Resting-state functional MRI predictors and correlates of response to rTMS in major depression

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

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Abstract

Major depressive disorder (MDD) is a debilitating and prevalent psychiatric illness for which an emerging treatment is repetitive transcranial magnetic stimulation (rTMS). Several recent studies have used functional brain imaging modalities to characterize neural predictors and correlates of rTMS response in MDD. Specifically, responders to rTMS may exhibit varying functional abnormalities among brain regions that play roles in cognition, attention, or reward response. Here, using a cohort of 303 patients who received one of two rTMS stimulation protocols, we aimed to identify functional MRI predictors and correlates of response to left dorsolateral prefrontal cortex (dIPFC)-rTMS. Based on baseline and post-treatment change in whole-brain functional connectivity to a selection of cortical, striatal, and network-wide seed regions, we identified several predictors and correlates of treatment response that comprise a ventromedial and orbitofrontal functional loop. This study represents a step to understanding maladaptive network interactions in MDD, the impact of non-invasive brain stimulation on improving inter-network function, and the role of functional brain networks in motivation, valuation, and behaviour.
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My own contributions included: checking and organizing neuroimaging IDs for all 303 patients; downloading and manually inspecting all pre-treatment and post-treatment structural and anatomical scans; selecting the dataset based on available clinical and neuroimaging data; preprocessing the neuroimaging data using FSL and MATLAB; performing statistical tests for analysis of clinical data; generating neuroimaging predictors and correlates of treatment, including selecting regions of interest (ROI), extracting ROI time series, performing first- and second-level time series regression analyses in FSL, and writing scripts to perform these tasks efficiently and automatically; constructing visualizations for neuroimaging results; comparing response and non-response groups; evaluating and interpreting results; and writing this thesis. This work was funded in part by the Institute of Medical Science Entrance Award, the Ontario Graduate Scholarship, and the Toronto General and Western Hospital Foundation.
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>aCompCor</td>
<td>Anatomical Components-Based Noise Correction</td>
</tr>
<tr>
<td>ATHF</td>
<td>Antidepressant Treatment History Form</td>
</tr>
<tr>
<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
</tr>
<tr>
<td>CEN</td>
<td>Central Executive Network</td>
</tr>
<tr>
<td>CST</td>
<td>Cortico-Striato-Thalamic</td>
</tr>
<tr>
<td>CSTC</td>
<td>Cortico-Striato-Thalamo-Cortical</td>
</tr>
<tr>
<td>cTBS</td>
<td>Continuous Theta Burst Stimulation</td>
</tr>
<tr>
<td>dACC</td>
<td>Dorsal Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>DBS</td>
<td>Deep Brain Stimulation</td>
</tr>
<tr>
<td>dIPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>DMN</td>
<td>Default Mode Network</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>HRSD</td>
<td>Hamilton Rating Scale for Depression-17</td>
</tr>
<tr>
<td>ICA</td>
<td>Independent Component Analysis</td>
</tr>
<tr>
<td>iTBS</td>
<td>Intermittent Theta Burst Stimulation</td>
</tr>
<tr>
<td>IOFC</td>
<td>Lateral Orbitofrontal Cortex</td>
</tr>
<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
</tr>
<tr>
<td>MINI</td>
<td>Mini International Neuropsychiatric Interview</td>
</tr>
<tr>
<td>MINI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>mOFC</td>
<td>Medial Orbitofrontal Cortex</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>OFC</td>
<td>Orbitofrontal Cortex</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal Components Analysis</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>RMT</td>
<td>Resting Motor Threshold</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>rs-FC</td>
<td>Resting State Functional Connectivity</td>
</tr>
<tr>
<td>rTMS</td>
<td>Repetitive Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>sgACC</td>
<td>Subgenual Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>SN</td>
<td>Salience Network</td>
</tr>
<tr>
<td>TBS</td>
<td>Theta Burst Stimulation</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>UBC</td>
<td>University of British Columbia</td>
</tr>
<tr>
<td>UHN</td>
<td>University Health Network</td>
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<tr>
<td>VBM</td>
<td>Voxel-Based Morphometry</td>
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<tr>
<td>VMN</td>
<td>Ventromedial Network</td>
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<tr>
<td>vmPFC</td>
<td>Ventromedial Prefrontal Cortex</td>
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1.0 Literature Review

