniosacral regions of the CNS, this has been difficult to establish for the higher regions of the CNS (for a meta-analysis, see (Beissner et al., 2013) and (Thayer et al., 2011); for overviews (Thayer and Lane, 2009; Thayer and Lane, 2000; Thayer et al., 2011). An overview is presented in figure 1 on page 167. A substantial amount of literature indicates that both the sympathetic and parasympathetic pathways have direct or indirect connections with the frontal cortex in primates and humans (Shoemaker and Goswami, 2015; van der Kooy et al., 1982; Chang et al., 2013). Furthermore, BOLD activity in, amongst others, the amygdala, ventromedial prefrontal cortex (VMPFC) and sgACC was found to be associated with HRV measures, indicative of connectivity between these structures and the heart (Ziegler et al., 2009; Lane et al., 2013).

HRV is the fluctuation around the mean heart rate, largely under influence of respiration, but also affected by blood pressure and arterial fluctuations, and is often used as an index for autonomic functioning. HRV represents the flexible shift between sympathetic and parasympathetic activity, thus how well the central nervous system can react and adapt to environmental cues (Thayer and Lane, 2000). There are three ways to measure HRV, a temporal-based, a frequency-based, or a non-linear approach, all measured using an electrocardiogram (ECG) (Shaffer and Ginsberg, 2017). For the time-based approach the time between two R-peaks (maximum deflection of the QRS complex)

is assessed. The standard deviation of all NN (average RR) intervals (SDNN) is the most used time-based measurement in the existing literature of HRV due to simplicity and ease of calculation. The frequency-based approach makes a distinction between low frequency HRV (LF, 0.04-0.15 Hz), very low frequency HRV (VLF, 0.00-0.04 Hz) and high frequency HRV (HF, 0.15-0.40 Hz) (Shaffer and Ginsberg, 2017). LF-HRV has been associated with sympathetic activity and HF-HRV with parasympathetic activity. Thus, the LF/HF HRV ratio is thought to reflect the interaction between the PNS and the SNS (McCraty and Shaffer, 2015). HF-HRV is a measure of the natural variation occurring in the HR during a breathing cycle (respiratory sinus arrhythmia (RSA)) (Porges 1995), however, during slow respiration or slow paced breathing, vagal activity can easily generate oscillations that cross over into the LF band. Thus, some research suggest that LF-HRV is not simply a reflection of sympathetic activity (Billman 2007; Reyes del Paso et al., 2013) and that LF/HF HRV is not an indication of PNS and SNS balance. More specifically, the LF band is believed to reflect baroreflex activity (MacKinnon et al., 2013; Porges 2007).

According to Lane et. al., (2009), higher HF-HRV is associated with cerebral blood flow increases in the prefrontal cortex. High prefrontal cortex activity was found in individuals with higher HF-HRV as observed by several neuroimaging studies (Smith et al., 2015; Lane et al., 2009; Nugent et al., 2011). A human study that directly studied the role of the prefrontal hemispheres on both HR and HRV, showed that the left and right prefrontal hemispheres have different effects on HR (Ahern et al., 2001). Amytal, a nonselective CNS depressant, was injected in the internal carotid artery that inactivates either the left or right anterior neural structures (i.e. the orbital and mesial prefrontal cortices). Both prefrontal cortices are linked to parasympathetic (vagal) activity (Ter Horst 1999), and 10 minutes after amytal administration HR was similarly increased with a peak after three minutes. Interestingly, they found a difference in the effects on HR after separate administration in either the left or the right carotid artery. HR increase was faster and greater in extent after right prefrontal cortex inactivation. In addition, HRV decreased more during right prefrontal cortex inactivation, relative to left prefrontal cortex inactivation. These results seem to indicate that the right prefrontal

cortex has a greater inhibitory role on the cardiac output in comparison to the left prefrontal cortex. What should be noted is that the study by Ahern et. al., (2001) used patients with epileptic foci and/or lesions in either the left or the right hemisphere who had a diverse variety in used medication thus results might not be generalizable to healthy subjects (Ahern et al., 2001). However, another study compared patients with lesions in the right hemisphere with healthy individuals and patients with left hemispheric lesions. They observed that anticipatory HR deceleration was blunted in patients with right hemispheric lesions, which also suggests hemispheric differences (Yokoyama et al., 1987). Moreover, patients with left or right hemispheric stroke experienced different types of cardiac arrhythmias (Lane et al., 1992). Strokes in both hemispheres led to a decrease of HRV, but this effect was significantly greater as a result of a right hemispheric stroke (Barron et al., 1994). These findings imply a greater right prefrontal influence over vagal mediated cardiac output.

The medial prefrontal cortex (mPFC) has strong connections with the anterior and posterior cingulate cortex, and the orbital prefrontal cortex (OFC) with the middle part of the cingulate cortex (Thayer and Lane, 2000). The anterior cingulate cortex can be divided into ventral (affective) and dorsal (cognitive) sections which have different connectivity and functions (Vogt et al., 2005). The ventral section consists of the subgenual and pregenual ACC and is associated with parasympathetic functioning, as indicated by a meta-analysis of (Thayer et al., 2011). The dorsal section is associated with sympathetic activity (Critchley et al., 2003; Paus et al., 1998) . Both the dACC and the vACC are connected to the insula (Deen et al., 2011) that controls both sympathetic and parasympathetic pathways (Nagai et al., 2010). HF-HRV correlated with emotion-specific cerebral blood flow in the left mid-insula (Lane et al., 2009), as well as in the medial prefrontal cortex, periaqueductal gray (PAG) and caudate nucleus. Strong positive correlations were found between cardiac vagal control and the BOLD signal intensity in the VMPFC (Ziegler et al., 2009) and the sgACC (Lane et al., 2013). Several studies demonstrated that activity of the sgACC/vACC in healthy humans positively correlated with cardiac vagal output during cognitive tasks (Lane et al., 2013; Gianaros et al., 2004; Matthews et al., 2004; Thayer et al., 2012). Moreover, RR inter-

vals (intervals between ventricular depolarizations) were positively related with VMPFC activity in healthy participants during rest (Ziegler et al., 2009), which confirms previous animal studies that indicated that this area is an important prefrontal structure in the ANS.

Voxel-based morphometry has shown in a sample of 185 healthy subjects, that HF-HRV is negatively correlated with grey matter volume in the right putamen, amygdala, insula, superior temporal gyrus, temporal pole and parahippocampal gyrus (Wei et al., 2018), indicating that these structures possibly are implicated in the parasympathetic regulation. A study in rodents, using retrograde viral staining, identified similar pathways to be specifically involved in the forebrain parasympathetic regulation of cardiac activity. Labeled cardiac vagal motorneurons and higher order command cells were found in the DVN, the NA, the NTS, the area postrema, the ventrolateral reticular formation, the locus coeruleus, parabrachial nucleus (PBN), PAG, several regions of the hypothalamus, the bed nucleus of the stria terminalis, the CeA, the anterior cingulate (ACC), the insula, and the frontal cortex (Ahern et al., 2001), showing that these physiologically identified areas overlap with human studies.

Prefrontal cortical areas such as the OFC and mPFC tonically inhibit the amygdala via GABAergic neurons in the amygdala (Barbas et al., 2003; Shekhar et al., 2003). According to Thayer & Lane (2009), a decreased suppression (activation of the amygdala) leads to increases in HR and decreases in HRV and three different pathways were proposed for this.

- Activation of neurons in the rostral RVLM after decreased inhibition from neurons in the CVLM, resulting in enhanced sympathetic activity.
- Inhibition of neurons in the NTS, resulting in inhibition of neurons in both the AMB and the DVN, which leads to suppression of parasympathetic activity.
- Direct activation of neurons in the RVLM resulting in increased sympathetic activity. However, it is stated that this is a less significant pathway due to a lower number of fibers that are connected with the amygdala and the medulla.

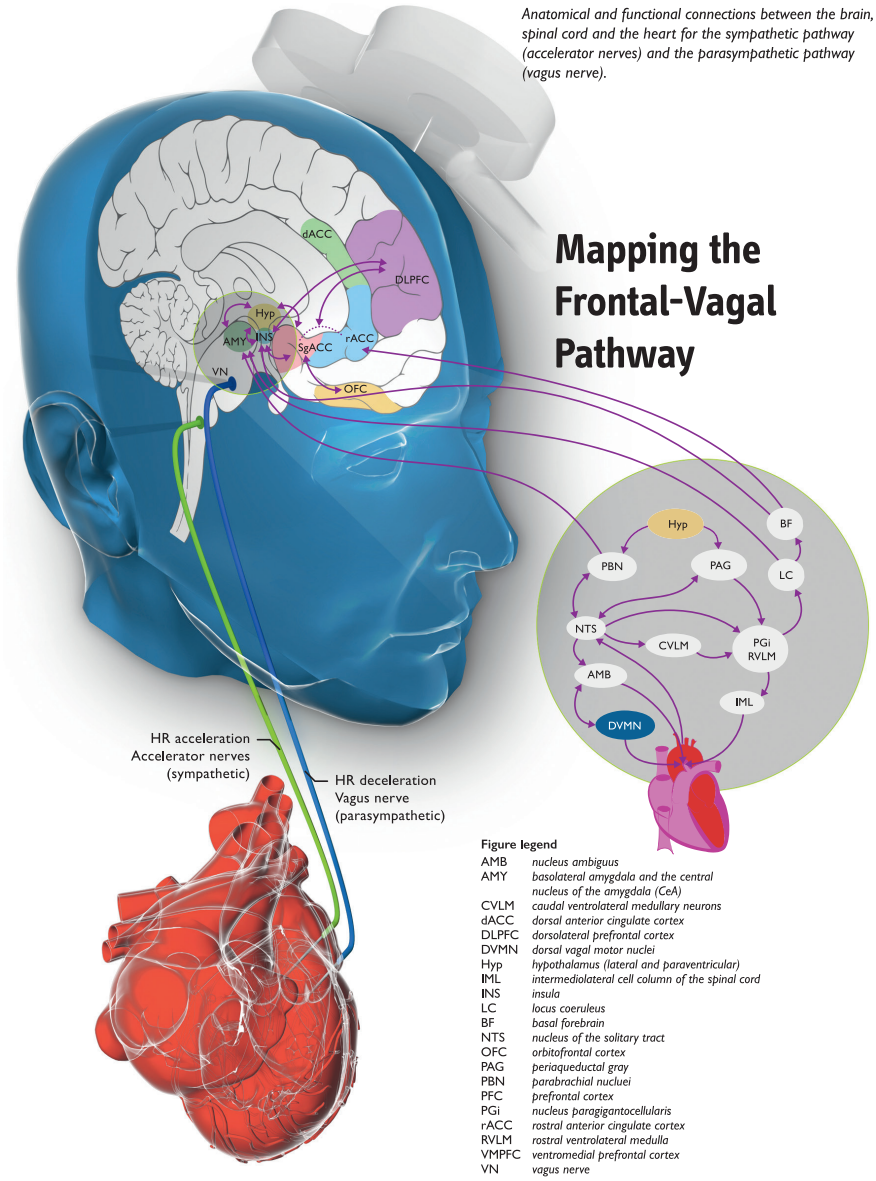


Figure 1: *Visual overview of sympathetic and parasympathetic brain-heart connections.*

Since MDD patients have increased HR and decreased HRV, the first and second pathway could be disturbed by decreased activation of the prefrontal cortex, leading to increased activity in the amygdala, resulting in disinhibition of sympathetic activity and inhibition of parasympathetic activity (Thayer and Lane, 2009). Since this central autonomic network is implicated in the whole brain, there is substantial overlap with important hubs in psychiatric disorders.

MAJOR DEPRESSIVE DISORDER AND THE AUTONOMIC NERVOUS SYSTEM

Previous research indicates comorbidity between cardiovascular disease and MDD which is a pertinent public health concern due to the fact that both diseases are leading causes of disability (Glassman 2007; Penninx et al., 2001; Musselman et al., 1998). Several studies have shown that depression increases risk for cardiovascular illness from two to fivefold. Moreover, autonomic regulation is already disturbed in depressed patients without heart disease, manifested in an overall higher HR, and lower HRV in comparison to healthy controls (Koenig et al., 2016; Ehrenthal et al., 2010; Udupa et al., 2007; Licht et al., 2008), which is more pronounced in patients with severe MDD (Stein et al., 2000), indicating overlap between the depression network and the heart-brain axis.

HRV is extensively studied in depressed patients and healthy individuals (for meta-analysis, see (Kemp et al., 2010)). Low HRV is related to maladaptive and hyper-vigilant processing of emotional stimuli (Park and Thayer, 2014), which is believed to be a right-hemispheric function. In addition, low HRV is linked to a hypoactive prefrontal regulation and is associated with disturbed processing of environmental changes. In contrast, higher HRV is associated with an effective functioning of inhibitory circuits between the prefrontal cortices and the subcortical areas, and enables flexible responses to environmental influences (Thayer and Lane, 2000). Higher HRV is also related to an adaptive and increased top-down and bottom up modulation of cognitive emotional processing and to effective processing of negative stimuli (Gross 1998).

Interestingly, HRV is related to several factors which are linked to depression as well, such as circadian rhythm, seasonality, gender, exercise and smoking (Rajendra Acharya et al., 2006; Kristal-Boneh et al., 2000). Furthermore, dysregulated HR patterns are also seen when sleep patterns are disturbed due to misalignment of the circadian rhythm (Morris et al., 2012), and for other somatic processes such as an increased appetite (de Jonge et al., 2007). Changes in appetite and insomnia are common symptoms of MDD and probably caused through increased sympathetic activity in depressed patients, relative to parasympathetic activity, which subsequently lowers vagal tone in the nerve branches that project to the visceral organs. Depression is found to be more common in women compared to men, in general, and HRV is lower in females as well (De Meersman and Stein, 2007). However, there are some mixed findings for this assertion as gender differences in HRV seem to be age and measure dependent, and age is an important modulator of HRV (Iseger et. al, 2019a; Chapter 3). Gender differences decrease with age, starting from 30 years old, and disappear around the age of 50, dependent on what measure for HRV is used (Umetani et al., 1998). This may be attributed to the level of estrogen (Liu et al., 2003). It appears that depressed men have lower HRV levels compared to nondepressed men. Yet, depressed women show higher HRV levels compared to nondepressed women, although this finding from one study had a small sample size (Thayer et al., 1998). Still, in another small sized study, the same trend was found in healthy subjects when investigating HRV and daily sadness (Verkuil et al., 2015), so this assertion is not settled yet. However, depressed women seem to have higher HRV levels than depressed men (Chambers and Allen, 2007) and this might explain the higher mortality rates for men (Cuijpers and Smit, 2002). Other studies have found reduced vagal activity (higher LF/HF ratios) and higher HR in depressed patients compared to healthy controls, when controlling for gender in a small sample (Agelink et al., 2002). HR and HRV are also under influence of a circadian rhythm, through the suprachiasmatic nucleus (SCN) (Scheer et al., 2004; Massin et al., 2000) and a substantial number of people suffering from mood disorders have significant disruptions in circadian rhythms and in the sleep/wake cycle (McClung 2013). Moreover, HRV seems to fluctuate during winter/summer (Kristal-Boneh et al., 2000) and a peak of patients with heart failure also occurs in

winter (Stewart et al., 2002). Contrary, increased vagal tone has been found in patients with seasonal affective disorder (SAD) during winter, however, the symptomology of this disorder (weight gain, increased appetite, hypersomnia) is different to that from depression (weight loss, decreased appetite, insomnia) (Austen and Wilson, 2001). Interestingly, season of birth seems to influence HRV later in life (Huang et al., 2015) and being born in winter seems to be cardioprotective (Sohn 2016). In line with this, there is a higher risk for depression when being born in spring, and this is lower in winter (Disanto et al., 2012; Torrey et al., 1996). Exercise tends to increase HRV (De Meersman and Stein, 2007), and lower HR. Several studies suggest that exercise has a preventive action on depression and may serve as treatment option (Kvam et al., 2016). Thus, HR and HRV is associated with many factors that are dysregulated in depression as well, further suggestive of a link between the depression network and the heart-brain axis.

A biofeedback technique called Heart Rate Variability Biofeedback (HRV-BF) has been shown to have anti-depressive effects, either by itself or when combined with evidence based behavioral treatments. During HRV-BF, patients are assessed to find a breathing frequency that produces the maximum effect on the vagal pacing of the heart (called respiratory sinus arrhythmia). A recent review on the effects of HRV-BF and emotion regulation, concludes that HR oscillations can enhance emotion by entraining brain rhythms in ways that enhance regulatory brain networks (Mather and Thayer, 2018). An open-label study where 11 depressed patients practiced HRV-BF as the sole treatment, indicated that by session 4, patients with mild depression showed improvements in sleep, hygiene, fatigue, and concentration. In addition, after receiving HRV-BF, there was a decrease in depression severity and an increase in HRV, after 10 sessions (Karavidas et al., 2007). The effects of HRV-BF in MDD patients were also compared to a control group, and to healthy subjects: After two weeks of treatment HRV increased for the HRV-BF group, but not in the active control group (relaxed rest) or in healthy subjects receiving biofeedback. Furthermore, at follow-up, mood was improved in depressed patients (Siepmann et al., 2008). Other studies have shown reductions in depressive symptoms in various patient groups (Patron et al., 2013; Zucker et al., 2009).

The mechanisms behind HRV-BF may parallel the pathways implicated in VN stimulation (VNS). By using Heart Beat Evoked Potentials (HEP), researchers discovered that perception of visceral phenomena such as HR may be mediated by vagal afferent pathways (Schandry et al., 1986). MacKinnon et al showed that the HEP (n250) responded to paced breathing at the optimal or resonance frequency used in HRV-BF (MacKinnon et al., 2013). 5 training sessions in HRV-BF vs. EMG relaxation, greatly increased the signal to the brain from the heart, further reinforcing this finding (Huang et al., 2017).

Thus, MDD, HR and HRV are linked to each other. Interestingly, different neuromodulation techniques, such as VNS, TMS, and potentially DBS, have proven to be successful and effective in treating depression in patients, and also have subsequent effects on cardiac measures such as HR and HRV, possibly (1) increasing parasympathetic activity, (2) decreasing sympathetic activity, or (3) both.

CARDIAC EFFECTS OF NEUROMODULATION ON MDD TREATMENT TARGETS

VAGUS NERVE

In the treatment of severely treatment-resistant depression, the VN has been considered a target for treatment, using Vagus Nerve stimulation (VNS). The VN is the most direct (parasympathetic) connection to the heart, thus during VNS HR consistently decreases (Lang and Levy, 1989; Buschman et al., 2006), with a maximum effect approximately within five seconds from onset of stimulation. VNS is an invasive technique that stimulates the left branch of the VN through electrical current (Schachter 2002; Daban et al., 2008), although new noninvasive techniques to target the VN are emerging, such as transcutaneous VNS (tVNS) (Fang et al., 2016), that also increased HRV (Clancy et al., 2014). VNS in MDD patients has been shown to produce changes in HRV measured by RMSSD (Root Mean Square of the Successive Differences) increase (Sperling et al., 2010). Animal research indicates that VNS can affect widespread brain regions, such as the orbital cortex, lateral frontal cortex, anterior rhinal sulcus and amygdala (George et al., 2000), and influence cardiac responses (Ojeda et al., 2016).

VMPFC

The VMPFC includes both the rostral anterior cingulate cortex (rACC) and the sgACC, which are subdivisions of the ACC. The sgACC has been implicated in depression and autonomic nervous system activity. This area is observed to be hyperactive in depressed adults and adolescents compared to healthy individuals, observed with positron emission tomography (PET) scans and with functional magnetic resonance imaging (fMRI) (Liston et al., 2014; Mayberg et al., 2005). The antidepressant response after deep brain stimulation (DBS) in the sgACC is mediated by the direct down regulation of this area (Mayberg et al., 2005). DBS is an invasive neuromodulatory technique that uses electrodes that need to be placed in different subcortical nuclei through surgery. Compared to healthy subjects, depressed patients show an altered emotional state shifting, due to abnormal sgACC activity, which subsequently leads to altered vagal control (Lane et al., 2013). There have not been many studies investigating the direct effect of deep brain stimulation on the VMPFC in relation to HR, although it was demonstrated in monkeys that electrical stimulation in the pregenual ACC was associated with cardiac slowing (Dua and MacLean, 1964). Furthermore, bradycardia was observed after electrical stimulation of the rostral cingular region in monkeys (Smith 1949). Stronger stimulation also produced increased slowing of the HR. The inhibitory effect on the heart was accentuated and prolonged in monkeys that have received eserine, indicating vagal activity, since eserine prevents the break-down of acetylcholine (Smith 1949). Also in rabbits, electrical stimulation of the medial frontal cortex led to bradycardia (Buchanan et al., 1985).

The studies on deep brain stimulation and HR effects in human subjects are limited and use often different targets: the PAG, subthalamic nucleus (STN), globus pallidum and hypothalamus, all showing an increase in HR (Rossi et al., 2016). Only recently, it was shown in 7 MDD patients that deep brain stimulation of the subgenual cingulate (SCC) white matter (which corresponds to the sgACC) was associated with larger HR acceleration than sham stimulation, in cases where an intraoperative behavior response was observed, but only after left hemispheric stimulation. Thus, this study indicates that with DBS, HR accelerates rather than decelerates after SCC stimulation. This

may be explained by the finding that across all 7 patients, there was a significant relationship between the estimated structural connectivity of the left SCC VTA to the mid-cingulate cortex and the change in HR: the greater the structural connectivity, the more the HR increased (Riva-Posse et al., 2019). Furthermore, it was suggested that the SCC has greater connectivity to the dorsal anterior cingulate which is thought to be linked to the sympathetic system (Crowell et al., 2015; Critchley et al., 2003), subsequently causing HR accelerations. Another important consideration is the true focality of DBS in these studies, since the electrical stimulation intensity in MDD DBS studies is often higher relative to Parkinson DBS studies, and more diffuse as demonstrated by electrical field modelling (Coenen et al., 2011). The latter SCC DBS study applied stimulation at 6mA (Riva-Posse et al., 2019), while for example in the rabbit study HR decelerations were observed at 40 μA (Buchanan et al., 1985), and 0.5-1 mA in monkeys (Dua and MacLean, 1964). Therefore, more human studies with adequate stimulation parameters are needed to confirm the sgACC to be the relay station in this frontal-vagal network.

DLPFC

rTMS is a non-invasive neuromodulation antidepressant treatment which has been shown to be able to influence HR, when aimed at the DLPFC (Makovac et al., 2016). The efficacy of rTMS in the treatment of MDD has been well established in recent years, especially for non-responders to conventional treatments. Currently, remission rates to rTMS are around 37% (Carpenter et al., 2012), and higher when combined with psychotherapy 56% (Donse et al., 2018). As rTMS is limited to cortical surfaces, it is hypothesized that DLPFC-rTMS might exert its antidepressant effect via trans-synaptic activation of deeper regions, such as the sgACC. The sgACC could carry the signal further to deeper brain structures and subsequently have an impact on HR via the VN. Fox et. al., (2012) demonstrated a negative correlation between BOLD activity in the sgACC and the DLPFC which was hypothesized to be associated to the antidepressant mechanism of rTMS (Fox et al., 2012), the higher the anticorrelation, the better the treatment response. In addition, DBS of the

sgACC which suppresses activity, results in an up regulation of the activity in the DLPFC (Mayberg et al., 2005). It is suggested that DLPFC-rTMS exerts its clinical effect via functional connectivity to the sgACC, the more negative the correlation between the two areas was, the better a patient responded to rTMS (Fox et al., 2012). In summary, irrespective of the direction and causality, there is an intricate interplay between the sgACC and DLPFC, and this interplay mediates antidepressant response to rTMS.

The DLPFC has been frequently selected as a target area in non-invasive neuromodulation research such as rTMS and tDCS due to the accessibility of this node in the depression network. For both rTMS and tDCS, several studies have investigated the effects on HR (for a complete overview see (Makovac et al., 2016)). In short, it was found that both tDCS and TMS reduced HR, but the cardiac effects of TMS were stronger relative to tDCS (Makovac et al., 2016). Furthermore, prefrontal stimulation was more effective in reducing HR relative to Motor Cortex stimulation. The DLPFC is usually targeted either on the right side with low (1 Hz) or on the left side with high frequency (10-20 Hz) stimulation. A study conducted in healthy subjects found reduced HR after rTMS over both the left and right DLPFC and decreased arterial pressure after left DLPFC-rTMS (Jenkins et al., 2002). A study by Udupa et. al., (2007) compared the effects of 2 weeks of rTMS with 4 weeks of SSRIs on HRV in antidepressant naïve MDD patients. This study did not implement a sham rTMS intervention in order to control for placebo effects, but measured HRV before and after each treatment. No difference was found on treatment outcome (all interventions were equally effective) and reduced sympathovagal balance was found in both groups. An interaction effect with treatment type was found for SDNN, RMSSD, LF and LF/HF ratio, which indicated a greater reduction in sympathovagal balance in the rTMS group. However, no significant correlation between clinical improvement and autonomic function parameters was found (Udupa et al., 2007). Another study, using intermittent theta burst stimulation (iTBS) in 15 MDD patients, showed that within the first minute of stimulation on the DLPFC a HR deceleration was observed that was significantly larger than during sham stimulation, thereby highlighting the acute effects on HR. More importantly, this study showed a

relationship between HR deceleration in the first 30 seconds (difference between sham and active) with HRSD reduction, indicating that the larger the HR deceleration, the better treatment outcome (Iseger et. al., 2019c; Chapter 7).

These studies suggest that prefrontal TMS is capable of decreasing HR, influencing HRV and possibly normalizing parasympathetic functioning in MDD. However, despite the promising clinical results of targeting the DLPFC, one outstanding problem involves identification of an individualized stimulation location (Rusjan et al., 2010; Mir-Moghtadaei et al., 2015).

TARGET ENGAGEMENT

In all, because of the overlap of the hubs in the depression network (DLPFC, sgACC, VN) with the heart-brain axis, stimulation of these hubs consequently leads to HR decelerations. This interaction might offer possibilities for improving localization of the stimulation target. As reviewed in the supplementary material, the effect of the VN on the heart is rapid; stimulation of the VN usually results in an immediate response of the heart, typically occurring within the cardiac cycle in which the stimulation occurred, with a peak in HR deceleration within 5 seconds (in pigs) (Buschman et al., 2006). The return to a normal HR is very quick after the activity of the VN is reduced (Hainsworth 1995; Shaffer et al., 2014), indicating that it could be verified within a few seconds whether coil or electrode positioning is optimal, taking the time for the signal to travel from the target location to the VN into account. This may be used for choosing an individual optimal target for, for example, TMS. In a proof-of-concept study, TMS was aimed to locate the DLPFC according to HR deceleration (Iseger et al., 2017; Chapter 4). HR data of 10 subjects was collected, while stimulating with 5 second trains of 10Hz TMS on various prefrontal locations (F4, FC4 and C4, F3, FC3 and C3) according to the 10-20 system. In this study, respiration was "filtered out" by converting the ECG to RR intervals and taking only the troughs of this signal (see figure 1). The rationale for this was that the troughs represent the highest HR and could thus show a deceleration more

clearly. In line with the hypothesis, it was found that on the group level, the locations that led best to the largest HR decelerations were F3 and F4, and these are conventionally used as rTMS targets (also referred to as the Beam-F3 method). However, individually, some subjects expressed larger HR decelerations at FC3 or FC4, indicating individual variation. On the other hand, no subject expressed the largest HR decelerations at C3 or C4 (Iseger et al., 2017; Chapter 4).

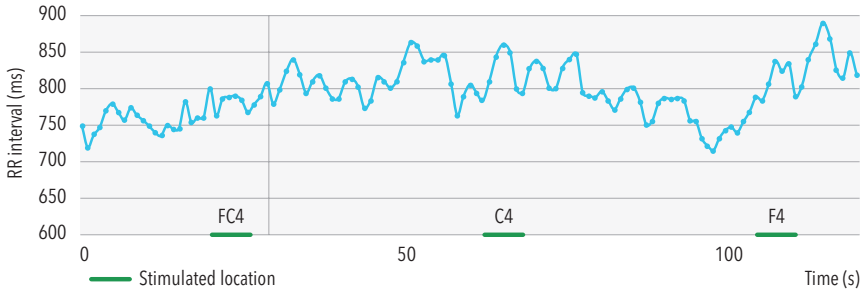


Figure 2: *RR intervals during a recording period with three trains aimed at different 10-20 system scalp locations. RR intervals represent the time within two successive R peaks in the ECG. Trains were applied at FC4 location, C4 and F4. Each train lasted 5 seconds, and between each train was a 30s interval. Increasing RR intervals represent lengthening of the interval, thus, decreases heart rates. Decreasing RR intervals represent shortening of the interval, thus increasing heart rates. For F4 and FC4, lengthening of the RR intervals is clearly visible.*

INDIVIDUAL PARTICIPANT DATA META-ANALYSIS

This method of Neuro-Cardiac-Guided TMS (NCG-TMS) has now been replicated in both a healthy control sample (n=30) and an MDD patient sample (n=33), where stimulation led only to HR decelerations at the F3/F4 and FC3/FC4 sites in both cohorts (healthy controls: F3-C3: d=.463, FC3-C3: d=.487). Up to 6 additional locations were tested, expressing only opposite patterns on the group level (Iseger et. al., 2019b; Chapter 5). Another independent replication was conducted in a sample of 20 healthy subjects. Here, only the left side was stimulated with the NCG-TMS method, on F3, FC3 and C3. Again, the largest HR deceleration was found for F3 (F3-C3: d=.597), and secondly for FC3 (FC3-C3: d=.433). On C3, an HR acceleration was found (Kaur et al., 2019; Chapter 6). In order to objectively test

and extend this NCG-TMS approach, an individual participant data meta-analysis was conducted. Here we aimed to 1) increase the power and, 2) to assess laterality differences. Four cohorts were included: the individual data from the original pilot study and the 3 replication cohorts. This resulted in a total of 99 NCG measurements from 89 subjects, 41 NCG measurements from the left hemisphere and 58 from the right hemisphere. Subjects from the pilot study were included twice since these individuals were tested on both hemispheres. However, the C3 or C4 location was not included for every measurement, leaving a total of 66 measurements (41 on the left and 25 on the right hemisphere). Earlier calculations for sample size (GPower 3.1.9.2) indicated that at least 22 subjects per group were required to replicate the results from the pilot study for both hemispheres.

All data were investigated for normality. A repeated measures ANOVA was conducted with location as within-subjects factor and hemisphere as between-subjects factor. No differences between left and right-sided stimulation were found on the amount of HR deceleration ($F(1, 64)=.837$, $p=.364$), nor an interaction of hemisphere with stimulation location ($F(1.715, 109)=.640$, $p=.506$). A main effect of location was found ($F(1.715, 109)=7.374$, $p=.002$), as hypothesized. Post-hoc analyses revealed that this main effect was due to a significant difference between F3/4 and C3/4 ($t(65)=3.515$, $p=.001$, $d=.56$) and FC3/4 and C3/4 ($t(65)=2.916$, $p=.005$, $d=.47$). No differences were found between F3/4 and FC3/4 in this sample ($n=66$, $p=.152$), nor in the full sample ($n=99$, $p=.08$).

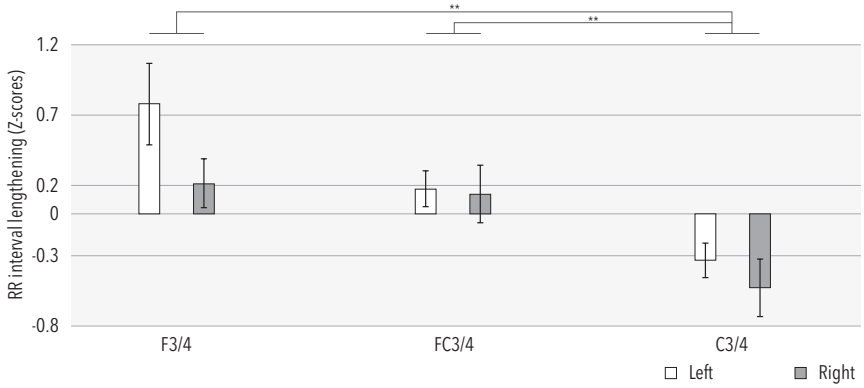


Figure 3: *RR interval lengthening (z-scores) during stimulation at different 10-20 system scalp locations (F3/4, FC3/4, C3/4), either at the left hemisphere (in white) or the right hemisphere (in grey). Increasing RR intervals represent lengthening of the interval, thus, a decrease in heart rate. Error bars represent standard errors of the mean (SEM).*

GENERAL DISCUSSION

We outlined a frontal-vagal brain-heart network that involves direct autonomic connections to the heart for both the SNS and the PNS from the first relay station of the CNS, showing overlap with several key nodes in depression, highlighted by various neuromodulation treatment aimed at these nodes. It has been shown that neuromodulation aimed at the DLPFC and the VN, directly influences this network by decreasing HR or increasing HRV, but it remains unclear what the effect of stimulation at the sgACC is. Interestingly, this framework for MDD is applicable to alternative treatments for depression as well, such as meditation, improving sleep quality, exercise, etc. These therapies have direct influences on autonomic functions such as HR and breathing, thus showing that the different therapies each exert their effect on different levels of the same network. This could imply that combining different treatments might be beneficial for treatment response, as was found for the combination of rTMS with psychotherapy (Donse et al., 2018) where psychotherapy specifically activates the sgACC (Marwood et al., 2018) and thus works synergistically with the DLPFC rTMS stimulation. With regard to antidepressant treatment, the exact working mechanism is unclear, but a review and meta-analysis by Kemp et. al., (2010) investigating the

effects of antidepressants on cardiac autonomic activity indicated that tricyclic antidepressants decrease HRV, and that SSRIs have no effect on HRV. A study by Olbrich et al (2016) showed that the antidepressant effects of SNRIs was accompanied by higher ANS arousal (e.g. HR) (Olbrich et al., 2016). These findings show that antidepressant medication shares overlap with the heart-brain network as well, but the mechanism and direction of these treatments remain unsettled.

We stated that autonomic functioning is disturbed in MDD (higher HR, lower HRV) by means of a shift between sympathetic and parasympathetic activity. This could be a result of either increased sympathetic activity, a decrease in parasympathetic activity or both, but the exact mechanism is still unclear. Also, it has yet to be determined whether high HR and low HRV are state or trait related, and thus, will normalize with response to treatment. Previous research suggests that an increase in HRV was related to successful pharmacological treatment (Khaykin et al., 1998; Balogh et al., 1993), and also with acupuncture treatment (Chambers and Allen, 2002) for depression. However, Udupa et. al., (Udupa et al., 2007) did not find significant correlations between clinical improvement and autonomic function parameters, for SSRI and rTMS treatment and Brunoni et. al., states that HRV is a trait marker of MDD (Brunoni et al., 2013), not a state marker. Recently, a study using iTBS also showed no differences over time for HR and several HRV indices (Iseger et. al., 2019c; Chapter 7). Differences between responders and non-responders in terms of HR and HRV have not been investigated to a great extent, although it was recently shown in a small sample that HR and HRV were not related to HRSD improvement (Iseger et. al., 2019c; Chapter 7).

As mentioned, multiple animal studies are available demonstrating HR decelerations after stimulation of the ventral regions of the ACC. However, there is no substantial evidence on the effect of DBS on HR in human subjects, since only a few studies investigated HR changes while stimulating the SCC/sgACC, albeit with relatively high stimulation intensities (Riva-Posse et al., 2019), relative to non-human studies (Dua and MacLean, 1964; Buchanan et al., 1985; Coenen et al., 2011). It also remains unclear how the findings from Fox et al (2012), that showed an association between high DLPFC-sgACC anticorrelation

and better treatment outcome, relate to the DBS findings, given that the DLPFC is associated with HR decreases. One difference is that the Fox 'method' makes use of a group sgACC seed region, while with DBS electrode placement is performed individually according to structural MRI's, and subjective impressions of the participant and there might be some discrepancy between both locations. This may explain why HR decelerations were found with TMS but not DBS, although both methods are similarly associated with MDD symptom improvement.

This review emphasized the opportunities of using HR and HRV metrics as a reliable marker of targeting the frontal-vagal depression network such as Neuro-Cardiac-Guided TMS, where the target is localized using HR. This method has been successfully replicated in 4 different samples, thereby the proof-of-concept has been validated. However, thus far only preliminary evidence for an association between the amount of HR-deceleration after a first session of iTBS and a better clinical response has been reported (Iseger et. al., 2019c; Chapter 7) therefore the relation to treatment outcome still needs to be assessed more thoroughly. Furthermore, the individual participant data meta-analysis indicated no differences between stimulation of the left or right hemisphere, although earlier studies suggest greater right prefrontal influence over vagal mediated cardiac output. Still, it was also found that both prefrontal cortices are linked to parasympathetic (vagal) activity (Ter Horst 1999), which is also found in the individual participant data meta-analysis.

Since the VN is involved in all parasympathetic functions, gastrointestinal functioning or blood pressure may also serve as markers of targeting the depression network, but, the adequacy and utility of these have yet to be established. Furthermore, the focus of this review was on MDD, but could be translated to other psychiatric disorders that impact the autonomic nervous system as well.

CONCLUSION

To summarize, autonomic functioning, such as HR and HRV, could serve as potential target engagement mechanism for optimizing and individualizing neuromodulation treatments in depression. Based on this, we emphasize the importance of including HR and HRV measurements during human depression studies, in particular those that conduct neuromodulation, to investigate to a better extent the acute effects of neuromodulation on autonomic functioning, and to establish the efficacy of ECG metrics in target engagement of the frontal-vagal network.

CHAPTER 9:

SUMMARY AND GENERAL DISCUSSION

KEY FINDINGS

In this thesis we investigated the heart brain pathway in Major Depressive Disorder. We found that the involvement of the heart in the depression network may be crucial, not only for the understanding of the underlying mechanism and pathology, but also to develop and refine adequate treatments.

Chapter 2 shows baseline differences in EEG theta connectivity between MDD patients and healthy controls. No differences between responders and non-responders to treatment were found, indicating no predictive value for response of theta connectivity. A change in alpha connectivity between the DLPFC and the sgACC was found, but only for male responders in response to treatment, indicating network changes in response to treatment, but also gender differences. Gender differences were observed for every analysis, which emphasizes the need for gender-controlled studies.

Chapter 3 shows that season of birth also influences heart rate variability later in life, which can predispose you for several psychiatric disease or other disorders. Furthermore, HRV is age and gender dependent.

Chapter 4 shows, in ten healthy controls, that rTMS stimulation at the F3 and F4 region (10-20 system EEG locations) lowers the heart rate, compared to stimulation at the C3 and C4 region, thereby confirming the extended depression network to also include the vagus-nerve in addition to the DLPFC and sgACC. This knowledge may be used for optimizing rTMS coil placement. This new method was termed Neuro-Cardiac-Guided TMS or NCG-TMS.

Chapter 5 and 6 demonstrated that the results of the NCG-TMS method replicated in a new sample in our own lab (chapter 5) but also in an independent lab at Monash University in Melbourne (Australia), in further support of the NCG-TMS principle. Furthermore, chapter 5 demonstrated a dose-response relationship: the higher the stimulation intensity, the higher the heart rate deceleration. Also, good test-retest reliability was demonstrated within subjects, demonstrating this method can be reliably applied on the individual level to localize the optimal prefrontal area for activating the whole MDD network.

Chapter 7 further demonstrated that using iTBS, a different stimulation protocol, similarly influences heart rate. Additionally, significants effect on HRV and blood pressure were found, confirming that the parasympathetic nervous system is activated. Furthermore, heart rate changes in the first minute were predictive of rTMS treatment response.

Chapter 8 finally reviewed the whole heart brain pathway and the dysregulation in depression and provides a summary of all the above chapters. In addition, the NCG-TMS method is reviewed by use of an individual participant data meta-analysis, thereby yielding increased power. Laterality differences were assessed, showing no differences between left and right sided stimulation on heart rate. This indicates that 10HZ rTMS impacts the parasympathetic nervous system similarly for both hemispheres.

DISCUSSION

Previous research indicates comorbidity between cardiovascular disease and MDD which is a pertinent public health concern due to the fact that both diseases are leading causes of disability (Glassman 2007; Penninx et al., 2001; Musselman et al., 1998). Several studies have shown that depression increases risk for cardiovascular illness from two to fivefold. Moreover, autonomic regulation is already disturbed in depressed patients without heart disease, manifested in an overall higher HR, and lower HRV in comparison to healthy controls (Koenig et al., 2016; Ehrenthal et al., 2010; Udupa et al., 2007; Licht et al., 2008), which is more pronounced in patients with severe MDD (Stein et al., 2000), indicating overlap between the depression network and the heart-brain axis.

Within this thesis we outlined a brain-heart network that involves direct autonomic connections to the heart for both the SNS and the PNS, showing involvement of several key nodes in depression, indicating various neuromodulation treatment opportunities. A summary of this heart-brain network is provided with **Chapter 8**.

In **Chapter 2** we investigated EEG alpha and theta differences between MDD patients and healthy controls, responders and non-responders. We found baseline differences within the theta band that differed between MDD patients and healthy controls. Furthermore, alpha connectivity changed for male responders to treatment only. Gender differences were found within every analysis. The rationale for this study was based on Fox et al (2012) in which it was found that the higher the anticorrelation between the sgACC and the DLPFC at baseline was, the better the treatment outcome (Fox et al., 2012). We did not find something similar in our data; no baseline differences between responders and non-responders were detected. The difference between Fox et al and our study is the used method: EEG vs. MRI (He and Liu, 2008). Both have their advantages and limitations. In general, fMRI has higher spatial resolution while EEG has better temporal resolution. EEG is based on true neuronal activity, while fMRI depends on blood oxygen flow (BOLD signal), of which the hypothesis is that active brain areas would need more oxygen. Thus, EEG gives real-time activity while for fMRI there is a time-de-

lay. These differences may explain some of the differences in results between the two studes. For example, EEG may not accurately detect activity in the sgACC, because this brain structures lies relatively deep in the brain, and EEG is more suitable for detecting superficial cortical brain activity. In contrast, fMRI may not register real-time activity nor the frequencies in which we were interested, namely in the alpha and theta range (since fMRI signals operate only on very slow time scales). Moreover, one study showed that individual reproducibility for sgACC-DLPFC fMRI connectivity was quite low and could differ a few centimeters between a morning and an afternoon scan (Ning et al., 2018). Still, the results have been replicated in 2 separate studies (Weigand et al., 2018; 45). Although the findings have not yet been prospectively validated in a randomized trial, more and more studies use this method in order to determine the individual stimulation target location.

Chapter 4 was also based on this DLPFC-sgACC connectivity. It was hypothesized that TMS signal could target the DLPFC and accordingly activate pathways to the sgACC, in turn activating the vagus nerve and lowering heart rate. This was indeed seen in a pilot study in 10 healthy volunteers and replicated in three separate cohorts in **Chapter 5** and **6**. Thus, 10 Hz stimulation of the DLPFC lowers heart rate on the group level, but little is known about the actual pathways leading to this HR decrease. Following the neurovisceral integration model and the theory of Fox et al (2012), it is likely that the signal travels form the DLPFC to the sgACC, and further to the vagus nerve. However, important to note is that only functional connectivity has been demonstrated between the DLPFC and the sgACC, but no structural connectivity and most studies investigated only autonomic functioning in relation to the vmPFC or the pregenual ACC. Non-human studies mostly indicate heart rate decelerations, but a recent study in humans showed that DBS applied to the sgACC was associated with HR accelerations (Riva-Posse et al., 2019). However, this study used a relatively high stimulation intensity, relative to non-human studies (Dua and MacLean, 1964; Buchanan et al., 1985; Coenen et al., 2011), as mentioned in **Chapter 8**. Also, this study indicated that their stimulation location had larger connections to the mid- and post-cingulate sections of the ACC (Riva-Posse et al.,

2019), regions that are associated with sympathetic activity (Crowell et al., 2015; Critchley et al., 2003; Paus et al., 1998). More studies are needed to establish the exact role of the sgACC. It remains unclear how the findings from Fox et al (2012) relate to these findings, assuming that the DLPFC is associated with heart rate decreases. One difference is that the Fox method makes use of a group sgACC seed region, while with DBS electrode placement is performed individually according to structural MRI's, so there might be some difference in locations across the two methods. This may explain why HR decelerations were found with TMS but not DBS. Another important remark is that the role of the insula was not investigated within this thesis. It may be the case that the DLPFC also has connections to the insula and that signals may be processed via the insula rather than the sgACC. One study showed that iTBS on the DLPFC interrupted fronto-insular effective connectivity and significantly impacted local GABA levels (Iwabuchi et al., 2017). The insula plays a role in neurovisceral integration, as well as in depression (Sliz and Hayley, 2012; Avery et al., 2014; Kandilarova et al., 2018), and has been associated with heart rate too (Thayer and Lane, 2009; Powell et al., 1985; Saleh and Connell, 1998; Pollatos et al., 2016). Taking the neurovisceral integration model into account, it is not unlikely that stimulation of the DLPFC also impacts the insula, but it is not clear to what extent.