1.1 Introduction

Major depressive disorder (MDD) is one of the leading causes of disability and disease burden worldwide, diminishing both quality of life and productivity (World Health Organization, 2001). Medication and psychotherapy are ineffective in at least one-third of patients, and even among responders, many experience relapse (Ferrari et al., 2013; Rush et al., 2006). Recently, functional brain imaging modalities have helped to characterize functional abnormalities throughout specific networks of the brain in patients with MDD. The emerging picture is that individuals with MDD may exhibit functional imbalances in brain regions that play a role in cognition, attention, and reward response (Downar and Daskalakis, 2013; Mulders et al., 2015).

Repetitive transcranial magnetic stimulation (rTMS) is an emerging therapeutic technique that can treat MDD by directly influencing and normalizing functional activity in affected brain networks (Concerto et al., 2015). Treatment guidelines increasingly recommend rTMS in cases where medications have failed (Lefaucheur et al., 2014; Patten et al., 2009). However, treatment outcomes vary, with recent reports suggesting distinct rTMS responder and non-responder subgroups (Bakker et al., 2015; Downar et al., 2014; Fitzgerald et al., 2016). Since rTMS treatment courses can be onerous, requiring 20-30 sessions of stimulation for optimal effect (Carpenter et al., 2012), a major goal in current rTMS research is to identify neurobiological markers that could identify, predict, or track rTMS treatment outcome (Silverstein et al., 2015).

Functional brain imaging techniques such as functional magnetic resonance imaging (fMRI) are potentially useful tools in understanding brain function in health and disease (Cole et al., 2010; Fox and Greicius, 2010a; Matthews and Hampshire, 2016). In particular, resting-state functional MRI studies over the last 20 years have helped to clarify both the abnormalities of brain activity associated with MDD (Craig et al., 2015; Dichter et al., 2014) and the mechanisms by which rTMS may remedy these abnormalities to treat MDD (Fitzgerald et al., 2016; Johnson et al., 2013). A better understanding of treatment mechanisms, as well as predictors of treatment response, is
necessary to improve clinical efficacy and optimize treatment outcomes. Therefore, using brain-imaging techniques such as fMRI to more thoroughly characterize brain activity in MDD patients could help to inform targeted and effective treatments, thereby raising treatment response rates and reducing the health burden of MDD.

This thesis project performed resting-state functional connectivity analyses on a cohort of 303 MDD patients who participated in a randomized clinical trial comparing two forms of rTMS. The specific aims of the project were to:

a) identify resting-state fMRI predictors and correlates of rTMS treatment response; and

b) compare the effects on clinical symptoms and resting-state brain activity for two forms of rTMS: 10 Hz stimulation and intermittent theta burst stimulation (iTBS).

This literature review aims to:

a) review the current state of the literature on anatomical and functional neuroimaging findings in MDD;

b) present an overview of the development of rTMS, its clinical efficacy in MDD, and current efforts to improve treatment outcomes;

c) review current literature on functional networks of the brain, including analytical methods for identifying and studying these networks, as well as their normal architecture and their role in psychiatric illness;

d) review the rationale and techniques for using resting-state functional connectivity analysis to investigate network-level predictors and correlates of rTMS response in MDD.
1.2 Depression

1.2.1 Overview

Depression has been recognized as a clinical disorder since antiquity (Telles-Correia and Marques, 2015). Over the last several decades, modern diagnostic systems have evolved to describe and codify its major symptom clusters and course (American Psychiatric Association, 2013; World Health Organization, 1992), and a suite of treatments have been developed, including psychotherapies, medication therapies, and brain stimulation therapies (Block and Nemeroff, 2014; Holtzheimer III and Nemeroff, 2006). In addition, over the last three decades, an extensive neuroimaging literature has helped to characterize the abnormalities of brain structure and function associated with depression (Dichter et al., 2014; Menon, 2011; Treadway and Pizzagalli, 2014).