Nevertheless, regardless of the pathway, it was shown that stimulation of the DLPFC influences heart rate (**Chapter 4,5,6,7,8**). In **Chapter 8**, an individual data meta-analysis was conducted, including data from Chapters 4,5 and 6. As expected, medium to large effect sizes were obtained for the difference between F3/4 (DLPFC) stimulation and C3/4 (motor strip). The increased power also demonstrated that there were no differences between stimulation on the left or the right hemisphere, regarding heart rate and 10Hz rTMS.

Although the NCG-TMS method has been successfully replicated in 3 different study samples, a relationship to treatment outcome still needs to be assessed. In **Chapter 7** we see strong indications for a relationship with treatment outcome, but here a different form of TMS is used (iTBS instead of 10hz) and this study sample was rather small. Chapter 7 also suggests that iTBS may be a more potent form of TMS

to be used for NCG-TMS, since the heart rate decelerations that were found were quite large, compared to sham stimulation. However, this was tested in a sample of 30 healthy controls. Subjects received 1 minute of iTBS on 7 different cortical locations while heart rate was recorded, and results actually suggest that not stimulation on F3/4, but on the FC5/6 and F5/6 region has most pronounced effects on heart rate (Chapter 5), thus does not seem useable for DLPFC localization. Interestingly, this area is in in close proximity to the insular cortex where it was previously shown that cTBS impacts the heart beat evoked potential (HBEP) (Pollatos et al., 2016). However, since stimulation on these cortical EEG locations also has profound effects on mastication muscles, it may also be the case that not the insular cortex is stimulated, but the trigeminal nerve, which also has been associated with depression treatments (Cook et al., 2016), and heart rate decelerations (Meuwly et al., 2015). More research is required in order to investigate this further, but these preliminary results may indicate stimulation frequency specific reactions on different cortical areas.

Next to target localization according to heart rate rather than fMRI DLFPC-sgACC connectivity, dosage may also be improved. The currently employed method to determine stimulation intensity is by using Motor Evoked Potentials (MEP), in which the Motor Cortex is stimulated with single pulses which in turn will lead to muscle reflexes in the hand. The intensity on which this reflex is found is assumed to be the right intensity for treatment. However, different brain areas may require different stimulation intensities. For example, the MEP may only indicate ideal stimulation intensity at the motor cortex but not for the DLPFC. The use of heart rate, instead of the MEP, may be more accurate for the DLPFC. In **Chapter 5**, we show that the amount of HR deceleration correlated with percentage machine output, but not with the individual motor threshold, suggesting HR may indeed not only serve as a localization method, but also for determining the ideal stimulation intensity.

Autonomic functioning is disturbed in MDD (higher HR, lower HRV) by means of a shift between sympathetic and parasympathetic activity. This could be a result of either increased sympathetic activity, a decrease in parasympathetic activity or both, but the exact mech-

anism is still unclear. Since the parasympathetic nervous system is dominant over the sympathetic system, it seems likely that decrease in parasympathetic activity is the main cause. Also, it has yet to be determined whether high HR and low HRV are state or trait related; in case of state it will normalize with response to treatment. Previous research suggests that an increase in HRV was related to successful pharmacological treatment (Khaykin et al., 1998; Balogh et al., 1993), and also with acupuncture treatment (Chambers and Allen, 2002) for depression. However, Udupa et. al., (Udupa et al., 2007) did not find significant correlations between clinical improvement and autonomic function parameters, for SSRI and rTMS treatment and Brunoni et. al., state that HRV is a trait marker of MDD (Brunoni et al., 2013), not a state marker. Differences between responders and non-responders in terms of HR and HRV have not been investigated to a great extent. In **Chapter 3**, we also did not find an association between DASS scores and HRV. However, it has to be noted that this was a healthy control sample, without depression. DASS scores where measured, but where mainly in the lower range. Thus, significant correlations may not be present for this reason. Chapter 3 also elaborates on the predisposition for certain HRV profiles. For males, lower HRV was found when born in winter, indicating that season of birth predisposes you for certain HRV profiles, and as a consequence, possibly for certain psychiatric or physical disorders. Furthermore, HRV seemed to be age and gender dependent, emphasizing the importance of taking these factors into account when investigating HRV. There are other confounding factors that interfere with the heart-brain network and may lead to obscured HR and HRV levels. These factors can be anything that influences heart rate such as antidepressant medication, or medication for heart related issues, and also substances such as nicotine are known for effects on the sympathetic system (Haass and Kübler, 1997). Moreover, factors like circadian rhythm, daily habits and exercise influence autonomic functioning (Sammito et al., 2016; Verkuil et al., 2015; Rajendra Acharya et al., 2006; Kristal-Boneh et al., 2000). Food intake and water seem to impact HR and HRV, in such way that about 30 minutes after water intake, HR decreased with approximately 4 BPM, and HRV was significantly increased (Routledge et al., 2002). It is unclear whether these factors would also interfere with the parasympathetic response to neurostimulation.

The framework for MDD as presented here, is applicable to alternative treatments for depression as well, such as meditation, improving sleep quality, exercise, etc. These therapies have influences on autonomic functions such as HR and breathing, suggesting that different therapies each exert their effect on different levels of the same network. This could also imply that combining different treatments might be beneficial for treatment response, as was suggested for the combination of rTMS with psychotherapy (Donse et al., 2018). With regard to antidepressant treatment, the exact working mechanism is unclear, but a review and meta-analysis by Kemp et. al., (2010) investigating the effects of antidepressants on cardiac autonomic activity indicated that tricyclic antidepressants decrease HRV, and that SSRIs have no effect on HRV. A study by Olbrich et al (2016) showed that the antidepressant effect of SNRIs was accompanied by higher ANS arousal (e.g. HR) (Olbrich et al., 2016). These findings show that antidepressant medication is active in the heart-brain network as well, but the mechanism and direction of these treatments is unsettled. It might be interesting to investigate how autonomic changes in response to antidepressant treatment relate to alpha connectivity between the DLPFC and the sgACC as was found to change over time for male responders to antidepressants (**Chapter 2**).

Since the vagus nerve is involved in other parasympathetic functions, stimulation of the DLPFC, sgACC or vagus nerve might also impact gastrointestinal function. Thus, these might also be dysregulated in depression, and may also serve as markers of targeting the depression network, but, the adequacy and utility of these have yet to be established. Interestingly, gastrointestinal function too has been related to depression (Cheung et al., 2019).

LIMITATIONS AND FUTURE RESEARCH

This chapter ends with the discussion of a number of issues that may be considered limitations of the present studies and/ or could be taken in account in future studies; the discussion is mostly in response to issues raised by reviewers of the thesis.

This thesis explores whether DLPFC localization can be improved using heart rate. The DLPFC is one of the main treatment locations for rTMS in MDD and the ultimate goal is to use NCG-TMS in the treatment of MDD. However, the DLPFC is not the only hub in the depression network. In sense, NCG-TMS may not only be a method to target the DLPFC, but rather a method to find the entry point to the depression network, characterized by a heart rate deceleration.

There are several ways to improve the method of NCG-TMS. In the studies presented in this thesis we have relied on the 10-20 system coordinates such as FC3 and F3, and these lie approximately 3-4 cm apart from each other. The electrical field focality induced with stimulation with the Magstim 70mm figure-of-eight coil at 100% MT is ~1.5 cm (Thielscher and Kammer, 2004; Deng et al., 2013), so we initially hypothesized the employed grid of 10-20 locations would mostly cover the prefrontal cortex well (as visualized in figure 5, p105), by means of interpolation). However, future studies should also explore finer grids (e.g. 1 cm. steps) surrounding the F3/F4 and FC3/FC4 sites, especially when using more focal approaches (i.e. lower % motor threshold) to map individual differences in this frontal-vagal-network in more detail.

Furthermore, as described earlier, NCG-TMS may provide an improved method over the fMRI based method by Fox and colleagues (Fox et al., 2012), since the latter method was shown to have low individual reproducibility (Ning et al., 2018). In Chapter 5, it was shown that NCG-TMS was reproducible. However, the interval between two sessions was about a week, and sessions were planned around the same time of day and week. It has yet to be established how reproducible the method is at other time intervals, what its dependence is on the circadian rhythm, which is known to influence the autonomic nervous system (Baschieri and Cortelli, 2019), and if test-retest reliability differs between cortical targets.

Another limitation is that it is yet unsure whether the obtained heart rate decelerations may be attributed to other factors than transsynaptic stimulation of the vagus nerve. For example, the obtained heart rate deceleration may be the result of direct stimulation of the vascular system rather than a cortical brain area activating a functional pathway. Several studies have shown that rTMS and tDCS elicit changes in cerebral blood flow velocity and vasomotor reactivity (the capability of cerebral vessels to dilate in response to stimuli) in branches of the middle cerebral artery (Sander et al., 1995; Pichiorri et al., 2012; Vernieri et al., 2010; Vernieri et al., 2014; Vernieri et al., 2009; Giorli et al., 2015). Though some studies observed HRV changes, no changes in heart rate were observed. This might be attributed to the fact that in these studies the primary motor cortex was targeted, and the motor cortex is not the best target to elicit a heart rate deceleration (Makovac et al., 2016). One study did asses cerebral blood flow velocity after rTMS of the DLPFC and found a decrease in flow velocity of the middle cerebral artery. Interestingly, this study did also not observe heart rate deceleration (Rollnik et al., 2002); note that in that study 1 Hz rTMS was used for 5 minutes, instead of repeated 10 Hz for 5 sec as in the present work. However, these results do indicate that cerebral blood flow and heart rate are not related, making it unlikely that the clear HR decelerations observed in our studies are solely the result of direct stimulation of the vascular system. Furthermore, in these studies, a cortical area was stimulated rather than solely the cerebral artery. Research on other arteries, for example the pulmonary artery, indicated that direct electrical vessel stimulation also does not elicit heart rate changes, only changes in blood pressure (Sun et al., 2015). Furthermore, it can be ruled out that the observed heart rate decelerations are the result of arterial baroreflex (which is an increase in arterial pressure that stimulates the arterial baroreceptors, causing sympathetic inhibition and parasympathetic activation, thus resulting in HR deceleration), since baroreceptors are only present in the vessel wall of the carotid sinus and aortic arch (Thomas 2011). Furthermore, studies assessing pharmacological blockade, lesions and neuroimaging implicate the PFC as a core region in regulation of cardiovascular autonomic activity (Ahern et al., 2001; Ter Horst and Postema, 1997; Lane et al., 2009; Buchanan et al., 1985; Thayer et al., 2012; Gianaros et al., 2004,

and as reviewed in great detail in chapter 8), further emphasizing that stimulation of the DLPFC activates a parasympathetic response pathway. Concluding, based on the above evidence the most likely explanation of the results presented in this thesis is transsynaptic activation of the vagus nerve.
Finally, TMS in most cases is discomforting for subjects. This may result an increased sympathetic tone, and thus heart rate acceleration. This emphasizes the specificity of the finding of heart rate deceleration for specific cortical areas, since accelerations would be expected. One exception could be that, due to anticipation of stimulation, higher pre-stimulation heart rates might occur that decrease during stimulation as a result of habituation. However, in our studies that was controlled for by the inclusion of multiple locations and the fact that every location was stimulated three times in a random order to rule out order effects. Also, the F3/4 location is not the most painful one (which was overall FC5/6) but also not the least painful one (which was in general F1/2).

To summarize, autonomic functioning, such as heart rate and heart rate variability, could serve as potential target engagement mechanism for treatment of depression. For this reason, we emphasize the importance of including heart rate and heart rate variability measurements during human depression studies, to investigate to a better extent baseline and treatments effects on autonomic functioning.

REFERENCES

Abhishekh H.A., Nisarga P., Kisan R., Meghana A., Chandran S., Trichur Raju, Sathyaprabha T. N., 2013. Influence of age and gender on autonomic regulation of heart. *J Clin Monit Comput.* **27**, 259-64.10.1007/s10877-012-9424-3

Agelink M.W., Boz C., Ullrich H., Andrich J., 2002. Relationship between major depression and heart rate variability. Clinical consequences and implications for antidepressive treatment. *Psychiatry Res*. **113**, 139-49.

Ahern G.L., Sollers J. J., Lane R. D., Labiner D. M., Herring A. M., Weinand M. E., Hutzler R., Thayer J. F., 2001. Heart rate and heart rate variability changes in the intracarotid sodium amobarbital test. *Epilepsia*. **42**, 912-21.

Alschuler D.M., Tenke C. E., Bruder G. E., Kayser J., 2014. Identifying electrode bridging from electrical distance distributions: a survey of publicly-available EEG data using a new method. *Clin Neurophysiol*. **125**, 484-90.10.1016/j.clinph.2013.08.024

Alvares G.A., Quintana D. S., Hickie I. B., Guastella A. J., 2016. Autonomic nervous system dysfunction in psychiatric disorders and the impact of psychotropic medications: a systematic review and meta-analysis. *J Psychiatry Neurosci*. **41**, 89-104.

Anand A., Li Y., Wang Y., Wu J., Gao S., Bukhari L., Mathews V. P., Kalnin A., Lowe M. J., 2005. Antidepressant effect on connectivity of the mood-regulating circuit: an FMRI study. *Neuropsychopharmacology*. **30**, 1334-44.10.1038/sj.npp.1300725

Anand A., Li Y., Wang Y., Gardner K., Lowe M. J., 2007. Reciprocal effects of antidepressant treatment on activity and connectivity of the mood regulating circuit: an FMRI study. *J Neuropsychiatry Clin Neurosci*. **19**, 274-82.10.1176/jnp.2007.19.3.274

Anderson I.M., Ferrier I. N., Baldwin R. C., Cowen P. J., Howard L., Lewis G., Matthews K., McAllister-Williams R. H., Peveler R. C., Scott J., Tylee A., 2008. Evidence-based guidelines for treating depressive disorders with antidepressants: a revision of the 2000 British Association for Psychopharmacology guidelines. *J Psychopharmacol*. **22**, 343-96.10.1177/0269881107088441

Arns M., 2010. Historical Archives: The Beginning. *Journal of Neurotherapy*. **14**, 291-292.

Arns M., Drinkenburg P., Fitzgerald P., Kenemans L., 2012. Neurophysiological predictors of non-response to rTMS in depression. *Brain Stimul.* **5**, 569-576.10.1016/j.brs.2011.12.003

Arns M., Gordon E., Boutros N. N., 2015. EEG Abnormalities Are Associated With Poorer Depressive Symptom Outcomes With Escitalopram and Venlafaxine-XR, but Not Sertraline: Results From the Multicenter Randomized iSPOT-D Study. *Clinical EEG and Neuroscience*.**10**.1177/1550059415621435

Arns M., Bruder G., Hegerl U., Spooner C., Palmer D. M., Etkin A., Fallahpour K., Gattt J. M., Hirshberg L., Gordon E., 2015. EEG alpha asymmetry as a gender-specific predictor of outcome to acute treatment with different antidepressant medications in the randomized iSPOT-D study. *Clinical Neurophysiology*.http://dx.doi.org/10.1016/j.clinph.2015.05.032

Arns M., Etkin A., Hegerl U., Williams L. M., DeBattista C., Palmer D. M., Fitzgerald P. B., Harris A., deBeuss R., Gordon E., 2015. Frontal and rostral anterior cingulate (rACC) theta EEG in depression: Implications for treatment outcome? *Eur Neuro psychopharmacol.***10**.1016/j.euroneuro.2015.03.007

Austen M.L., Wilson G. V., 2001. Increased vagal tone during winter in subsyndromal seasonal affective disorder. *Biol Psychiatry*. **50**, 28-34.

Avery D.H., Holtzheimer P. E., Fawaz W., Russo J., Neumaier J., Dunner D. L., Haynor

D. R., Claypoole K. H., Wajdik C., Roy-Byrne P., 2006. A controlled study of repetitive transcranial magnetic stimulation in medication-resistant major depression. *Biol Psychiatry*. **59**, 187-94.10.1016/j.biopsych.2005.07.003

Avery J.A., Drevets W. C., Moseman S. E., Bodurka J., Barcalow J. C., Simmons W. K., 2014. Major depressive disorder is associated with abnormal interoceptive activity and functional connectivity in the insula. *Biol Psychiatry*. **76**, 258-66.10.1016/j.biopsych.2013.11.027

Baek H.J., Cho C. -H., Cho J., Woo J. -M., 2015. Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability. *Telemed J E Health*. **21**, 404-14.10.1089/tmj.2014.0104

Bai X., Li J., Zhou L., Li X., 2009. Influence of the menstrual cycle on nonlinear properties of heart rate variability in young women. *Am J Physiol Heart Circ Physiol*. **297**, H765-74.10.1152/ajpheart.01283.2008

Bakker N., Shahab S., Giacobbe P., Blumberger D. M., Daskalakis Z. J., Kennedy S. H., Downar J., 2014. rTMS of the Dorsomedial Prefrontal Cortex for Major Depression: Safety, Tolerability, Effectiveness, and Outcome Predictors for 10 Hz Versus Intermittent Theta-burst Stimulation. *Brain Stimul.***10**.1016/j.brs.2014.11.002

Balogh S., Fitzpatrick D. F., Hendricks S. E., Paige S. R., 1993. Increases in heart rate variability with successful treatment in patients with major depressive disorder. *Psychopharmacol Bull*. **29**, 201-6.

Banegas J.R., Rodríguez-Artalejo F., de la Cruz J. J., Graciani A., Villar F., del Rey-Calero J., 2000. Adult men born in spring have lower blood pressure. *J Hypertens*. **18**, 1763-6.

Barbas H., Saha S., Rempel-Clower N., Ghashghaei T., 2003. Serial pathways from primate prefrontal cortex to autonomic areas may influence emotional expression. *BMC Neurosci.* **4**, 25.10.1186/1471-2202-4-25

Barker A.T., Jalinous R., Freeston I. L., 1985. Non-invasive magnetic stimulation of human motor cortex. *Lancet.* **1**, 1106-7.

Barron S.A., Rogovski Z., Hemli J., 1994. Autonomic consequences of cerebral hemisphere infarction. *Stroke*. **25**, 113-6.

Baschieri F., Cortelli P., 2019. Circadian rhythms of cardiovascular autonomic function: Physiology and clinical implications in neurodegenerative diseases. *Autonomic neuroscience* : basic & clinical. **217**, 91-101.10.1016/j.autneu.2019.01.009

Beam W., Borckardt J. J., Reeves S. T., George M. S., 2009. An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. *Brain Stimulation.* **2**, 50-54.10.1016/j.brs.2008.09.006

Beissner F., Meissner K., Bär K. -J., Napadow V., 2013. The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. *J Neurosci*. **33**, 10503-11.10.1523/JNEUROSCI.1103-13.2013

Berlim M.T., Van den Eynde F., Daskalakis Z. J., 2013. Efficacy and acceptability of high frequency repetitive transcranial magnetic stimulation (rTMS) versus electroconvulsive therapy (ECT) for major depression: a systematic review and meta-analysis of randomized trials. *Depress Anxiety.* **30**, 614-23.10.1002/da.22060

Berlim M.T., Van den Eynde F., Daskalakis Z. J., 2013. Efficacy and acceptability of high frequency repetitive transcranial magnetic stimulation (rTMS) versus electroconvulsive therapy (ECT) for major depression: a systematic review and meta-analysis of randomized trials. *Depress Anxiety.* **30**, 614-23.10.1002/da.22060