Despite these advances, MDD remains a major public health issue, due to both its high prevalence and its high burden of disability on patients, caregivers, and society as a whole (World Health Organization, 2001). As a result, new treatments are needed, as well as more reliable approaches to selecting the optimal treatment for any given patient (Downar and Daskalakis, 2013). This section will review the prevalence of MDD and its common presenting symptoms, current system of classification, and etiology as understood from a neurobiological perspective. Finally, this section will conclude with an introduction to the utility of non-invasive brain stimulation techniques in treatment-resistant depression.

1.2.2 Prevalence, Symptoms, and Comorbidities

Depression is a leading cause of worldwide disease burden, affecting over four percent of Canadian adults annually (Murray and Lopez, 1997). In coming decades, MDD is projected to be one of the most prevalent and burdensome psychiatric or neurobiological disorders in the world (World Health Organization, 2001). Almost one in five Canadians will experience a depressive episode at least once in their lifetime, often occurring for the first time in young adulthood (John and Antai-Otong, 2016); in Ontario, an estimated 650,000 individuals experience a major depressive episode each year.
(Patten et al., 2006). Some subgroups of the population are at an especially high risk for depression, including those of low socioeconomic status and individuals of racial, ethnic, or social minority groups (Perrino et al., 2015). Additionally, clinical populations facing chronic and terminal diseases such as cardiovascular disease, chronic pain, or cancer are at higher risk for major depression (Hegeman et al., 2016). Increased risk of depression in individuals with comorbid physical disease may also be attributed to disease-related personal trauma, cognitive impairments, or aging—all of which have separately been associated with increased risk of depression (John and Antai-Otong, 2016).

The core symptoms of major depression are currently considered to be low mood and loss of interest or pleasure in activities, or anhedonia; often, physical symptoms including fatigue, weight or appetite change, and decreased libido accompany psychological and emotional symptoms. In severe cases, suicidal thoughts and behaviours may be present (Kendler, 2016). MDD is often comorbid with other psychiatric disorders, including anxiety disorders, personality disorders, and substance use disorders (Hasin et al., 2005). Specific symptoms required for a diagnosis are explored in further detail below (Section 1.2.3).

The symptoms of major depression affect both quality of life and level of function in daily activities. For example, anhedonia may lead to withdrawal from normal hobbies or social activities, and cognitive and physical symptoms can affect individuals’ self-efficacy and ability to work. This impact on productivity also exerts societal costs, as afflicted individuals are often unable to function at normal levels in the workplace: some studies have reported that up to half of mood disorder sufferers remain away from work for at least six months following their first depression-related absence (Sylvain et al., 2016).

1.2.3 Diagnosis

A formal diagnosis of depression may be made if symptoms are enduring and have a significant impact on daily function. To meet diagnostic criteria for a major depressive
episode, the most recent version of the Diagnostic and Statistical Manual of Psychiatric Disorders (DSM-5) requires symptoms to be experienced for a minimum of two weeks and to cause significant distress and/or functional impairment (American Psychiatric Association, 2013). During this two week period, the distressing symptoms must include either consistent depressed mood or anhedonia in addition to at least four other symptoms, which can include: significant change in weight or appetite, significant sleep disturbance (insomnia or hypersomnia), psychomotor agitation or retardation, fatigue or loss of energy, excessive feelings of guilt or worthlessness, impaired concentration or decision making, or suicidal ideation, plan, or attempt (American Psychiatric Association, 2013). Major depression is considered distinct from other related disorders of low mood which have slightly different diagnostic criteria in the DSM-5, including persistent depressive disorder, postpartum depression, and seasonal affective disorder (American Psychiatric Association, 2013).