Bermpohl F., Walter M., Sajonz B., Lücke C., Hägele C., Sterzer P., Adli M., Heinz A., Northoff G., 2009. Attentional modulation of emotional stimulus processing in pa-

tients with major depression--alterations in prefrontal cortical regions. *Neurosci Lett.* **463**, 108-13.10.1016/j.neulet.2009.07.061
Bickford R.G., Guidi M., Fortesque P., Swenson M., 1987. Magnetic stimulation of human peripheral nerve and brain: response enhancement by combined magnetoelectrical technique. *Neurosurgery.* **20**, 110-6.
Billman G.E., 2013. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol*. **4**, 26.
Blumberger D.M., Mulsant B. H., Fitzgerald P. B., Rajji T. K., Ravindran A. V., Young L. T., Levinson A. J., Daskalakis Z. J., 2012. A randomized double-blind sham-controlled comparison of unilateral and bilateral repetitive transcranial magnetic stimulation for treatment-resistant major depression. *World J Biol Psychiatry*. **13**, 423-35.10.3109/15622975.2011.579163
Blumberger D.M., Vila-Rodriguez F., Thorpe K. E., Feffer K., Noda Y., Giacobbe P., Knyahnytska Y., Kennedy S. H., Lam R. W., Daskalakis Z. J., Downar J., 2018. Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. *Lancet.* **391**, 1683-1692.10.1016/S0140-6736(18)30295-2
Brakemeier E.-L., Luborzewski A., Danker-Hopfe H., Kathmann N., Bajbouj M., 2007. Positive predictors for antidepressive response to prefrontal repetitive transcranial magnetic stimulation (rTMS). *J Psychiatr Res*. **41**, 395-403.10.1016/j.jpsychires.2006.01.013
Brakemeier E.-L., Wilbertz G., Rodax S., Danker-Hopfe H., Zinka B., Zwanzger P., Grossheinrich N., Várkuti B., Rupprecht R., Bajbouj M., Padberg F., 2008. Patterns of response to repetitive transcranial magnetic stimulation (rTMS) in major depression: replication study in drug-free patients. *J Affect Disord*. **108**, 59-70.10.1016/j.jad.2007.09.007
Brewerton T.D., Putnam K. T., Lewine R. R. J., Risch S. C., 2018. Seasonality of cerebrospinal fluid monoamine metabolite concentrations and their associations with meteorological variables in humans. *J Psychiatr Res*. **99**, 76-82.10.1016/j.jpsychires.2018.01.004
Bruder G.E., Sedoruk J. P., Stewart J. W., McGrath P. J., Quitkin F. M., Tenke C. E., 2008. Electroencephalographic alpha measures predict therapeutic response to a selective serotonin reuptake inhibitor antidepressant: pre- and post-treatment findings. *Biol Psychiatry.* **63**, 1171-7.10.1016/j.biopsych.2007.10.009
Brunoni A.R., Kemp A. H., Dantas E. M., Goulart A. C., Nunes M. A., Boggio P. S., Mill J. G., Lotufo P. A., Fregni F., Benseñor I. M., 2013. Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs. electric current therapy to treat depression clinical study. *International Journal of Neuropsychopharmacology*. **16**, 1937-1949.
Brunoni A.R., Chaimani A., Moffa A. H., Razza L. B., Gattaz W. F., Daskalakis Z. J., Carvalho A. F., 2017. Repetitive Transcranial Magnetic Stimulation for the Acute Treatment of Major Depressive Episodes: A Systematic Review With Network Meta-analysis. *JAMA Psychiatry*. **74**, 143-152.10.1001/jamapsychiatry.2016.3644
Brunoni A.R., Moffa A. H., Sampaio-Júnior B., Gálvez V., Loo C. K., 2017. Treatment-emergent mania/hypomania during antidepressant treatment with transcranial direct current stimulation (tDCS): A systematic review and meta-analysis. *Brain Stimul.* **10**, 260-262.10.1016/j.brs.2016.11.005
Buchanan S.L., Valentine J., Powell D. A., 1985. Autonomic responses are elicited by

electrical stimulation of the medial but not lateral frontal cortex in rabbits. *Behav Brain Res*. **18**, 51-62.
Buschman H.P., Storm C. J., Duncker D. J., Verdouw P. D., van der Aa H. E., van der Kemp P., 2006. Heart rate control via vagus nerve stimulation. N*euromodulation: Technology at the Neural Interface*. **9**, 214-220.
Canpolat U., Özcan F., Özeke, Turak O., Yayla, Açıkgöz S. K., Çay S., Topaloğlu S., Aras D., Aydoğdu S., 2015. Impaired cardiac autonomic functions in apparently healthy subjects with vitamin D deficiency. *Ann Noninvasive Electrocardiol*. **20**, 378-85.10.1111/anec.12233
Carpenter L.L., Janicak P. G., Aaronson S. T., Boyadjis T., Brock D. G., Cook I. A., Dunner D. L., Lanocha K., Solvason H. B., Demitrack M. A., 2012. Transcranial magnetic stimulation (TMS) for major depression: a multisite, naturalistic, observational study of acute treatment outcomes in clinical practice. *Depress Anxiety*. **29**, 587-96.10.1002/da.21969
Cash RFH, Zalesky A, Thomson RH, Tian Y, Cocchi L, and Fitzgerald PB. Subgenual Functional Connectivity Predicts Antidepressant Treatment Response to Transcranial Magnetic Stimulation: Independent Validation and Evaluation of Personalization. *Biol Psychiatry*. **2019**. doi:10.1016/j.biopsych.2018.12.002.
Castaldo R., Melillo P., Bracale U., Caserta M., Triassi M., Pecchia L., 2015. Acute mental stress assessment via short term HRV analysis in healthy adults: A systematic review with meta-analysis. *Biomedical Signal Processing and Control*. **18**, 370-377.
Chalmers J.A., Quintana D. S., Abbott M. J. -A., Kemp A. H., 2014. Anxiety Disorders are Associated with Reduced Heart Rate Variability: A Meta-Analysis. *Front Psychiatry.* **5**, 80.10.3389/fpsyt.2014.00080
Chambers A.S., Allen J. J. B., 2002. Vagal tone as an indicator of treatment response in major depression. *Psychophysiology.* **39**, 861-4.10.1017/S0048577202010442
Chambers A.S., Allen J. J., 2007. Sex differences in cardiac vagal control in a depressed sample: implications for differential cardiovascular mortality. *Biological psychology*. **75**, 32-36.
Chang C., Metzger C. D., Glover G. H., Duyn J. H., Heinze H. -J., Walter M., 2013. Association between heart rate variability and fluctuations in resting-state functional connectivity. *Neuroimage.* **68**, 93-104.10.1016/j.neuroimage.2012.11.038
Clancy J.A., Mary D. A., Witte K. K., Greenwood J. P., Deuchars S. A., Deuchars J., 2014. Non-invasive Vagus Nerve Stimulation in Healthy Humans Reduces Sympathetic Nerve Activity. *Brain Stimul.* **7**, 871-7.10.1016/j.brs.2014.07.031
Clark C.R., Paul R. H., Williams L. M., Arns M., Fallahpour K., Handmer C., Gordon E., 2006. Standardized assessment of cognitive functioning during development and aging using an automated touchscreen battery. *Arch Clin Neuropsychol.* **21**, 449-67.10.1016/j.acn.2006.06.005
Coenen V.A., Schlaepfer T. E., Maedler B., Panksepp J., 2011. Cross-species affective functions of the medial forebrain bundle-implications for the treatment of affective pain and depression in humans. *Neurosci Biobehav Rev*. **35**, 1971-81.10.1016/j.neubiorev.2010.12.009
Cook I.A., Leuchter A. F., Witte E., Abrams M., Uijtdehaage S. H., Stubbeman W., Rosenberg-Thompson S., Anderson-Hanley C., Dunkin J. J., 1999. Neurophysiologic predictors of treatment response to fluoxetine in major depression. *Psychiatry Res*. **85**, 263-73.
Cook I.A., Abrams M., Leuchter A. F., 2016. Trigeminal Nerve Stimulation for Comor-

bid Posttraumatic Stress Disorder and Major Depressive Disorder. *Neuromodulation.* **19**, 299-305.10.1111/ner.12399
Critchley H.D., Mathias C. J., Josephs O., O'Doherty J., Zanini S., Dewar B. -K., Cipolotti L., Shallice T., Dolan R. J., 2003. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain.* **126**, 2139-52.10.1093/brain/awg216
Crossman AR, and Neary D. *Neuroanatomy.* **5th**. Churchill Livingstone; 2014.
Crowell A.L., Garlow S. J., Riva-Posse P., Mayberg H. S., 2015. Characterizing the therapeutic response to deep brain stimulation for treatment-resistant depression: a single center long-term perspective. *Front Integr Neurosci.* **9**, 41.10.3389/fnint. 2015. 00041
Cuijpers P., Smit F., 2002. Excess mortality in depression: a meta-analysis of community studies. *J Affect Disord*. **72**, 227-36.
Cuijpers P., Karyotaki E., Weitz E., Andersson G., Hollon S. D., van Straten A., 2014. The effects of psychotherapies for major depression in adults on remission, recovery and improvement: a meta-analysis. *J Affect Disord*. **159**, 118-26.10.1016/j.jad.2014.02.026
Cummings J.L., 1993. The neuroanatomy of depression. *J Clin Psychiatry*. **54** Suppl, 14-20.
Čukić I., Bates T.C., 2014. Openness to experience and aesthetic chills: Links to heart rate sympathetic activity. *Personality and Individual Differences*. **64**, 152-156.10.1016/j.paid.2014.02.012
Daban C., Martinez-Aran A., Cruz N., Vieta E., 2008. Safety and efficacy of Vagus Nerve Stimulation in treatment-resistant depression. A systematic review. *J Affect Disord*. **110**, 1-15.10.1016/j.jad.2008.02.012
Deakin J., 1998. The role of serotonin in depression and anxiety. *Eur Psychiatry*. **13** Suppl 2, 57s-63s.10.1016/S0924-9338(98)80015-1
Deen B., Pitskel N. B., Pelphrey K. A., 2011. Three systems of insular functional connectivity identified with cluster analysis. *Cereb Cortex*. **21**, 1498-506.10.1093/cercor/bhq186
Deng Z.D., Lisanby S. H., Peterchev A. V., 2013. Electric field depth-focality tradeoff in transcranial magnetic stimulation: simulation comparison of 50 coil designs. B*rain Stimul.* **6**, 1-13.10.1016/j.brs.2012.02.005
Disanto G., Morahan J. M., Lacey M. V., DeLuca G. C., Giovannoni G., Ebers G. C., Ramagopalan S. V., 2012. Seasonal distribution of psychiatric births in England. *PLoS One*. **7**, e34866.10.1371/journal.pone.0034866
Donse L., Sack A. T., Fitzgerald P. B., Arns M., 2017. Sleep disturbances in obsessive-compulsive disorder: Association with non-response to repetitive transcranial magnetic stimulation (rTMS). *Journal of Anxiety Disorders*. **49**, 31-39.10.1016/j.janxdis.2017.03.006
Donse L., Padberg F., Sack A. T., Rush A. J., Arns M., 2018. Simultaneous rTMS and psychotherapy in major depressive disorder: Clinical outcomes and predictors from a large naturalistic study. *Brain Stimul.* **11**, 337-345.10.1016/j.brs.2017.11.004
Downar J., Daskalakis Z. J., 2013. New targets for rTMS in depression: a review of convergent evidence. *Brain Stimul.* **6**, 231-40.10.1016/j.brs.2012.08.006
Downar J., Geraci J., Salomons T. V., Dunlop K., Wheeler S., McAndrews M. P., Bakker N., Blumberger D. M., Daskalakis Z. J., Kennedy S. H., Flint A. J., Giacobbe P., 2014. Anhedonia and reward-circuit connectivity distinguish nonresponders from responders to dorsomedial prefrontal repetitive transcranial magnetic stimulation in major depression. *Biol Psychiatry.* **76**, 176-85.10.1016/j.biopsych.2013.10.026
Drevets W.C., Price J. L., Simpson J. R., Todd R. D., Reich T., Vannier M., Raichle M.

E., 1997. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature.* **386**, 824-827.
Drevets W.C., Price J. L., Furey M. L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct*. **213**, 93-118.10.1007/s00429-008-0189-x
Dua S., Maclean P. D., 1964. Localization for penile erection in medial frontal lobe. *Am J Physiol*. **207**, 1425-34.
Dunlop K., Hanlon C. A., Downar J., 2015. Noninvasive brain stimulation treatments for addiction and major depression. *Annals of the New York Academy of sciences*.
Dunner D.L., Aaronson S. T., Sackeim H. A., Janicak P. G., Carpenter L. L., Boyadjis T., Brock D. G., Bonneh-Barkay D., Cook I. A., Lanocha K., Solvason H. B., Demitrack M. A., 2014. A multisite, naturalistic, observational study of transcranial magnetic stimulation for patients with pharmacoresistant major depressive disorder: durability of benefit over a 1-year follow-up period. *J Clin Psychiatry*. **75**, 1394-401.10.4088/JCP.13m08977
Ehrenthal J.C., Herrmann-Lingen C., Fey M., Schauenburg H., 2010. Altered cardiovascular adaptability in depressed patients without heart disease. *World J Biol Psychia try.* **11**, 586-93.10.3109/15622970903397714
Eranti S., Mogg A., Pluck G., Landau S., Purvis R., Brown R. G., Howard R., Knapp M., Philpot M., Rabe-Hesketh S., Romeo R., Rothwell J., Edwards D., McLoughlin D. M., 2007. A randomized, controlled trial with 6-month follow-up of repetitive transcranial magnetic stimulation and electroconvulsive therapy for severe depression. *Am J Psychiatry.* **164**, 73-81.10.1176/appi.ajp.164.1.73
Fadini T., Matthäus L., Rothkegel H., Sommer M., Tergau F., Schweikard A., Paulus W., Nitsche M. A., 2009. H-coil: Induced electric field properties and input/output curves on healthy volunteers, comparison with a standard figure-of-eight coil. *Clin Neurophysiol.* **120**, 1174-82.10.1016/j.clinph.2009.02.176
Fang J., Rong P., Hong Y., Fan Y., Liu J., Wang H., Zhang G., Chen X., Shi S., Wang L., Liu R., Hwang J., Li Z., Tao J., Wang Y., Zhu B., Kong J., 2016. Transcutaneous Vagus Nerve Stimulation Modulates Default Mode Network in Major Depressive Disorder. *Biol Psychiatry.* **79**, 266-73.10.1016/j.biopsych.2015.03.025
Feffer K., Fettes P., Giacobbe P., Daskalakis Z. J., Blumberger D. M., Downar J., 2018. 1Hz rTMS of the right orbitofrontal cortex for major depression: Safety, tolerability and clinical outcomes. *Eur Neuropsychopharmacol.* **28**, 109-117.10.1016/j.euroneuro.2017.11.011
Fitzgerald P.B., Brown T. L., Marston N. A. U., Daskalakis Z. J., de Castella A., Kulkarni J., 2003. Transcranial magnetic stimulation in the treatment of depression: a double-blind, placebo-controlled trial. *Archives of General Psychiatry*. **60**, 1002.
Fitzgerald P.B., Oxley T. J., Laird A. R., Kulkarni J., Egan G. F., Daskalakis Z. J., 2006. An analysis of functional neuroimaging studies of dorsolateral prefrontal cortical activity in depression. *Psychiatry Res.* **148**, 33-45.10.1016/j.pscychresns.2006.04.006
Fitzgerald P.B., Fountain S., Daskalakis Z. J., 2006. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin Neurophysiol*. **117**, 2584-96.10.1016/j.clinph.2006.06.712
Fitzgerald P.B., Benitez J., de Castella A., Daskalakis Z. J., Brown T. L., Kulkarni J., 2006. A randomized, controlled trial of sequential bilateral repetitive transcranial magnetic stimulation for treatment-resistant depression. *Am J Psychiatry.* **163**, 88-94.10.1176/appi.ajp.163.1.88

Fitzgerald P.B., Huntsman S., Gunewardene R., Kulkarni J., Daskalakis Z. J., 2006. A randomized trial of low-frequency right-prefrontal-cortex transcranial magnetic stimulation as augmentation in treatment-resistant major depression. *Int J Neuro psychopharmacol.* **9**, 655-66.10.1017/S1461145706007176

Fitzgerald P.B., Maller J. J., Hoy K. E., Thomson R., Daskalakis Z. J., 2009. Exploring the optimal site for the localization of dorsolateral prefrontal cortex in brain stimulation experiments. *Brain Stimul.* **2**, 234-7.10.1016/j.brs.2009.03.002

Fitzgerald P.B., Maller J. J., Hoy K. E., Thomson R., Daskalakis Z. J., 2009. Exploring the optimal site for the localization of dorsolateral prefrontal cortex in brain stimulation experiments. *Brain Stimulation*.**10**.1016/j.brs.2009.03.002

Fitzgerald P.B., Hoy K., Daskalakis Z. J., Kulkarni J., 2009. A randomized trial of the anti-depressant effects of low- and high-frequency transcranial magnetic stimulation in treatment-resistant depression. *Depress Anxiety*. **26**, 229-34.10.1002/da.20454

Fitzgerald P.B., Hoy K., Gunewardene R., Slack C., Ibrahim S., Bailey M., Daskalakis Z. J., 2010. A randomized trial of unilateral and bilateral prefrontal cortex transcranial magnetic stimulation in treatment-resistant major depression. *Psychol Med*.**1**-10.10.1017/S0033291710001923

Fitzgerald P.B., Hoy K. E., Anderson R. J., Daskalakis Z. J., 2016. A study of the pattern of response to rTMS in depression. *Depress Anxiety*. **33**, 746-753.10.1002/da.22503

Foerster A., Schmitz J. M., Nouri S., Claus D., 1997. Safety of rapid-rate transcranial magnetic stimulation: heart rate and blood pressure changes. *Electroencephalogr Clin Neurophysiol*. **104**, 207-12.

Fox M.D., Buckner R. L., White M. P., Greicius M. D., Pascual-Leone A., 2012. Efficacy of Transcranial Magnetic Stimulation Targets for Depression Is Related to Intrinsic Functional Connectivity with the Subgenual Cingulate. *Biol Psychiatry.***10**.1016/j.biopsych.2012.04.028

Fox M.D., Liu H., Pascual-Leone A., 2013. Identification of reproducible individualized targets for treatment of depression with TMS based on intrinsic connectivity. *NeuroImage.* **66**, 151-160.10.1016/j.neuroimage.2012.10.082

Fox M.D., Buckner R. L., Liu H., Chakravarty M. M., Lozano A. M., Pascual-Leone A., 2014. Resting-state networks link invasive and noninvasive brain stimulation across diverse psychiatric and neurological diseases. *Proceedings of the National Academy of Sciences.* **111**, E4367-E4375.10.1073/pnas.1405003111

Fregni F., Marcolin M. A., Myczkowski M., Amiaz R., Hasey G., Rumi D. O., Rosa M., Rigonatti S. P., Camprodon J., Walpoth M., Heaslip J., Grunhaus L., Hausmann A., Pascual-Leone A., 2006. Predictors of antidepressant response in clinical trials of transcranial magnetic stimulation. *Int J Neuropsychopharmacol.* **9**, 641-54.10.1017/S1461145705006280

Frenneaux M.P., 2004. Autonomic changes in patients with heart failure and in post-myocardial infarction patients. *Heart*. **90**, 1248-55.10.1136/hrt.2003.026146

Frodl T., Bokde A. L. W., Scheuerecker J., Lisiecka D., Schoepf V., Hampel H., Möller H. -J., Brückmann H., Wiesmann M., Meisenzahl E., 2010. Functional connectivity bias of the orbitofrontal cortex in drug-free patients with major depression. *Biol Psychiatry.* **67**, 161-7.10.1016/j.biopsych.2009.08.02298.

Garcia-Toro M, Pascual-Leone A, Romera M, Gonzalez A, Mico J, Ibarra O, Arnillas H, Capllonch I, Mayol A, and Tormos JM. *British Medical Journal.* British Medical Journal. 2001; 71(4):546-548.

George M.S., Wassermann E. M., Williams W. A., Callahan A., Ketter T. A., Basser P.,

Hallett M., Post R. M., 1995. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. *Neuroreport.* **6**, 1853-6.
George M.S., Wassermann E. M., Kimbrell T. A., Little J. T., Williams W. E., Danielson A. L., Greenberg B. D., Hallett M., Post R. M., 1997. Mood improvement following daily left prefrontal repetitive transcranial magnetic stimulation in patients with depression: a placebo-controlled crossover trial. *Am J Psychiatry*. **154**, 1752-6.
George M.S., Sackeim H. A., Rush A. J., Marangell L. B., Nahas Z., Husain M. M., Lisanby S., Burt T., Goldman J., Ballenger J. C., 2000. Vagus nerve stimulation: a new tool for brain research and therapy. *Biol Psychiatry.* **47**, 287-95.
George M.S., Lisanby S. H., Avery D., McDonald W. M., Durkalski V., Pavlicova M., Anderson B., Nahas Z., Bulow P., Zarkowski P., Holtzheimer P. E., Schwartz T., Sackeim H. A., 2010. Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. *Arch Gen Psychiatry.* **67**, 507-16.10.1001/archgenpsychiatry.2010.46
George M.S., Taylor J. J., Short E. B., 2013. The expanding evidence base for rTMS treatment of depression. *Curr Opin Psychiatry*. **26**, 13-8.10.1097/YCO.0b013e32835ab46d
Gerwig M., Kastrup O., Meyer B. -U., Niehaus L., 2003. Evaluation of cortical excitability by motor and phosphene thresholds in transcranial magnetic stimulation. *J Neurol Sci.* **215**, 75-8.
Gianaros P.J., Van Der Veen F. M., Jennings J. R., 2004. Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: Implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology.* **41**, 521-30.10.1111/1469-8986.2004.00179.x
Giorli E., Tognazzi S., Briscese L., Bocci T., Mazzatenta A., Priori A., Orlandi G., Del Sette M., Sartucci F., 2015. Transcranial Direct Current Stimulation and Cerebral Vasomotor Reserve: A Study in Healthy Subjects. *J Neuroimaging.* **25**, 571-4.10.1111/jon.12162
Glassman A.H., 2007. Depression and cardiovascular comorbidity. *Dialogues Clin Neurosci.* **9**, 9-17.
Gordan R., Gwathmey J. K., Xie L. -H., 2015. Autonomic and endocrine control of cardiovascular function. *World J Cardiol*. **7**, 204-14.10.4330/wjc.v7.i4.204
Gorman J.M., 2006. Gender differences in depression and response to psychotropic medication. *Gender Medicine*. **3**, 93-109.
Gratton G., Coles M. G., Donchin E., 1983. A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol*. **55**, 468-84.
Grieve S.M., Korgaonkar M. S., Koslow S. H., Gordon E., Williams L. M., 2013. Widespread reductions in gray matter volume in depression. *Neuroimage Clin*. **3**, 332-9.10.1016/j.nicl.2013.08.016
Griffiths K.R., Quintana D. S., Hermens D. F., Spooner C., Tsang T. W., Clarke S., Kohn M. R., 2017. Sustained attention and heart rate variability in children and adolescents with ADHD. *Biol Psychol.* **124**, 11-20.10.1016/j.biopsycho.2017.01.004
Gross J.J., 1998. Antecedent- and response-focused emotion regulation: divergent consequences for experience, expression, and physiology. *J Pers Soc Psychol*. **74**, 224-37.
Gross M., Nakamura L., Pascual-Leone A., Fregni F., 2007. Has repetitive transcranial magnetic stimulation (rTMS) treatment for depression improved? A systematic review and meta-analysis comparing the recent vs. the earlier rTMS studies. *Acta Psychiatr Scand*. **116**, 165-73.10.1111/j.1600-0447.2007.01049.x

Grunhaus L., Schreiber S., Dolberg O. T., Polak D., Dannon P. N., 2003. A randomized controlled comparison of electroconvulsive therapy and repetitive transcranial magnetic stimulation in severe and resistant nonpsychotic major depression. *Biol Psychiatry.* **53**, 324-31.

Gudayol-Ferré E., Peró-Cebollero M., González-Garrido A. A., Guàrdia-Olmos J., 2015. Changes in brain connectivity related to the treatment of depression measured through fMRI: a systematic review. *Frontiers in human neuroscience*. **9**,

Haass M., Kübler W., 1997. Nicotine and sympathetic neurotransmission. *Cardiovasc Drugs Ther*. **10**, 657-65.

Hainsworth R. The control and physiological importance of heart rate. In: : *Heart Rate Variability.* Futura Publishing Company, Inc.; 1995:3-19.

Hallett M., 2007. Transcranial magnetic stimulation: a primer. *Neuron.* **55**, 187-99.10.1016/j.neuron.2007.06.026

He B., Liu Z., 2008. Multimodal functional neuroimaging: integrating functional MRI and EEG/MEG. IEEE *Rev Biomed Eng*. **1**, 23-40.10.1109/RBME.2008.2008233

Henriques J.B., Davidson R. J., 1990. Regional brain electrical asymmetries discriminate between previously depressed and healthy control subjects. *J Abnorm Psychol.* **99**, 22-31.

Henry J.D., Crawford J. R., 2005. The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative data in a large non-clinical sample. *Br J Clin Psychol.* **44**, 227-39.10.1348/014466505X29657

Herbsman T., Avery D., Ramsey D., Holtzheimer P., Wadjik C., Hardaway F., Haynor D., George M. S., Nahas Z., 2009. More lateral and anterior prefrontal coil location is associated with better repetitive transcranial magnetic stimulation antidepressant response. *Biol Psychiatry*. **66**, 509-15.10.1016/j.biopsych.2009.04.034

Hillebrand S., Gast K. B., de Mutsert R., Swenne C. A., Jukema J. W., Middeldorp S., Rosendaal F. R., Dekkers O. M., 2013. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace*. **15**, 742-9.10.1093/europace/eus341

Hoflich G, Kasper S, Hufnagel A, Ruhrmann S, Moller HJ, 1993. Application of transcranial magnetic stimulation in the treatment of drug-resistant major depression: a report of two cases. Application of transcranial magnetic stimulation in the treatment of drug-resistant major depression: a report of two cases.. *Hum Psychopharmacol* **8**, 361-365.

Huang C., Gevirtz R. N., Onton J., Criado J. R., 2017. Investigation of vagal afferent functioning using the Heartbeat Event Related Potential. *Int J Psychophysiol*.**10**.1016/j.ijpsycho.2017.06.007

Huang W.-L., Chang L. -R., Kuo T. B. J., Lin Y. -H., Chen Y. -Z., Yang C. C. H., 2013. Gender differences in personality and heart-rate variability. *Psychiatry Res*. **209**, 652-7.10.1016/j.psychres.2013.01.031

Huang W.-L., Hwang B. -T., Lai C. -T., Li J. -Y., Kuo T. B. J., Yang C. C. H., 2015. Is Heart Rate Variability Related to Season of Birth?. *Clin Cardiol*. **38**, 407-12.10.1002/clc.22410

Huang Y.-Z., Edwards M. J., Rounis E., Bhatia K. P., Rothwell J. C., 2005. Theta burst stimulation of the human motor cortex. *Neuron*. **45**, 201-6.10.1016/j.neuron.2004.12.033

Huang Y.-Z., Rothwell J. C., Lu C. -S., Wang J., Weng Y. -H., Lai S. -C., Chuang W. -L., Hung J., Chen R. -S., 2009. The effect of continuous theta burst stimulation over

premotor cortex on circuits in primary motor cortex and spinal cord. *Clin Neurophysiol.* **120**, 796-801.10.1016/j.clinph.2009.01.003

Husain M.M., Rush A. J., Fink M., Knapp R., Petrides G., Rummans T., Biggs M. M., O'Connor K., Rasmussen K., Litle M., Zhao W., Bernstein H. J., Smith G., Mueller M., McClintock S. M., Bailine S. H., Kellner C. H., 2004. Speed of response and remission in major depressive disorder with acute electroconvulsive therapy (ECT): a Consortium for Research in ECT (CORE) report. *J Clin Psychiatry*. **65**, 485-91.

Ingalhalikar M., Smith A., Parker D., Satterthwaite T. D., Elliott M. A., Ruparel K., Hakonarson H., Gur R. E., Gur R. C., Verma R., 2014. Sex differences in the structural connectome of the human brain. *Proceedings of the National Academy of Sciences*. **111**, 823-828.

Iseger T.A., Korgaonkar M. S., Kenemans J. L., Grieve S. M., Baeken C., Fitzgerald P. B., Arns M., 2017. EEG connectivity between the subgenual anterior cingulate and prefrontal cortices in response to antidepressant medication. *European Neuropsychopharmacology*. **27**(4):301-312. 10.1016/j.euroneuro.2017.02.002

Iseger T.A., Padberg F., Kenemans J. L., Gevirtz R., Arns M., 2017. Neuro-Cardiac-Guided TMS (NCG-TMS): Probing DLPFC-sgACC-vagus nerve connectivity using heart rate--First results. *Brain Stimulation*. **10**(5):1006-1008. 10.1016/j.brs.2017.05.002.

Iseger, T.A., Vollebregt, M.A., Krepel, N., Arns., M. 2019a. Heart rate variability related to season of birth: A Replication Study. *Psychophysiology*. e13419. Epub ahead of print. 10.1111/psyp.13419.

Isenberg K., Downs D., Pierce K., Svarakic D., Garcia K., Jarvis M., North C., Kormos T. C., 2005. Low frequency rTMS stimulation of the right frontal cortex is as effective as high frequency rTMS stimulation of the left frontal cortex for antidepressant-free, treatment-resistant depressed patients. *Ann Clin Psychiatry*. **17**, 153-9.

Ishikawa S., Matsunaga K., Nakanishi R., Kawahira K., Murayama N., Tsuji S., Huang Y. Z., Rothwell J. C., 2007. Effect of theta burst stimulation over the human sensorimotor cortex on motor and somatosensory evoked potentials. *Clin Neurophysiol*. **118**, 1033-43.10.1016/j.clinph.2007.02.003

Iwabuchi S.J., Raschke F., Auer D. P., Liddle P. F., Lankappa S. T., Palaniyappan L., 2017. Targeted transcranial theta-burst stimulation alters fronto-insular network and prefrontal GABA. *Neuroimage*. **146**, 395-403.10.1016/j.neuroimage.2016.09.043

Jahanshahi M., Ridding M. C., Limousin P., Profice P., Fogel W., Dressler D., Fuller R., Brown R. G., Brown P., Rothwell J. C., 1997. Rapid rate transcranial magnetic stimulation--a safety study. *Electroencephalogr Clin Neurophysiol*. **105**, 422-9.

Jain F.A., Cook I. A., Leuchter A. F., Hunter A. M., Davydov D. M., Ottaviani C., Tartter M., Crump C., Shapiro D., 2014. Heart rate variability and treatment outcome in major depression: A pilot study. *Int J Psychophysiol*. **93**, 204-10.10.1016/j.ijpsycho.2014.04.006

Januel D., Dumortier G., Verdon C. -M., Stamatiadis L., Saba G., Cabaret W., Benadhira R., Rocamora J. -F., Braha S., Kalalou K., Vicaut P. E., Fermanian J., 2006. A double-blind sham controlled study of right prefrontal repetitive transcranial magnetic stimulation (rTMS): therapeutic and cognitive effect in medication free unipolar depression during 4 weeks. *Prog Neuropsychopharmacol Biol Psychiatry*. **30**, 126-30.10.1016/j.pnpbp.2005.08.016

Jenkins J., Shajahan P. M., Lappin J. M., Ebmeier K. P., 2002. Right and left prefrontal transcranial magnetic stimulation at 1 Hz does not affect mood in healthy volunteers. *BMC Psychiatry*. 2, 1.

Johnson K.A., Baig M., Ramsey D., Lisanby S. H., Avery D., McDonald W. M., Li X., B ernhardt E. R., Haynor D. R., Holtzheimer P. E., Sackeim H. A., George M. S., Nahas Z., 2013. Prefrontal rTMS for treating depression: location and intensity results from the OPT-TMS multi-site clinical trial. *Brain Stimul*. **6**, 108-17.10.1016/j.brs.2012.02.003

de Jonge P., Mangano D., Whooley M. A., 2007. Differential association of cognitive and somatic depressive symptoms with heart rate variability in patients with stable coronary heart disease: findings from the Heart and Soul Study. *Psychosom Med*. **69**, 735-9.10.1097/PSY.0b013e31815743ca

Kandilarova S., Stoyanov D., Kostianev S., Specht K., 2018. Altered Resting State Effective Connectivity of Anterior Insula in Depression. *Front Psychiatry*. **9**, 83.10.3389/fpsyt.2018.00083

Karavidas M.K., Lehrer P. M., Vaschillo E., Vaschillo B., Marin H., Buyske S., Malinovsky I., Radvanski D., Hassett A., 2007. Preliminary results of an open label study of heart rate variability biofeedback for the treatment of major depression. *Appl Psychophysiol Biofeedback*. **32**, 19-30.10.1007/s10484-006-9029-z

Keller M.B., McCullough J. P., Klein D. N., Arnow B., Dunner D. L., Gelenberg A. J., Markowitz J. C., Nemeroff C. B., Russell J. M., Thase M. E., Trivedi M. H., Zajecka J., 2000. A comparison of nefazodone, the cognitive behavioral-analysis system of psychotherapy, and their combination for the treatment of chronic depression. *N Engl J Med.* **342**, 1462-70.

Kemp A.H., Quintana D. S., Gray M. A., Felmingham K. L., Brown K., Gatt J. M., 2010. Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol Psychiatry.* **67**, 1067-74.10.1016/j.biopsych.2009.12.012

Kessler R.C., Petukhova M., Sampson N. A., Zaslavsky A. M., Wittchen H. -U., 2012. Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int J Methods Psychiatr Res*. **21**, 169-84.10.1002/mpr.1359

Kessler R.C., Bromet E. J., 2013. The epidemiology of depression across cultures. *Annu Rev Public Health*. **34**, 119-38.10.1146/annurev-publhealth-031912-114409

Khaykin Y., Dorian P., Baker B., Shapiro C., Sandor P., Mironov D., Irvine J., Newman D., 1998. Autonomic correlates of antidepressant treatment using heart-rate variability analysis. *Can J Psychiatry*. **43**, 183-6.10.1177/070674379804300209

Kiernan JA, and Rajakumar N. Barr's The Human Nervous System: an anatomical viewpoint. *10th. Lipincott Williams and Wilkins*; 2013.

Kirsch I., Deacon B. J., Huedo-Medina T. B., Scoboria A., Moore T. J., Johnson B. T., 2008. Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. *PLoS Med*. **5**, e45.10.1371/journal.pmed.0050045

Klein E., Kreinin I., Chistyakov A., Koren D., Mecz L., Marmur S., Ben-Shachar D., Feinsod M., 1999. Therapeutic efficacy of right prefrontal slow repetitive transcranial magnetic stimulation in major depression: a double-blind controlled study. *Arch Gen Psychiatry*. **56**, 315-20.

Knotkova H., Rosedale M., Strauss S. M., Horne J., Soto E., Cruciani R. A., Malaspina D., Malamud D., 2012. Using Transcranial Direct Current Stimulation to Treat Depression in HIV-Infected Persons: The Outcomes of a Feasibility Study. *Front Psychiatry*. **3**, 59.10.3389/fpsyt.2012.00059

Koenig J., Kemp A. H., Beauchaine T. P., Thayer J. F., Kaess M., 2016. Depression and

ʃresting state heart rate variability in children and adolescents - A systematic review and meta-analysis. *Clin Psychol Rev*. **46**, 136-50.10.1016/j.cpr.2016.04.013
Koenig J., Thayer J. F., 2016. Sex differences in healthy human heart rate variability: A meta-analysis. *Neurosci Biobehav Rev*. **64**, 288-310.10.1016/j.neubiorev.2016.03.007
Koenigs M., Grafman J., 2009. The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav Brain Res*. **201**, 239-43.10.1016/j.bbr.2009.03.004
Korgaonkar M.S., Grieve S. M., Koslow S. H., Gabrieli J. D. E., Gordon E., Williams L. M., 2010. Loss of white matter integrity in major depressive disorder: Evidence using tract-based spatial statistical analysis of diffusion tensor imaging. *Hum Brain Mapp*.**10**.1002/hbm.21178
Korgaonkar M.S., Grieve S. M., Etkin A., Koslow S. H., Williams L. M., 2012. Using Standardized fMRI Protocols to Identify Patterns of Prefrontal Circuit Dysregulation that are Common and Specific to Cognitive and Emotional Tasks in Major Depressive Disorder: First Wave Results from the iSPOT-D Study. *Neuropsychopharmacology*.**10**.1038/npp.2012.252
Kozlowska K., Palmer D. M., Brown K. J., Scher S., Chudleigh C., Davies F., Williams L. M., 2015. Conversion disorder in children and adolescents: a disorder of cognitive control. *J Neuropsychol*. **9**, 87-108.10.1111/jnp.12037
Krepel N., Sack A. T., Kenemans J. L., Fitzgerald P. B., Drinkenburg W. H., Arns M., 2018. Non-replication of neurophysiological predictors of non-response to rTMS in depression and neurophysiological data-sharing proposal. *Brain Stimul*.**10**.1016/j.brs.2018.01.032
Kreuzer P.M., Schecklmann M., Lehner A., Wetter T. C., Poeppl T. B., Rupprecht R., de Ridder D., Landgrebe M., Langguth B., 2015. The ACDC pilot trial: targeting the anterior cingulate by double cone coil rTMS for the treatment of depression. *Brain Stimul*. **8**, 240-6.10.1016/j.brs.2014.11.014
Kristal-Boneh E., Froom P., Harari G., Malik M., Ribak J., 2000. Summer-winter differences in 24 h variability of heart rate. *J Cardiovasc Risk*. **7**, 141-6.
Kuder T., Nowak E., 2015. Autonomic cardiac nerves: literature review. *Folia Morphol (Warsz)*. **74**, 1-8.10.5603/FM.2015.0003
Kvam S., Kleppe C. L., Nordhus I. H., Hovland A., 2016. Exercise as a treatment for depression: A meta-analysis. *Journal of affective disorders*. **202**, 67-86.
Lacey B.C., Lacey J. I., 1978. Two-way communication between the heart and the brain. Significance of time within the cardiac cycle. *Am Psychol*. **33**, 99-113.
Lane R.D., Wallace J. D., Petrosky P. P., Schwartz G. E., Gradman A. H., 1992. Supraventricular tachycardia in patients with right hemisphere strokes. *Stroke*. **23**, 362-6.
Lane R.D., McRae K., Reiman E. M., Chen K., Ahern G. L., Thayer J. F., 2009. Neural correlates of heart rate variability during emotion. *Neuroimage*. **44**, 213-22.10.1016/j.neuroimage.2008.07.056
Lane R.D., Weidenbacher H., Smith R., Fort C., Thayer J. F., Allen J. J. B., 2013. Subgenual anterior cingulate cortex activity covariation with cardiac vagal control is altered in depression. *J Affect Disord*. **150**, 565-70.10.1016/j.jad.2013.02.005
Lang S.A., Levy M. N., 1989. Effects of vagus nerve on heart rate and ventricular contractility in chicken. *Am J Physiol*. **256**, H1295-302.
Lefaucheur J.-P., André-Obadia N., Antal A., Ayache S. S., Baeken C., Benninger D. H., Cantello R. M., Cincotta M., de Carvalho M., De Ridder D., Devanne H., Di Lazzaro V., Filipović S. R., Hummel F. C., Jääskeläinen S. K., Kimiskidis V. K., Koch G., Lang-

guth B., Nyffeler T., Oliviero A., et al., 2014. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin Neurophysiol*. **125**, 2150-2206.10.1016/j.clinph.2014.05.021

Lett H.S., Blumenthal J. A., Babyak M. A., Sherwood A., Strauman T., Robins C., Newman M. F., 2004. Depression as a risk factor for coronary artery disease: evidence, mechanisms, and treatment. *Psychosom Med*. **66**, 305-15.

Leuchter A.F., Cook I. A., Lufkin R. B., Dunkin J., Newton T. F., Cummings J. L., Mackey J. K., Walter D. O., 1994. Cordance: a new method for assessment of cerebral perfusion and metabolism using quantitative electroencephalography. *Neuroimage*. **1**, 208-19.10.1006/nimg.1994.1006

Leuchter A.F., Cook I. A., Gilmer W. S., Marangell L. B., Burgoyne K. S., Howland R. H., Trivedi M. H., Zisook S., Jain R., Fava M., Iosifescu D., Greenwald S., 2009. Effectiveness of a quantitative electroencephalographic biomarker for predicting differential response or remission with escitalopram and bupropion in major depressive disorder. *Psychiatry Res*. **169**, 132-8.10.1016/j.psychres.2009.04.004

Leuchter A.F., Cook I. A., Hunter A. M., Korb A. S., 2009. A new paradigm for the prediction of antidepressant treatment response. *Dialogues Clin Neurosci*. **11**, 435-46.

Levkovitz Y., Harel E. V., Roth Y., Braw Y., Most D., Katz L. N., Sheer A., Gersner R., Zangen A., 2009. Deep transcranial magnetic stimulation over the prefrontal cortex: evaluation of antidepressant and cognitive effects in depressive patients. *Brain Stimul.* **2**, 188-200.10.1016/j.brs.2009.08.002

Levkovitz Y., Isserles M., Padberg F., Lisanby S. H., Bystritsky A., Xia G., Tendler A., Daskalakis Z. J., Winston J. L., Dannon P., Hafez H. M., Reti I. M., Morales O. G., Schlaepfer T. E., Hollander E., Berman J. A., Husain M. M., Sofer U., Stein A., Adler S., et al., 2015. Efficacy and safety of deep transcranial magnetic stimulation for major depression: a prospective multicenter randomized controlled trial. *World Psychiatry.* **14**, 64-73.10.1002/wps.20199

Levy M.N., 1984. Cardiac sympathetic-parasympathetic interactions. *Fed Proc*. **43**, 2598-602.

Levy M.N., 1990. Autonomic interactions in cardiac control. *Ann N Y Acad Sci*. **601**, 209-21.

Levy M.N., 1997. Neural control of cardiac function. B*aillieres Clin Neurol.* **6**, 227-44.

Licht C.M.M., de Geus E. J. C., Zitman F. G., Hoogendijk W. J. G., van Dyck R., Penninx B. W. J. H., 2008. Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). *Arch Gen Psychiatry.* **65**, 1358-67.10.1001/archpsyc.65.12.1358

Liston C., Chen A. C., Zebley B. D., Drysdale A. T., Gordon R., Leuchter B., Voss H. U., Casey B. J., Etkin A., Dubin M. J., 2014. Default mode network mechanisms of transcranial magnetic stimulation in depression. *Biol Psychiatry*. **76**, 517-26.10.1016/j.biopsych.2014.01.023

Liu C.C., Kuo T. B. J., Yang C. C. H., 2003. Effects of estrogen on gender-related autonomic differences in humans. *Am J Physiol Heart Circ Physiol*. **285**, H2188-93.10.1152/ajpheart.00256.2003

Losonczy M.F., Mohs R. C., Davis K. L., 1984. Seasonal variations of human lumbar CSF neurotransmitter metabolite concentrations. *Psychiatry Res*. **12**, 79-87.

MacKinnon S., Gevirtz R., McCraty R., Brown M., 2013. Utilizing heartbeat evoked potentials to identify cardiac regulation of vagal afferents during emotion and resonant breathing. *Appl Psychophysiol Biofeedback*. **38**, 241-55.10.1007/s10484-013-9226-5

Makovac E., Thayer J. F., Ottaviani C., 2016. A meta-analysis of non-invasive brain stimulation and autonomic functioning: Implications for brain-heart pathways to cardiovascular disease. *Neurosci Biobehav Rev*.**10**.1016/j.neubiorev.2016.05.001

Manes F., Jorge R., Morcuende M., Yamada T., Paradiso S., Robinson R. G., 2001. A controlled study of repetitive transcranial magnetic stimulation as a treatment of depression in the elderly. *Int Psychogeriatr*. **13**, 225-31.

Mann M.C., Exner D. V., Hemmelgarn B. R., Sola D. Y., Turin T. C., Ellis L., Ahmed S. B., 2013. Vitamin D levels are associated with cardiac autonomic activity in healthy humans. *Nutrients*. **5**, 2114-27.10.3390/nu5062114

Martényi F., Dossenbach M., Mraz K., Metcalfe S., 2001. Gender differences in the efficacy of fluoxetine and maprotiline in depressed patients: a double-blind trial of antidepressants with serotonergic or norepinephrinergic reuptake inhibition profile. *Eur Neuropsychopharmacol*. **11**, 227-32.

Marwood L., Wise T., Perkins A. M., Cleare A. J., 2018. Meta-analyses of the neural mechanisms and predictors of response to psychotherapy in depression and anxiety. *Neurosci Biobehav Rev*. **95**, 61-72.10.1016/j.neubiorev.2018.09.022

Massin M.M., Maeyns K., Withofs N., Ravet F., Gérard P., 2000. Circadian rhythm of heart rate and heart rate variability. *Arch Dis Child*. **83**, 179-82.

Mather M., Thayer J., 2018. How heart rate variability affects emotion regulation brain networks. *Curr Opin Behav Sci.* **19**, 98-104.10.1016/j.cobeha.2017.12.017

Matthews S.C., Paulus M. P., Simmons A. N., Nelesen R. A., Dimsdale J. E., 2004. Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *Neuroimage*. **22**, 1151-6.10.1016/j.neuroimage.2004.03.005

Mayberg H.S., Liotti M., Brannan S. K., McGinnis S., Mahurin R. K., Jerabek P. A., Silva J. A., Tekell J. L., Martin C. C., Lancaster J. L., Fox P. T., 1999. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Am J Psychiatry*. **156**, 675-82.

Mayberg H.S., Lozano A. M., Voon V., McNeely H. E., Seminowicz D., Hamani C., Schwalb J. M., Kennedy S. H., 2005. Deep brain stimulation for treatment-resistant depression. *Neuron*. **45**, 651-60.10.1016/j.neuron.2005.02.014

McClung C.A., 2013. How might circadian rhythms control mood? Let me count the ways. *Biol Psychiatry*. **74**, 242-9.10.1016/j.biopsych.2013.02.019

McCraty R., Shaffer F., 2015. Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health risk. *Glob Adv Health Med*. **4**, 46-61.10.7453/gahmj.2014.073

De Meersman R.E., Stein P. K., 2007. Vagal modulation and aging. *Biological psychology.* **74**, 165-173.

Meuwly C., Golanov E., Chowdhury T., Erne P., Schaller B., 2015. Trigeminal cardiac reflex: new thinking model about the definition based on a literature review. *Medicine* (*Baltimore).* **94**, e484.10.1097/MD.0000000000000484

Miniussi C., Bonato C., Bignotti S., Gazzoli A., Gennarelli M., Pasqualetti P., Tura G. B., Ventriglia M., Rossini P. M., 2005. Repetitive transcranial magnetic stimulation (rTMS) at high and low frequency: an efficacious therapy for major drug-resistant depression?. *Clin Neurophysiol*. **116**, 1062-71.10.1016/j.clinph.2005.01.002

Mir-Moghtadaei, Caballero R., Fried P., Fox M. D., Lee K., Giacobbe P., Daskalakis Z. J., Blumberger D. M., Downar J., 2015. Concordance between BeamF3 and MRI-neuro navigated target sites for repetitive transcranial magnetic stimulation of the left

dorsolateral prefrontal cortex. *Brain Stimulation*.**10**.1016/j.brs.2015.05.008
Mir-Moghtadaei A., Caballero R., Fried P., Fox M. D., Lee K., Giacobbe P., Daskalakis Z. J., Blumberger D. M., Downar J., 2015. Concordance Between BeamF3 and MRI-neuronavigated Target Sites for Repetitive Transcranial Magnetic Stimulation of the Left Dorsolateral Prefrontal Cortex. *Brain Stimul.* **8**, 965-73.10.1016/j.brs.2015.05.008
Mogg A., Pluck G., Eranti S. V., Landau S., Purvis R., Brown R. G., Curtis V., Howard R., Philpot M., McLoughlin D. M., 2008. A randomized controlled trial with 4-month follow-up of adjunctive repetitive transcranial magnetic stimulation of the left prefrontal cortex for depression. *Psychol Med.* **38**, 323-33.10.1017/S0033291707001663
Monnard C.R., Grasser E. K., 2017. Water ingestion decreases cardiac workload time-dependent in healthy adults with no effect of gender. *Sci Rep*. **7**, 7939.10.1038/s41598-017-08446-4
Morris C.J., Yang J. N., Scheer F. A. J. L., 2012. The impact of the circadian timing system on cardiovascular and metabolic function. *Prog Brain Res*. **199**, 337-58.10.1016/B978-0-444-59427-3.00019-8
Musselman D.L., Evans D. L., Nemeroff C. B., 1998. The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. *Arch Gen Psychiatry.* **55**, 580-92.
Nagai M., Hoshide S., Kario K., 2010. The insular cortex and cardiovascular system: a new insight into the brain-heart axis. *J Am Soc Hypertens.* **4**, 174-82.10.1016/j.jash.2010.05.001
Nibuya M., Nestler E. J., Duman R. S., 1996. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci.* **16**, 2365-72.
Nimptsch K., Platz E. A., Willett W. C., Giovannucci E., 2012. Association between plasma 25-OH vitamin D and testosterone levels in men. *Clin Endocrinol (Oxf).* **77**, 106-12.10.1111/j.1365-2265.2012.04332.x
Ning L., Makris N., Camprodon J. A., Rathi Y., 2018. Limits and reproducibility of resting-state functional MRI definition of DLPFC targets for neuromodulation. *Brain Stimul.***10**.1016/j.brs.2018.10.004
Nonaka K., Imaizumi Y., 2000. Deaths from cerebrovascular diseases correlated to month of birth: elevated risk of death from subarachnoid hemorrhage among summer-born. *Int J Biometeorol*. **44**, 182-5.
Nugent A.C., Bain E. E., Thayer J. F., Sollers J. J., Drevets W. C., 2011. Heart rate variability during motor and cognitive tasks in females with major depressive disorder. *Psychiatry Res*. **191**, 1-8.10.1016/j.pscychresns.2010.08.013
Ojeda D., Le Rolle V., Romero-Ugalde H. M., Gallet C., Bonnet J. -L., Henry C., Bel A., Mabo P., Carrault G., Hernández A. I., 2016. Sensitivity Analysis of Vagus Nerve Stimulation Parameters on Acute Cardiac Autonomic Responses: Chronotropic, Inotropic and Dromotropic Effects. *PLoS One*. **11**, e0163734.10.1371/journal.pone.0163734
Olbrich S., Tränkner A., Surova G., Gevirtz R., Gordon E., Hegerl U., Arns M., 2016. CNS- and ANS-arousal predict response to antidepressant medication: Findings from the randomized iSPOT-D study. *Journal of Psychiatric Research.* **73**, 108-115.10.1016/j.jpsychires.2015.12.001
O'Reardon J.P., Solvason H. B., Janicak P. G., Sampson S., Isenberg K. E., Nahas Z., McDonald W. M., Avery D., Fitzgerald P. B., Loo C., Demitrack M. A., George M. S., Sackeim H. A., 2007. Efficacy and safety of transcranial magnetic stimulation in

the acute treatment of major depression: a multisite randomized controlled trial. *Biol Psychiatry.* **62**, 1208-16.10.1016/j.biopsych.2007.01.018

Padberg F., Zwanzger P., Thoma H., Kathmann N., Haag C., Greenberg B. D., Hampel H., Möller H. J., 1999. Repetitive transcranial magnetic stimulation (rTMS) in pharmacotherapy-refractory major depression: comparative study of fast, slow and sham rTMS. *Psychiatry Res.* **88**, 163-71.

Padberg F., Zwanzger P., Keck M. E., Kathmann N., Mikhaiel P., Ella R., Rupprecht P., Thoma H., Hampel H., Toschi N., Möller H. -J., 2002. Repetitive transcranial magnetic stimulation (rTMS) in major depression: relation between efficacy and stimulation intensity. *Neuropsychopharmacology.* **27**, 638-45.10.1016/S0893-133X(02)-00338-X

Padberg F., George M. S., 2009. Repetitive transcranial magnetic stimulation of the prefrontal cortex in depression. *Exp Neurol.* **219**, 2-13.10.1016/j.expneurol.-2009.04.020

Pallanti S., Bernardi S., Rollo A. D., Antonini S., Quercioli L., 2010. Unilateral low frequency versus sequential bilateral repetitive transcranial magnetic stimulation: is simpler better for treatment of resistant depression?. *Neuroscience*.**10**.1016/j.neuroscience.2010.01.063

Pardini B.J., Lund D. D., Schmid P. G., 1989. Organization of the sympathetic postganglionic innervation of the rat heart. *J Auton Nerv Syst*. **28**, 193-201.

Park G., Thayer J. F., 2014. From the heart to the mind: cardiac vagal tone modulates top-down and bottom-up visual perception and attention to emotional stimuli. *Front Psychol.* **5**, 278.10.3389/fpsyg.2014.00278

Pascual-Leone A., Tarazona F., Keenan J., Tormos J. M., Hamilton R., Catala M. D., 1999. Transcranial magnetic stimulation and neuroplasticity. *Neuropsychologia*. **37**, 207-17.

Pascual-Marqui R.D., Michel C. M., Lehmann D., 1994. Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *Int J Psychophysiol.* **18**, 49-65.

Pascual-Marqui R.D., 2002. Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. Methods Find *Exp Clin Pharmacol*. **24**, 5-12.

Pascual-Marqui R.D., 2007. Discrete, 3D distributed, linear imaging methods of electric neuronal activity. Part 1: exact, zero error localization. *arXiv preprint* arXiv:0710.3341.

Pascual-Marqui R.D., Lehmann D., Koukkou M., Kochi K., Anderer P., Saletu B., Tanaka H., Hirata K., John E. R., Prichep L., Biscay-Lirio R., Kinoshita T., 2011. Assessing interactions in the brain with exact low-resolution electromagnetic tomography. *Philos Trans A Math Phys Eng Sci*. **369**, 3768-84.10.1098/rsta.2011.0081

Patron E., Messerotti Benvenuti S., Favretto G., Valfrè C., Bonfà C., Gasparotto R., Palomba D., 2013. Biofeedback assisted control of respiratory sinus arrhythmia as a biobehavioral intervention for depressive symptoms in patients after cardiac surgery: a preliminary study. *Appl Psychophysiol Biofeedback*. **38**, 1-9.10.1007/s10484-012-9202-5

Paul R.H., Gunstad J., Cooper N., Williams L. M., Clark C. R., Cohen R. A., Lawrence J. J., Gordon E., 2007. Cross-cultural assessment of neuropsychological performance and electrical brain function measures: additional validation of an international brain database. *Int J Neurosci*. **117**, 549-68.10.1080/00207450600773665

Paul R.H., Gunstad J., Cooper N., Williams L. M., Clark C. R., Cohen R. A., Lawrence J. J., Gordon E., 2007. Cross-cultural assessment of neuropsychological performance

and electrical brain function measures: additional validation of an international brain database. *Int J Neurosci.* **117**, 549-68.10.1080/00207450600773665
Paulus W., 2005. Toward establishing a therapeutic window for rTMS by theta burst stimulation. *Neuron.* **45**, 181-3.10.1016/j.neuron.2005.01.008
Paus T., Koski L., Caramanos Z., Westbury C., 1998. Regional differences in the effects of task difficulty and motor output on blood flow response in the human anterior cingulate cortex: a review of 107 PET activation studies. *Neuroreport*. **9**, R37-47.
Paus T., Barrett J., 2004. Transcranial magnetic stimulation (TMS) of the human frontal cortex: implications for repetitive TMS treatment of depression. *J Psychiatry Neurosci.* **29**, 268-79.
Pawlowski M. Abstract Book IPEG Meeting Zurich 2018. *Abstract Book IPEG Meeting Zurich* 2018. 2018.
Peciña M., Bohnert A. S. B., Sikora M., Avery E. T., Langenecker S. A., Mickey B. J., Zubieta J. -K., 2015. Association Between Placebo-Activated Neural Systems and Antidepressant Responses: Neurochemistry of Placebo Effects in Major Depression. *JAMA Psychiatry*. **72**, 1087-94.10.1001/jamapsychiatry.2015.1335
Penninx B.W., Beekman A. T., Honig A., Deeg D. J., Schoevers R. A., van Eijk J. T., van Tilburg W., 2001. Depression and cardiac mortality: results from a community-based longitudinal study. *Arch Gen Psychiatry*. **58**, 221-7.
Pichiorri F., Vicenzini E., Gilio F., Giacomelli E., Frasca V., Cambieri C., Ceccanti M., Di Piero V., Inghilleri M., 2012. Effects of intermittent theta burst stimulation on cerebral blood flow and cerebral vasomotor reactivity. *J Ultrasound Med*. **31**, 1159-67.10.7863/jum.2012.31.8.1159
Pollatos O., Herbert B. M., Mai S., Kammer T., 2016. Changes in interoceptive processes following brain stimulation. *Philos Trans R Soc Lond B Biol Sci*. **371**, 10.1098/rstb.2016.0016
Porges S.W., 1995. Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory. *Psychophysiology*. **32**, 301-18.
Porges S.W., 2007. The polyvagal perspective. *Biological psychology.* **74**, 116-143.
Powell D.A., Buchanan S., Hernàndez L., 1985. Electrical stimulation of insular cortex elicits cardiac inhibition but insular lesions do not abolish conditioned bradycardia in rabbits. *Behav Brain Res.* **17**, 125-44.
Pridmore S., Bruno R., Turnier-Shea Y., Reid P., Rybak M., 2000. Comparison of unlimited numbers of rapid transcranial magnetic stimulation (rTMS) and ECT treatment sessions in major depressive episode. *Int J Neuropsychopharmacol*. **3**, 129-134.10.1017/S1461145700001784
Rajendra Acharya U., Paul Joseph K., Kannathal N., Lim C. M., Suri J. S., 2006. Heart rate variability: a review. *Med Biol Eng Comput.* **44**, 1031-51.10.1007/s11517-006-0119-0
Reffelmann T., Ittermann T., Empen K., Dörr M., Felix S. B., 2011. Is cardiovascular mortality related to the season of birth? Evidence from more than 6 million cardiovascular deaths between 1992 and 2007. *J Am Coll Cardiol*. **57**, 887-8.10.1016/j.jacc.2010.10.021
Renner F., Penninx B. W. J. H., Peeters F., Cuijpers P., Huibers M. J. H., 2013. Two-year stability and change of neuroticism and extraversion in treated and untreated persons with depression: findings from the Netherlands Study of Depression and Anxiety (NESDA). *J Affect Disord*. **150**, 201-8.10.1016/j.jad.2013.03.022
Reyes del Paso G.A., Langewitz W., Mulder L. J. M., van Roon A., Duschek S., 2013. The utility of low frequency heart rate variability as an index of sympathetic cardiac

tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology.* **50**, 477-87.10.1111/psyp.12027
Ridding M.C., Rothwell J. C., 2007. Is there a future for therapeutic use of transcranial magnetic stimulation?. *Nat Rev Neurosci.* **8**, 559-67.10.1038/nrn2169
Riva-Posse P., Inman C. S., Choi K. S., Crowell A. L., Gross R. E., Hamann S., Mayberg H. S., 2019. Autonomic arousal elicited by subcallosal cingulate stimulation is explained by white matter connectivity. Brain Stimulation: Basic, Translational, and Clinical Research in *Neuromodulation.* **0**, 10.1016/j.brs.2019.01.015
Robertson H., Pryor R., 2006. Memory and cognitive effects of ECT: informing and assessing patients†. *Advances in Psychiatric Treatment.* **12**, 228-237.10.1192/apt.12.3.228
Rollnik J.D., Düsterhöft A., Däuper J., Kossev A., Weissenborn K., Dengler R., 2002. Decrease of middle cerebral artery blood flow velocity after low-frequency repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex. *Clin Neurophysiol.* **113**, 951-5.
Rosa M.A., Gattaz W. F., Pascual-Leone A., Fregni F., Rosa M. O., Rumi D. O., Myczkowski M., Silva M. F., Mansur C., Rigonatti S. P., Jacobsen Teixeira M., Marcolin M. A., 2006. Comparison of repetitive transcranial magnetic stimulation and electroconvulsive therapy in unipolar non-psychotic refractory depression: a randomized, single-blind study. *Int J Neuropsychopharmacol.* **9**, 667-76.10.1017/S1461145706007127
Rossini D., Lucca A., Zanardi R., Magri L., Smeraldi E., 2005. Transcranial magnetic stimulation in treatment-resistant depressed patients: a double-blind, placebo-controlled trial. *Psychiatry Res.* **137**, 1-10.10.1016/j.psychres.2005.06.008
Rossi S., Rossini P. M., 2004. TMS in cognitive plasticity and the potential for rehabilitation. *Trends Cogn Sci.* **8**, 273-9.10.1016/j.tics.2004.04.012
Rossi S., Hallett M., Rossini P. M., Pascual-Leone A., The Safety of TMS Consensus Group, 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol.* **120**, 2008-39.10.1016/j.clinph.2009.08.016
Rossi S., Santarnecchi E., Valenza G., Ulivelli M., 2016. The heart side of brain neuromodulation. *Philos Trans A Math Phys Eng Sci.* **374**, 10.1098/rsta.2015.0187
Routledge H.C., Chowdhary S., Coote J. H., Townend J. N., 2002. Cardiac vagal response to water ingestion in normal human subjects. *Clin Sci (Lond).* **103**, 157-62.10.1042/
Rumi D.O., Gattaz W. F., Rigonatti S. P., Rosa M. A., Fregni F., Rosa M. O., Mansur C., Myczkowski M. L., Moreno R. A., Marcolin M. A., 2005. Transcranial magnetic stimulation accelerates the antidepressant effect of amitriptyline in severe depression: a double-blind placebo-controlled study. *Biol Psychiatry.* **57**, 162-6.10.1016/j.biopsych.2004.10.029
Rush A.J., George M. S., Sackeim H. A., Marangell L. B., Husain M. M., Giller C., Nahas Z., Haines S., Simpson R. K., Goodman R., 2000. Vagus nerve stimulation (VNS) for treatment-resistant depressions: a multicenter study. *Biological psychiatry.* **47**, 276-286.
Rush A.J., Trivedi M. H., Wisniewski S. R., Nierenberg A. A., Stewart J. W., Warden D., Niederehe G., Thase M. E., Lavori P. W., Lebowitz B. D., McGrath P. J., Rosenbaum J. F., Sackeim H. A., Kupfer D. J., Luther J., Fava M., 2006. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry.* **163**, 1905-17.10.1176/appi.ajp.163.11.1905
Rusjan P.M., Barr M. S., Farzan F., Arenovich T., Maller J. J., Fitzgerald P. B., Daskalakis

Z. J., 2010. Optimal transcranial magnetic stimulation coil placement for targeting the dorsolateral prefrontal cortex using novel magnetic resonance image-guided neuronavigation. *Hum Brain Mapp*.**10**.1002/hbm.20964
Saleh T.M., Connell B. J., 1998. Role of the insular cortex in the modulation of baroreflex sensitivity. *Am J Physiol.* **274**, R1417-24.
Salomons T.V., Dunlop K., Kennedy S. H., Flint A., Geraci J., Giacobbe P., Downar J., 2014. Resting-state cortico-thalamic-striatal connectivity predicts response to dorsomedial prefrontal rTMS in major depressive disorder. *Neuropsychopharmacology.* **39**, 488-98.10.1038/npp.2013.222
Sammito S., Sammito W., Böckelmann I., 2016. The circadian rhythm of heart rate variability. *Biological Rhythm Research.***1**-34.
Sampaio L.A.N.P.C., Fraguas R., Lotufo P. A., Benseñor I. M., Brunoni A. R., 2012. A systematic review of non-invasive brain stimulation therapies and cardiovascular risk: implications for the treatment of major depressive disorder. *Front Psychiatry*. **3**, 87.10.3389/fpsyt.2012.00087
Sander D., Meyer B. U., Röricht S., Klingelhöfer J., 1995. Effect of hemisphere-selective repetitive magnetic brain stimulation on middle cerebral artery blood flow velocity. *Electroencephalogr Clin Neurophysiol.* **97**, 43-8.10.1016/0924-980x(94)00247-5
Sato J.R., Rondinoni C., Sturzbecher M., de Araujo D. B., Amaro E., 2010. From EEG to BOLD: brain mapping and estimating transfer functions in simultaneous EEG-fMRI acquisitions. *Neuroimage.* **50**, 1416-26.10.1016/j.neuroimage.2010.01.075
Saveanu R., Etkin A., Duchemin A. -M., Goldstein-Piekarski A., Gyurak A., Debattista C., Schatzberg A. F., Sood S., Day C. V. A., Palmer D. M., Rekshan W. R., Gordon E., Rush A. J., Williams L. M., 2014. The International Study to Predict Optimized Treatment in Depression (iSPOT-D): Outcomes from the acute phase of antidepressant treatment. *J Psychiatr Res.***10**.1016/j.jpsychires.2014.12.018
Schachter S.C., 2002. Vagus nerve stimulation therapy summary: five years after FDA approval. *Neurology.* **59**, S15-20.
Schandry R., Sparrer B., Weitkunat R., 1986. From the heart to the brain: a study of heartbeat contingent scalp potentials. *Int J Neurosci.* **30**, 261-75.
Scheer F.A.J.L., Van Doornen L. J. P., Buijs R. M., 2004. Light and diurnal cycle affect autonomic cardiac balance in human; possible role for the biological clock. *Auton Neurosci.* **110**, 44-8.10.1016/j.autneu.2003.03.001
Schlaepfer T.E., Bewernick B. H., Kayser S., Mädler B., Coenen V. A., 2013. Rapid Effects of Deep Brain Stimulation for Treatment-Resistant Major Depression. *Biol Psychiatry*.**10**.1016/j.biopsych.2013.01.034
Schmidt S., 2009. Shall We Really Do It Again? The Powerful Concept of Replication Is Neglected in the Social Science. *Review of General Psychology.* **13**, 90-100.
Schutter D., 2009. Transcraniale magnetische stimulatie en cerebrale fysiologische processen bij psychiatrische stoornissen. *Tijdschrift voor Psychiatrie.* **51**, 97-105.
Schutter D.J.L.G., 2010. Quantitative review of the efficacy of slow-frequency magnetic brain stimulation in major depressive disorder. *Psychol Med.* **40**, 1789-1795.10.1017/S003329171000005X
Sen S., Duman R., Sanacora G., 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry.* **64**, 527-32.10.1016/j.biopsych.2008.05.005
Shaffer F., McCraty R., Zerr C. L., 2014. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in psychology.* **5**,

Shaffer F., Ginsberg J. P., 2017. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health.* **5**, 258.10.3389/fpubh.2017.00258
Sheehan D.V., Lecrubier Y., Sheehan K. H., Amorim P., Janavs J., Weiller E., Hergueta T., Baker R., Dunbar G. C., 1998. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry.* **59**, 22-33.
Shekhar A., Sajdyk T. J., Gehlert D. R., Rainnie D. G., 2003. The amygdala, panic disorder, and cardiovascular responses. *Ann N Y Acad Sci*. **985**, 308-25.
Sheline Y.I., Price J. L., Yan Z., Mintun M. A., 2010. Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. *Proc Natl Acad Sci U S A.* **107**, 11020-5.10.1073/pnas.1000446107
Shepherd D., Mulgrew J., Hautus M. J., 2015. Exploring the autonomic correlates of personality. *Auton Neurosci.* **193**, 127-31.10.1016/j.autneu.2015.05.004
Shoemaker J.K., Goswami R., 2015. Forebrain neurocircuitry associated with human reflex cardiovascular control. *Front Physiol.* **6**, 240.10.3389/fphys.2015.00240
Siepmann M., Aykac V., Unterdörfer J., Petrowski K., Mueck-Weymann M., 2008. A pilot study on the effects of heart rate variability biofeedback in patients with depression and in healthy subjects. *Appl Psychophysiol Biofeedback*. **33**, 195-201.10.1007/s10484-008-9064-z
Silverstein W.K., Noda Y., Barr M. S., Vila-Rodriguez F., Rajji T. K., Fitzgerald P. B., Downar J., Mulsant B. H., Vigod S., Daskalakis Z. J., Blumberger D. M., 2015. Neurobiological predictors of response to dorsolateral prefrontal cortex repetitive transcranial magnetic stimulation in depression: A systematic review. *Depress Anxiety.* **32**, 871-91.10.1002/da.22424
Sliz D., Hayley S., 2012. Major depressive disorder and alterations in insular cortical activity: a review of current functional magnetic imaging research. *Front Hum Neurosci.* **6**, 323.10.3389/fnhum.2012.00323
Smith R., Allen J. J. B., Thayer J. F., Lane R. D., 2015. Altered functional connectivity between medial prefrontal cortex and the inferior brainstem in major depression during appraisal of subjective emotional responses: A preliminary study. *Biol Psychol.* **108**, 13-24.10.1016/j.biopsycho.2015.03.007
Smith W.K., 1949. The functional significance of the rostral cingular cortex as revealed by its responses to electrical excitation. *Journal of Neurophysiology*. **12**, 385-392.
Sohn K., 2016. The influence of birth season on mortality in the United States. *Am J Hum Biol.* **28**, 662-70.10.1002/ajhb.22848
Sperling W., Reulbach U., Bleich S., Padberg F., Kornhuber J., Mueck-Weymann M., 2010. Cardiac effects of vagus nerve stimulation in patients with major depression. *Pharmacopsychiatry.* **43**, 7-11.10.1055/s-0029-1237374
Spronk D., Arns M., Barnett K., Cooper N., Gordon E., 2011. An investigation of EEG, genetic and cognitive markers of treatment response to antidepressant medication in patients with major depressive disorder: a pilot study. *J Affect Disord.* **128**, 41-48.10.1016/j.jad.2010.06.021
Sridharan D., Levitin D. J., Menon V., 2008. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci U S A.* **105**, 12569-74.10.1073/pnas.0800005105
Stein J., Talcott J., Walsh V., 2000. Controversy about the visual magnocellular deficit in developmental dyslexics. *Trends Cogn Sci.* **4**, 209-211.
Stewart L.M., Walsh V., Rothwell J. C., 2001. Motor and phosphene thresholds: a trans-

cranial magnetic stimulation correlation study. *Neuropsychologia.* **39**, 415-9.
Stewart S., McIntyre K., Capewell S., McMurray J. J. V., 2002. Heart failure in a cold climate. Seasonal variation in heart failure-related morbidity and mortality. *J Am Coll Cardiol.* **39**, 760-6.
Sun J., Scherlag B. J., He B. O., Shen X., Gao M., Zhang L., Li Y., Po S. S., 2015. Electrical Stimulation of Vascular Autonomic Nerves: Effects on Heart Rate, Blood Pressure, and Arrhythmias. *Pacing Clin Electrophysiol.* **38**, 825-30.10.1111/pace.12603
Takase B., Akima T., Satomura K., Ohsuzu F., Mastui T., Ishihara M., Kurita A., 2004. Effects of chronic sleep deprivation on autonomic activity by examining heart rate variability, plasma catecholamine, and intracellular magnesium levels. *Biomed Pharmacother.* **58** Suppl 1, S35-9.
Taylor E.W., Jordan D., Coote J. H., 1999. Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiol Rev.* **79**, 855-916.10.1152/physrev.1999.79.3.855
Ter Horst G.J., 1999. Central autonomic control of the heart, angina, and pathogenic mechanisms of post-myocardial infarction depression. *Eur J Morphol.* **37**, 257-66.
Ter Horst G.J., Postema F., 1997. Forebrain parasympathetic control of heart activity: retrograde transneuronal viral labeling in rats. *Am J Physiol*. **273**, H2926-30.10.1152/ajpheart.1997.273.6.H2926
Thayer J.F., Smith M., Rossy L. A., Sollers J. J., Friedman B. H., 1998. Heart period variability and depressive symptoms: gender differences. *Biol Psychiatry.* **44**, 304-6.
Thayer J.F., Lane R. D., 2000. A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord.* **61**, 201-16.
Thayer J.F., Lane R. D., 2009. Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci Biobehav Rev.* **33**, 81-8.10.1016/j.neubiorev.2008.08.004
Thayer J.F., Yamamoto S. S., Brosschot J. F., 2010. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int J Cardiol.* **141**, 122-31.10.1016/j.ijcard.2009.09.543
Thayer J.F., Ahs F., Fredrikson M., Sollers J. J., Wager T. D., 2012. A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neurosci Biobehav Rev.***10**.1016/j.neubiorev.2011.11.009
Thielscher A., Kammer T., 2004. Electric field properties of two commercial figure-8 coils in TMS: calculation of focality and efficiency. *Clin Neurophysiol.* **115**, 1697-708.10.1016/j.clinph.2004.02.019
Thomas G.D., 2011. Neural control of the circulation. *Adv Physiol Educ.* **35**, 28-32.10.1152/advan.00114.2010
Torrey E.F., Rawlings R. R., Ennis J. M., Merrill D. D., Flores D. S., 1996. Birth seasonality in bipolar disorder, schizophrenia, schizoaffective disorder and stillbirths. *Schizophr Res.* **21**, 141-9.
Udupa K., Sathyaprabha T. N., Thirthalli J., Kishore K. R., Lavekar G. S., Raju T. R., Gangadhar B. N., 2007. Alteration of cardiac autonomic functions in patients with major depression: a study using heart rate variability measures. *J Affect Disord.* **100**, 137-41.10.1016/j.jad.2006.10.007
Udupa K., Sathyaprabha T. N., Thirthalli J., Kishore K. R., Raju T. R., Gangadhar B. N., 2007. Modulation of cardiac autonomic functions in patients with major depression treated with repetitive transcranial magnetic stimulation. *J Affect Disord.* **104**,

231-6.10.1016/j.jad.2007.04.002
Uijtdehaage S.H., Thayer J. F., 2000. Accentuated antagonism in the control of human heart rate. *Clin Auton Res.* **10**, 107-10.
Umetani K., Singer D. H., McCraty R., Atkinson M., 1998. Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J Am Coll Cardiol.* **31**, 593-601.
van der Kooy D., McGinty J. F., Koda L. Y., Gerfen C. R., Bloom F. E., 1982. Visceral cortex: a direct connection from prefrontal cortex to the solitary nucleus in rat. *Neurosci Lett.* **33**, 123-7.
van der Vinne N., Vollebregt M. A., van Putten M. J., Arns M., 2017. Frontal alpha asymmetry as a diagnostic marker in depression: Fact or fiction? A meta-analysis. *Neuro Image: Clinical.*
van Dinteren R., Arns M., Kenemans L., Jongsma M. L., Kessels R. P., Fitzgerald P., Fallahpour K., Debattista C., Gordon E., Williams L. M., 2015. Utility of event-related potentials in predicting antidepressant treatment response: An iSPOT-D report. *European Neuropsychopharmacology.***10**.1016/j.euroneuro.2015.07.022
Verkuil B., Brosschot J. F., Marques A. H., Kampschroer K., Sternberg E. M., Thayer J. F., 2015. Gender differences in the impact of daily sadness on 24-h heart rate variability. *Psychophysiology.* **52**, 1682-8.10.1111/psyp.12541
Vernieri F., Maggio P., Tibuzzi F., Filippi M. M., Pasqualetti P., Melgari J. M., Altamura C., Palazzo P., Di Giorgio M., Rossini P. M., 2009. High frequency repetitive transcranial magnetic stimulation decreases cerebral vasomotor reactivity. *Clin Neurophysiol.* **120**, 1188-94.10.1016/j.clinph.2009.03.021
Vernieri F., Assenza G., Maggio P., Tibuzzi F., Zappasodi F., Altamura C., Corbetto M., Trotta L., Palazzo P., Ercolani M., Tecchio F., Rossini P. M., 2010. Cortical neuromodulation modifies cerebral vasomotor reactivity. S*troke.* **41**, 2087-90.10.1161/STROKEAHA.110.583088
Vernieri F., Altamura C., Palazzo P., Altavilla R., Fabrizio E., Fini R., Melgari J. M., Paolucci M., Pasqualetti P., Maggio P., 2014. 1-Hz repetitive transcranial magnetic stimulation increases cerebral vasomotor reactivity: a possible autonomic nervous system modulation. *Brain Stimul.* **7**, 281-6.10.1016/j.brs2013.12.014
Vogt B.A., Vogt L., Farber N. B., Bush G., 2005. Architecture and neurocytology of monkey cingulate gyrus. *J Comp Neurol.* **485**, 218-39.10.1002/cne.20512
Voss A., Schulz S., Schroeder R., Baumert M., Caminal P., 2009. Methods derived from nonlinear dynamics for analysing heart rate variability. *Philos Trans A Math Phys Eng Sci.* **367**, 277-96.10.1098/rsta.2008.0232
Wang Y., Hensley M. K., Tasman A., Sears L., Casanova M. F., Sokhadze E. M., 2015. Heart Rate Variability and Skin Conductance During Repetitive TMS Course in Children with Autism. *Appl Psychophysiol Biofeedback.***10**.1007/s10484-015-9311-z
Wassermann E.M., 1998. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroen cephalogr Clin Neurophysiol.* **108**, 1-16.
Wassermann E.M., Lisanby S. H., 2001. Therapeutic application of repetitive transcranial magnetic stimulation: a review. *Clin Neurophysiol.* **112**, 1367-77.
Weigand A., Horn A., Caballero R., Cooke D., Stern A. P., Taylor S. F., Press D., Pascual-Leone A., Fox M. D., 2018. Prospective Validation That Subgenual Connectivity Predicts Antidepressant Efficacy of Transcranial Magnetic Stimulation Sites. *Biol*

Psychiatry. **84**, 28-37.10.1016/j.biopsych.2017.10.028
Wei L., Chen H., Wu G. -R., 2018. Heart rate variability associated with grey matter volumes in striatal and limbic structures of the central autonomic network. *Brain Res.* **1681**, 14-20.10.1016/j.brainres.2017.12.024
Whiteford H.A., Degenhardt L., Rehm J., Baxter A. J., Ferrari A. J., Erskine H. E., Charlson F. J., Norman R. E., Flaxman A. D., Johns N., Burstein R., Murray C. J., Vos T., 2013. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *The Lancet.* **382**, 1575-1586.10.1016/S0140-6736(13)61611-6
Williams D.P., Cash C., Rankin C., Bernardi A., Koenig J., Thayer J. F., 2015. Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. *Front Psychol.* **6**, 261.10.3389/fpsyg.2015.00261
Williams L.M., Simms E., Clark C. R., Paul R. H., Rowe D., Gordon E., 2005. The test-retest reliability of a standardized neurocognitive and neurophysiological test battery: "neuromarker". *Int J Neurosci.* **115**, 1605-30.10.1080/00207450590958475
Williams L.M., Rush A. J., Koslow S. H., Wisniewski S. R., Cooper N. J., Nemeroff C. B., Schatzberg A. F., Gordon E., 2011. International Study to Predict Optimized Treatment for Depression (iSPOT-D), a randomized clinical trial: rationale and protocol. *Trials.* **12**, 4.10.1186/1745-6215-12-4
Wranicz J.K., Rosiak M., Cygankiewicz I., Kula P., Kula K., Zareba W., 2004. Sex steroids and heart rate variability in patients after myocardial infarction. *Ann Noninvasive Electrocardiol.* **9**, 156-61.10.1111/j.1542-474X.2004.92539.x
Yokoyama K., Jennings R., Ackles P., Hood P., Boller F., 1987. Lack of heart rate changes during an attention-demanding task after right hemisphere lesions. *Neurology.* **37**, 624-30.
Young H., Benton D., 2015. We should be using nonlinear indices when relating heart-rate dynamics to cognition and mood. *Sci Rep.* **5**, 16619.10.1038/srep16619
Zangen A., Roth Y., Voller B., Hallett M., 2005. Transcranial magnetic stimulation of deep brain regions: evidence for efficacy of the H-coil. *Clin Neurophysiol.* **116**, 775-9.10.1016/j.clinph.2004.11.008
Ziegler G., Dahnke R., Yeragani V. K., Bär K. -J., 2009. The relation of ventromedial prefrontal cortex activity and heart rate fluctuations at rest. *European Journal of Neuroscience.* **30**, 2205-2210.
Zucker T.L., Samuelson K. W., Muench F., Greenberg M. A., Gevirtz R. N., 2009. The effects of respiratory sinus arrhythmia biofeedback on heart rate variability and posttraumatic stress disorder symptoms: a pilot study. *Appl Psychophysiol Biofeedback.* **34**, 135-43.10.1007/s10484-009-9085-2

ARTICLES IN PREPARATION/SUBMITTED/IN PRESS

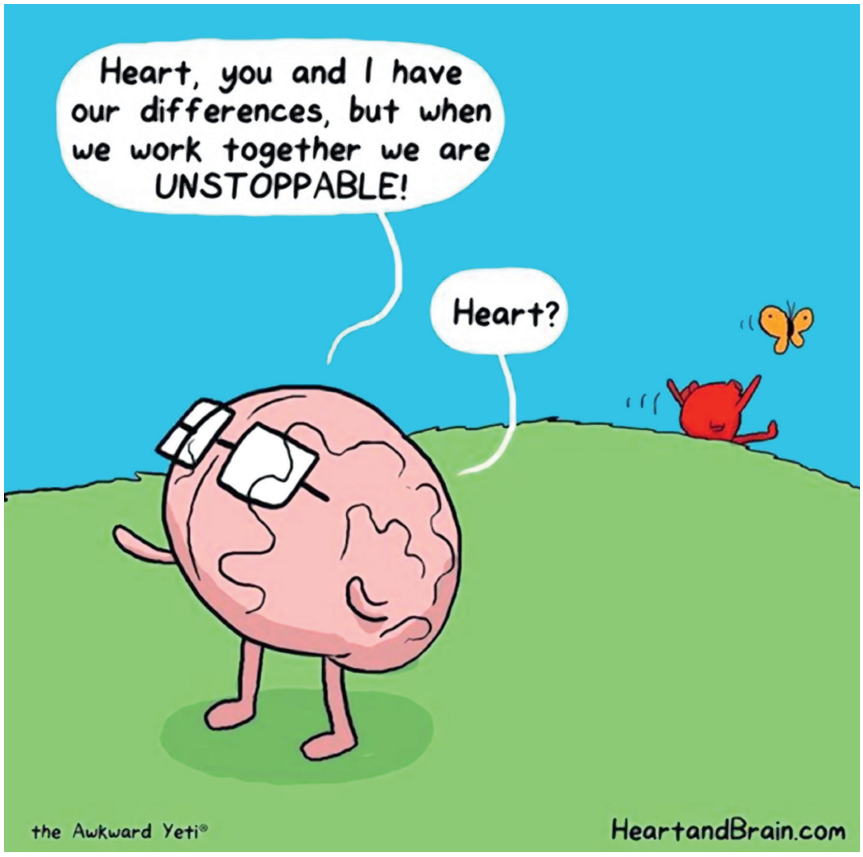
Iseger, T.A., Padberg, F., Kenemans, J.L., van Dijk, H., Arns, M. 2019b. Neuro-cardiac-guided TMS: a validation study (under review for publication in *Neuroimage*).

Kaur, M., Michael, J.A., Hoy, K.E., Fitzgibbon, B.M., Ross, M., Iseger, T.A., Arns, M., Hudaib, A.R., Fitzgerald, P. 2019. Neuro-cardio-guided TMS: Targeting the brain-heart connection to personalise and optimise rTMS treatment for depression. An independent replication. (under review for publication in *Brain Stimulation*).

Iseger, T.A., Arns, M., Downar, J., Blumberger, D., Daskalakis, Z., Vila-Rodriguez, F. 2019c. Cardiovascular differences between sham and active iTBS related to treatment response in MDD. *Brain Stimulation* (in press).

Iseger, T.A., van Bueren, N.E.R., Kenemans, J.L., Gevirtz, R., Arns, M. 2019d. A frontal-vagal network theory for Major Depressive Disorder: implications for optimizing neuromodulation techniques. *Brain Stimulation* (in press).

NEDERLANDSE SAMENVATTING



Figuur 1: *Hart-brein cartoon. Gebruikt met toestemming van: http://theawkwardyeti.com/chapter/heart-and-brain-2/*

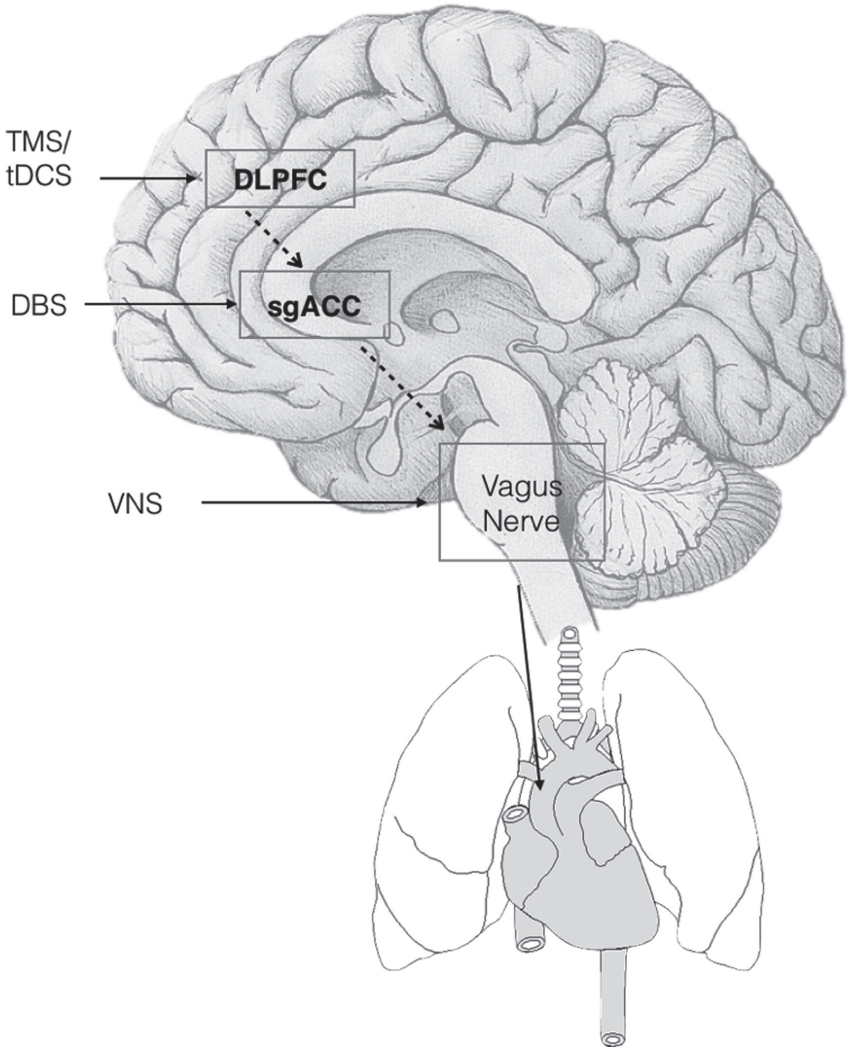
De hersenen en het hart hebben vaak een andere kijk op het leven. Het hart wordt gezien als meer emotioneel, terwijl de hersenen vaak als meer rationeel worden gezien. Ze luisteren niet altijd naar elkaar. Maar, toch is het erg belangrijk dat er balans is tussen de twee (zie figuur 1). Als dat niet zo is, heeft dat consequenties, en dat is bijvoorbeeld zo bij mensen met een depressie. Mensen met een depressie hebben over het algemeen een verhoogde hartslag en lagere variatie in de hartslag (verminderd aanpassingsvermogen naar externe factoren, zoals stress, ademhaling, etc). Ook hebben deze mensen een 5 keer hogere kans op hartaandoeningen. Dit laat zien dat er iets niet in balans is binnen dit brein-hart netwerk, voor mensen met een depressie.

Een depressieve stoornis is een chronische psychiatrische aandoening die gekenmerkt wordt door een verloop van verbetering en terugval en beïnvloedt zo'n 4.7 procent van de wereldbevolking op elk moment (Kessler et al., 2012). Behandeling gebeurt over het algemeen met medicatie (Anderson et al, 2008), maar neuromodulatie technieken worden ook steeds vaker toegepast. Deze technieken zijn bijvoorbeeld: transcraniële magnetische stimulatie (*transcranial magnetic stimulation*; TMS), diepe hersenstimulatie (*deep brain stimulation*; DBS), en nervus vagus stimulatie (*vagal nerve stimulation*; VNS). Deze technieken zijn gericht op verschillende gebieden uit het depressie netwerk zoals de dorsolaterale prefrontale cortex (DLPFC) (George et al., 2010; O'Reardon et al., 2007), de dorsomediale prefrontale cortex (DMPFC) (Downar and Daskalakis, 2013; Downar et al., 2014), de subgenuale cingulate cortex (sgACC) (Mayberg et al., 2005) en de nervus vagus (VN) en hebben daarbij laten zien dat stimulatie van die gebieden leidden tot klinische verbetering. Maar, zelfs met dit brede aanbod van behandelingen reageert 40-50% van de depressieve mensen niet (Kessler and Bromet, 2013). Vandaar dat optimalisatie en individualisatie van de huidige behandeling hard nodig zijn.

Nieuwe inzichten laten zien dat er waarschijnlijk netwerk veranderingen plaatsvinden in de hersenen om zo de klinische verbetering te bewerkstelligen (Liston et al., 2014; Fox et al., 2012).

De nervus vagus is in feite een zenuw, die verbonden is met het hart. Het autonome zenuwstelsel bestaat uit 2 takken: het sympathische en het parasympatische zenuwstelsel. Het sympathische zenuwstelsel is actief in stresssituaties en het parasympatische zenuwstelsel in rust. De nervus vagus is onderdeel van de laatste, en daarom leid stimulatie van de nervus vagus tot een hartslagverlaging (Lang and Levy, 1989; Buschman et al., 2006). Dit is altijd een kortdurend effect. Interessant genoeg, hebben verscheidene studies ook laten zien dat stimulatie van de DLPFC leidde tot een hartslagverlaging (Makovac et al., 2016). Dit suggereert sterk dat er inderdaad een netwerkverbinding is tussen de DLPFC en de nervus vagus (zie figuur 2 op bladzijde 223). Naar aanleiding van een studie van Fox et al., (2012), wordt gedacht dat signalen via de DLPFC worden overgebracht naar de sgACC en vervolgens naar de nervus vagus. Er werd hierin namelijk gevonden dat, hoe sterker de anticorrelatie in activiteit tussen de DLPFC en de sgACC was, hoe beter mensen reageerden op behandeling. Specifieker, voor een groep mensen is bekeken hoe groot de anticorrelatie was tussen het gebied waarop gestimuleerd werd, en de sgACC. Hieruit trokken zij de conclusie dat je de ideale locatie van de DLPFC zou kunnen bepalen aan de hand van de anticorrelatie met de sgACC. Ter verduidelijking: momenteel zijn er verschillende methoden om de DLPFC te lokaliseren, maar deze werken vaak alleen op groepsniveau, omdat elk hoofd en hersenen verschillend zijn. Voorbeelden hiervan zijn de 5 cm regel (waarin eerst op de motor strip gestimuleerd wordt totdat er een response van de hand zichtbaar is, vervolgens zou 5 cm naar voren de DLPFC moeten liggen), of de BEAM-methode (aan de hand van bepaalde afmetingen wordt de locatie bepaald). Tegenwoordig wordt er ook meer gedaan met MRI, zo ook de methode van Fox et al (2012). Met deze methode zou je per individu kunnen zeggen op welke locatie zij het beste gestimuleerd zouden kunnen worden (Fox et al, 2012).

Sindsdien is deze methode in 2 studies gerepliceerd (Weigand et al., 2018; 45) . Maar, MRI blijft kostbaar en de individuele reproduceerbaarheid van functionele MRI's blijft lastig. Zo kon er gemakkelijk 2 centimeter verschil zitten tussen een ochtend en een middag scan (Ning et al., 2018). Er is dus nog behoefte aan andere, wellicht betere methoden.



Figuur 2: *Hart-brein connectie. Te zien zijn verschillende hersenstructuren (DLPFC, sgACC en de nervus vagus) die met verschillende neuromodulatie technieken (TMS, tDCS, DBS, VNS). Wanneer de DLPFC gestimuleerd wordt, zou dit via via ook het hart kunnen inhibiteren, leidend tot een hartslagvertraging.*

Gezien de betrokkenheid van het hart, zou het ook mogelijk kunnen zijn om de hartslag te gebruiken om individueel te bepalen of er op de juiste plek gestimuleerd wordt. In dit proefschrift wordt het hart-brein netwerk nader bekeken om meer te weten te komen over behandelopties, individualiseren van behandeling en mogelijk

te kunnen voorspellen of iemand wel of niet gaat reageren op behandeling. Dit proefschrift bestaat uit 5 empirische hoofdstukken (Hoofdstuk 2 t/m 7) en 1 review (Hoofdstuk 8). De resultaten van deze hoofstukken zullen hieronder kort worden beschreven.

In **Hoofdstuk 2** werd er met behulp van EEG scans (deze meten de hersenactiviteit), gekeken of er verschillen zaten in activiteit tussen gezonde mensen en mensen met een depressie, maar ook of er verschillen zaten tussen mensen die wel reageerden op behandeling (in deze studie was dat medicatie) en mensen die niet reageerden. EEG hersenactiviteit is op te delen in verschillende frequenties (bandbreedtes) en tijdens deze studie is gekeken naar de alpha frequenties (tussen 8 en 13 Hz) en de theta frequenties (tussen 4 en 7,5 Hz). Dit zijn de tragere hersengolven. Het studiesample was afkomstig van de internationale iSPOT studie en had daarmee een steekproefgrootte van 1008 depressieve mensen en 336 gezonde controles. We hebben gekeken naar verschillen in correlatie in de activiteit tussen de DLPFC en de sgACC en zagen dat op baseline, gezonde mensen verschilden van depressieve mensen op de lage theta frequenties. In alpha frequenties werden geen verschillen gevonden. Tussen de mensen die wel of niet reageerden op behandeling werden ook geen verschillen gevonden. Daarnaast vonden we wel verschillen tussen mannen en vrouwen. Na behandeling vonden we dat de correlatie in activiteit tussen de DLPFC en de sgACC was afgenomen voor mannen die reageerden op behandeling, maar daarmee meer gingen afwijken van gezonde controles. Deze resultaten laten zien dat op basis van het EEG het niet mogelijk was om vooraf te voorspellen wie wel en niet zou gaan reageren op behandeling. De correlatie tussen de DLPFC en de sgACC was dus niet verbonden aan klinische verbetering, in ieder geval niet voor medicatie en middels EEG. Wel lieten ze zien dat het belangrijk was om geslacht mee te nemen als variabele in het onderzoek, omdat hier grote verschillen tussen kunnen zitten.

In **Hoofdstuk 3** werd gekeken naar factoren die een invloed kunnen hebben op de variatie in de hartslag. De hoeveelheid variatie in de hartslag wordt namelijk gelinkt aan verschillende psychiatrische en fysieke aandoeningen. Oorspronkelijk diende deze studie als replicatie van een eerder onderzoek waarin gevonden werd dat jongens

die geboren waren in de winter een hogere hartslagvariabiliteit hadden. Dit was inderdaad wat wij ook vonden, al was onze steekproef 5 maal groter (1871 gezonde proefpersonen) in de leeftijd van 6 tot 87 jaar, i.p.v. 6 tot 12. Daarnaast vonden we dat deze variatie in de hartslag ook leeftijds- en geslachtsgebonden was. Deze resultaten lieten zien dat het belangrijk is om leeftijd en geslacht, en mogelijk ook geboorteseizoen mee te nemen in onderzoek naar hartslagvariabiliteit.

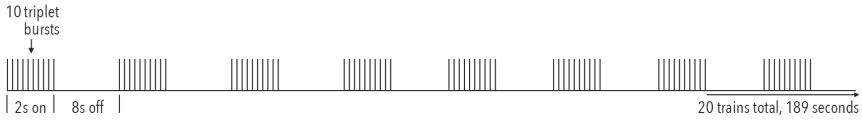
In **Hoofdstuk 4** werd met behulp van een zogeheten pilot-studie getest of de eerdergenoemde methode om de hartslag te gebruiken voor het bepalen van de ideale stimulatie locatie inderdaad werkt. Hiervoor werden 10 gezonde mensen gebruikt, die vervolgens gestimuleerd werden met TMS op 7 verschillende hersengebieden (3 aan de linkerkant, 3 aan de rechterkant en 1 controle locatie bovenop het hoofd). Daarin zaten de hersengebieden die op groepsniveau de DLPFC zouden moeten zijn, en een paar locaties verder die daar verder vanaf lagen. Tijdens stimulatie werd de hartslag gemeten. We vonden dat, op groepsniveau, er tijdens stimulatie van DLPFC een grotere hartslagverlaging plaatsvond dan tijdens stimulatie van de andere locaties. Er zaten wel wat individuele verschillen die lieten zien dat voor sommige proefpersonen, de exacte locatie van DLPFC waarschijnlijk iets meer naar achter lag.

In **Hoofdstuk 5** hebben we dit onderzoek herhaald, maar nu in een grotere steekproef van 30 gezonde mensen en 33 depressieve patiënten. In beide groepen werden op groepsniveau op dezelfde locaties hartslagverlagingen gevonden als in de pilot-studie, en bevestigden daarmee de eerder gevonden resultaten. Hiernaast wordt er in Hoofdstuk 5 ook gekeken naar de individuele reproduceerbaarheid. Dit werd alleen bekeken in de groep gezonde mensen. Zij kregen 2 sessies en in plaats van in sessie 2 weer verschillende locaties te stimuleren werd er in deze sessie langzaam de stimulatie intensiteit verhoogd op de locatie die op groepsniveau de DLPFC was, maar ook op de locatie die voor dat individu de grootste hartslagverlaging liet zien. Voor het bepalen van de individuele reproduceerbaarheid werd de hartslagreactie na stimulatie van de groeps-DLPFC tijdens sessie 1 vergeleken met de hartslagreactie na stimulatie van de groeps-DLP-

FC op normale intensiteit tijdens sessie 2. Deze 2 sessies bleken significant met elkaar te correleren. Als volgende validatiestap werd bekeken of de hartslagverlaging groter is bij een hogere stimulatie intensiteit. Dit bleek inderdaad het geval te zijn. Deze resultaten laten zien dat het inderdaad mogelijk lijkt te zijn om aan de hand van de hartslagverlaging te bepalen of er wel of niet op de DLPFC wordt gestimuleerd. Echter, deze resultaten laten nog niet zien of lokalisatie aan de hand van hartslag ook leidt tot betere behandeluitkomsten. Hier is verder onderzoek voor nodig.

Hoofdstuk 6 is een studie van een onafhankelijke onderzoeksgroep uit Australië, die ook de resultaten van de pilot-studie gerepliceerd hebben. Ook zij vonden de grootste hartslagverlaging op de groeps-DLPFC in 18 gezonde vrijwilligers. Daarnaast hebben zij gekeken naar het effect van een mildere vorm van stimulatie (1 Hz in plaats van 10 Hz). Hierbij worden enkele pulsen gegeven op een ritme van 1 Hz en dit werd gedurende 1 minuut per locatie toegediend terwijl de hartslag gemeten werd. Vervolgens is de gemiddelde hartslag per locatie vergeleken om te kijken of deze ook gemiddeld lager lag tijdens stimulatie op de groeps-DLPFC. Dit was echter niet het geval, het lag zelfs hoger op die locatie. Ook is er hierin gekeken naar hartslagvariabiliteit maar er waren geen significante verschillen tussen de verschillende locaties. Anders dan in het volgende hoofdstuk hebben zij echter niet gekeken naar de daadwerkelijke hartslagverlaging tijdens de minuut van stimulatie, wat misschien kan verklaren waarom er met 1Hz tegenovergestelde effecten worden gevonden.

In **Hoofdstuk 7** wordt een andere vorm van TMS gebruikt. Waar in Hoofdstuk 4 en 5 TMS op een ritme van 10Hz werd toegediend, is dit in Hoofdstuk 7 in een theta burst ritme (zie figuur 3).



Figuur 3: *Voorbeeld van een theta burst ritme. In dit protocol worden er gedurende 2 seconden 30 pulsen afgevoerd in series van 3. Vervolgens volgt er 8 seconden rust, waarna er weer 2 seconden van stimulatie volgen. Dit protocol duurt in totaal 189 seconden.*

In deze studie werd de hartslag en bloeddruk gemeten tijdens behandeling van 15 depressieve mensen. Deze mensen kregen in totaal 30 sessies, maar kregen actieve behandeling én een schijnbehandeling. We bekeken of we tijdens de eerste sessie al een verschil konden zien in de hartslag tussen wanneer iemand actieve behandeling kreeg en wanneer iemand de schijnbehandeling kreeg. Dit bleek het geval te zijn. Al tijdens de eerste minuut van behandeling trad er een grotere hartslagverlaging op tijdens actieve behandeling dan tijdens de schijnbehandeling. Hiermee konden we met 91% zekerheid voorspellen welke opname genomen was tijdens actieve behandeling en welke tijdens schijnbehandeling. Daarbij correleerde het verschil in hartslagverlaging tussen schijn- en actieve behandeling met behandeluitkomst. Dat betekent dat dit verschil mogelijk zou kunnen voorspellen of iemand wel of niet zo reageren op behandeling. Naast deze bevinding vonden we ook dat tijdens de actieve behandeling mensen een lagere bloeddruk hadden dan tijdens schijnbehandeling en ook hadden ze een hogere variatie in de hartslag, wat dus suggereert dat het hart een beter aanpassingsvermogen had. Deze resultaten laten dus zien dat hersenstimulatie een grote invloed kan hebben op de hartslag, maar omdat deze steekproef nog zo klein was zullen er eerst meerdere onderzoeken moeten komen.

Hoofdstuk 8 is een review en vat eigenlijk dit proefschrift grotendeels samen. Het belicht allereerst de anatomie van het hart-brein netwerk. Vervolgens maakt deze review een link tussen depressie en het hart-brein netwerk. Er wordt zicht geboden op de verschillende neuromodulatietechnieken die worden gebruikt om depressie te verlichten en we lieten zien dat deze technieken effect hebben op de hartslag. Daarna wordt het principe van 'target engagement' uitgelegd. Target engagement is het gebruiken van een functionele connectie om daarmee de streeflokatie te vinden. Een voorbeeld daarvan is de eerder genoemde methode van Fox et al, maar ook de NCG-TMS methode waarin hartslag wordt gebruikt om de DLFPC te vinden is er één. Het voordeel van deze methode is dat hij makkelijk in gebruik te nemen is en er geen MRI scans nodig zijn.

In **Hoofdstuk 9** worden de belangrijkste bevindingen op een rij gezet en bediscussieerd.

- Hoofdstuk 2 laat baseline verschillen in EEG theta connectiviteit zien tussen MDD patienten en gezonde controles, en een verandering in connectiviteit tussen de DLPFC en de sgACC voor mannelijke responders op behandeling.
- In Hoofdstuk 3 zien we dat geboorteseizoen de hartslagvariabiliteit later in het leven kan beïnvloeden, maar dat de huidige leeftijd en geslacht hierin ook meespelen.
- Hoofdstuk 4 laat zien dat TMS stimulatie op de DLPFC regio de hartslag kan verlagen.
- Hoofdstuk 5 laat zien dat deze resultaten repliceerden in een grotere steekproef, in 2 verschillende groepen. Ook dat de resultaten een goede individuele reproduceerbaarheid hadden en de hartslagreactie leek samen te hangen met de intensiteit van stimulatie.
- In Hoofdstuk 6 heeft een onafhankelijke onderzoeksgroep de resultaten van de pilot-studie ook succesvol gerepliceerd en daarnaast gekeken naar de effecten van een 1Hz stimulatieritme. Hier zijn echter nog geen conclusies aan te verbinden.
- In Hoofdstuk 7 zien we dat een nieuwe vorm van TMS, iTBS, ook grote invloed heeft op de hartslag, maar ook op bloeddruk. De hartslagverlaging tijdens actieve iTBS was vele malen groter dan tijdens schijnstimulatie en dit verschil hing samen met behandeleffectiviteit.
- Hoofdstuk 8 is een review die tevens alle bovenstaande studies samenvat en poogt om verbanden te leggen tussen het hart-brein netwerk, depressie en neuromodulatie.

Bij elkaar geven deze hoofdstukken aan dat er een 'depressie netwerk' bestaat in de hersenen, waar verschillende hersenstructuren bij betrokken zijn, maar waar vooral ook het hart bij betrokken is. Deze kennis kan gebruikt worden om bestaande neuromodulatietechnieken te verbeteren. De methode van NCG-TMS (gebruik van hartslag voor lokalisatie) is veelbelovend maar het is belangrijk om te weten dat het nog niet getest is in het kader van behandeleffectiviteit. Het is dus nog belangrijk om te testen of individuele lokalisatie aan de hand van de hartslag ook leidt tot een betere behandeluitkomst.

Daarnaast gaan we er in dit proefschrift vanuit dat de sgACC een grote rol speelt in het doorgeven van signalen vanuit stimulatie van de DLPFC, maar dat zou betekenen dat stimulatie van de sgACC ook zou moeten leiden tot hartslagverlagingen. Echter, hierover zijn wisselende resultaten. Het is dus nog niet bewezen dat de sgACC de verbinding is tussen de DLPFC en het hart.

Naast de invloed op de hartslag reguleert de nervus vagus onder andere ook de pupilgrootte, maag-darm stelsel en speekselproductie. Het zou kunnen dat ook deze functies kunnen laten zien of er op de juiste locatie wordt gestimuleerd. Ook hebben we alleen gekeken naar de hartslag, maar er zouden ook aanwijzingen kunnen zitten in de hartslagvariabiliteit. Dit is gebleken uit de studie waarbij iTBS gebruikt werd. Echter, om een betrouwbare hartslagvariabiliteit te meten zijn er opnames van minimaal 2 minuten nodig.

Er zijn diverse factoren die mogelijk ook van invloed kunnen zijn op de hartslag, en op de hartslagvariabiliteit. Dat zijn bijvoorbeeld op de lange termijn conditie, slaap, en ademhaling en op korte termijn de tijd van de dag waarop gemeten wordt, wanneer iemand voor het laatst gegeten en water gedronken heeft e.d. Vandaar dat ter voorkoming van depressieve stoornissen vaak ook wordt aangeraden om te bewegen. En zijn meditatie en aanpassingen op het gebied van slaap ook effectief. We hebben ook gezien dat iemands geboorteseizoen invloed kan hebben op de hartslagvariabiliteit.

Ook blijkt gender een grote rol te spelen in Hoofdstuk 2 en 6. Allereerst laten mannen en vrouwen een verschil zien op het gebied van verbindingen in de hersenen. Maar ook laten ze een verschil in hartslag zien. Dat benadrukt hoe belangrijk het is om gender mee te nemen in onderzoek.

Met dit proefschrift laten we zien dat een belangrijk onderdeel van het depressie netwerk het hart is, en dat deze kennis mogelijk gebruikt kan worden om behandeling te individualiseren en mogelijk te optimaliseren.

DANKWOORD

Dan zijn we nu gekomen bij het deel waar ik iedereen bedank voor zijn of haar steun, begeleiding, gezelschap of hulp bij het tot stand komen van dit proefschrift.

Allereerst natuurlijk dank aan mijn copromotor Martijn. Zonder zijn inspiratie, geloof en ondernemendheid was het idee van NCG-TMS nooit tot stand gekomen en zou de rode lijn in dit proefschrift er heel anders uit zien. Je enthousiasme wist je over te brengen en ik heb afgelopen jaren met plezier aan het onderzoek gewerkt. Ik ben enorm gegroeid als onderzoeker in de afgelopen jaren en heb mij erg op mijn plek gevoeld bij Brainclinics.

Uiteraard droeg de uitbreiding van Brainclinics, de komst van nieuwe collega's, daar erg aan mee. Noralie, Hanneke, Madelon, Nikita, Tommy en Mark, bedankt voor de gezellige tijd, de koffie-breaks, taart, kerst en paaslunches, borrels, congressen (en het ontdekken van de stad), de leuke gesprekjes, de grapjes maar ook zeker voor de hulp bij mijn onderzoeken, het sparren en het brainstormen.

Daarnaast waren er natuurlijk ook de collega's van neuroCare, welk team ook steeds groter werd. Dank aan de psychologen Vera, Myrthe en Joris die altijd openstonden om mijn ideeën, na zorgvuldige afweging van baten en risico's, toe te passen bij cliënten. Maar bovenal ook door zichzelf steeds aan te bieden als proefpersoon (al dan niet met een beetje mopperen). Dit laatste geldt overigens voor alle collega's van neuroCare en Brainclinics, bedankt.

Ook mijn promotor Leon heeft een grote rol gespeeld in het tot stand komen van dit proefschrift, maar bovenal mijn ontwikkeling als onderzoeker. Zijn kritische blik en vragen dwongen mij goed na te denken en vooral ook een eigen mening te ontwikkelen. Over de opzet van het onderzoek, over het verwoorden in de manuscripten en de statistische analyses. Ik kan wel zeggen dat de manuscripten en dit proefschrift sterk verbeterd zijn onder jouw begeleiding.

Dank aan mijn familie, voor alle steun door de jaren heen. In het bijzonder dank aan Boris, voor het aanhoren van mijn 'problemen' en ervoor zorgen dat ik het rustig aan deed, althans, dat probeerde je, en

mijn dochter Ruby, die zich, al dan niet bewust, pas aandiende op de dag na het officiële indienen van dit proefschrift.

Dank aan Nienke die als stagiaire bijdroeg aan de review - and thanks to Fiamma who as an intern helped in setting up the NCG-TMS replication study. Mark, bedankt voor de lay-out van dit boekje en het ontwerp van het omslag. Thanks to the people from the other neuroCare institutes, in particular Klaus for the development of the NCG-ENGAGE and Eliana for selling the idea. Alle co-auteurs, proefpersonen, the people from BRC, enorm bedankt voor jullie bijdragen en hulp. En aan iedereen die ik geheid vergeten ben, bedankt!

CURRICULUM VITAE

Tabitha Amanda Iseger werd geboren op 21 mei 1989 te Alphen aan de Rijn. Na haar middelbare school vertrok ze naar Leiden om daar vanaf 2008 Biologie te studeren om vervolgens in 2011 een onderzoeksmaster in Neurowetenschappen te starten aan de VU in Amsterdam. Tijdens die master heeft ze haar eindstage gelopen aan King's College in Londen.

Bij terugkomst studeerde ze af en begon ze te solliciteren. Ze kwam terecht bij het Amsterdam Medisch Centrum op de afdeling Radiologie en Psychopharmacologie waar ze als onderzoeksassistent onderzoek deed naar de invloed van ADHD medicatie en antidepressiva op de hersenontwikkeling. De volgende stap was een promotietraject en dit werd mogelijk bij Onderzoeksinstituut Brainclinics, waar ook onderzoek werd gedaan naar ADHD en depressie, maar nu vooral gericht op het voorspellen en verbeteren van behandeluitkomsten. Hier startte ze in 2015 onder leiding van prof. Dr. Leon Kenemans en Dr. Martijn Arns. Dat heeft geleid tot dit proefschrift over het gebruik van hartslag om rTMS behandelingen te individualiseren en te optimaliseren.

Tabitha Amanda Iseger was born on May 21, 1989, in Alphen aan de Rijn. After finishing high-school, she went to Leiden in 2008 to study Biology. In 2011 she started a research master in Neuroscience at the VU University in Amsterdam. During that master she also completed her final internship at King's College in London.

Upon her return, she graduated and started searching for jobs. This led her to the department of Radiology and Psychopharmacology at the Amsterdam Medical Center, where she, as a research assistant, investigated de influence of ADHD medication and antidepressants on the brain development. The next step was to start a PhD trajectory and this became possible at Research Institute Brainclinics, where also researched was performed toward ADHD and depression, but now more focusing on the prediction and improvement of treatment outcome. In 2015 she started her PhD trajectory under supervision of prof. Dr. Leon Kenemans and Dr. Martijn Arns. This led to this thesis on the use of heart rate for optimization and individualization of rTMS treatment.

Brainclinics®
INSIGHTS

ISBN 978-90-830013-1-9