Although the DSM-5 is the primary diagnostic tool used by clinicians, it has been criticized for not capturing other common criteria—including anxiety, slowed speech, and changes in motivation—that are historically linked to depression (Kendler, 2016). For this reason, clinicians and researchers are encouraged to use both clinician-rated and patient-rated scales to appraise relevant clinical information that may not be fully captured with either type of assessment individually (Schneibel et al., 2012). Some commonly used assessment tools include the clinician-rated Hamilton Rating Scale for Depression (HRSD) or the self-rated Beck Depression Inventory (BDI), which assess depression severity based on the range and intensity of symptoms. For example, the 17 interview items of the HRSD assess symptoms relating to depressed mood (1), feelings of self-criticism or guilt (2), suicidal thoughts or behaviours (3), sleep (4-6), impact on work and activities (7), psychic or motor slowness (8), psychic or motor anxiety (9-11), appetite (12), energy (13), sexual interest (14), hypochondriasis (15), weight change (16), and insight (17). Although the validity of some items in this scale has been questioned (Rehm and O’Hara, 1985), it remains in common use in major randomized trials of both medications (Rush et al., 2006) and brain stimulation treatments (Bakker et al., 2015; Concerto et al., 2015; Liu et al., 2015).
1.2.4 Etiology and Neural Abnormalities

Depression has several biological risk factors. For the purpose of this review, the etiology of MDD will be outlined from a neurobiological perspective, with a focus on structural and functional abnormalities.

1.2.4.1 Structural

MDD is associated with subtle neuroanatomical abnormalities detectable using structural MRI. A recent meta-analysis reported that reduced gray matter volume in the anterior insula and dorsal anterior cingulate cortex (dACC) is a common substrate of a variety of psychiatric illnesses, including MDD (Goodkind et al., 2015). In addition to structural changes in these common regions, MDD is uniquely characterized by volumetric reductions in the medial and lateral prefrontal cortex and limbic regions, such as the amygdala, hippocampus, and orbitofrontal cortex (Bora et al., 2012; Greicius et al., 2007; Lener and Iosifescu, 2015; Petrovic et al., 2015). Notable but less commonly reported regions of gray matter volume reduction include the caudate nucleus and putamen (Bora et al., 2012; Goodkind et al., 2015; Shepherd, 2013). Volumetric disturbances in these subcortical striatal regions have been linked to abnormal signalling within dopaminergic pathways, and are hypothesized to contribute to symptoms that encompass maladaptive reward response, including anhedonia and decreased reinforcement learning (Pizzagalli, 2014).

1.2.4.2 Functional

Combinations of neuroimaging and lesion studies in humans, primates, and other animals suggest that depressive symptoms involve widespread dysfunction throughout individual regions and among interconnected networks, and can be investigated using functional imaging techniques. For example, resting-state functional magnetic resonance imaging (rs-fMRI) provides a whole-brain view of functional connectivity in the absence of a focused task (for a more detailed review of rs-fMRI, see Section 1.6). Historically, the default mode network (DMN)—a functional network implicated in
internally-directed thought—has been established as an overactive resting state network in individuals with MDD (Greicius et al., 2007). In depressed individuals compared to controls, the DMN also shows increased connectivity to additional brain regions, including the subgenual cingulate cortex (sgCC) and thalamus; the sgCC also shows higher regional metabolism and greater resting activity in depressed patients (Greicius et al., 2007; Mayberg et al., 2005).

In addition to subgenual hyperconnectivity with the DMN, MDD is characterized by abnormal activity in nearby medial prefrontal regions and associated subcortical structures that extend throughout the ‘limbic loop,’ including the striatum, thalamus, and brainstem (Price and Drevets, 2012). Investigations of resting-state functional connectivity (rs-FC) also reveal abnormalities throughout cortico-striatal-thalamic (CST) loops that are linked to emotional and cognitive symptoms of MDD (Bora et al., 2012; Kerestes et al., 2015). While MDD has been classically described in terms of dysfunctional ventral corticostriatal loops that play a role in affect, recent reports have also identified abnormalities throughout dorsal, cognitive corticostriatal loops in MDD patients (Kerestes et al., 2015). Specifically, individuals with depression show increased rs-FC between the dorsal caudate nucleus and the right dIPFC, with disease severity correlated to increased connectivity. Hyperactivation in these regions may indicate a pathological compensation of cognitive processing over negative emotions and stimuli (Furman et al., 2011; Kerestes et al., 2015). Conversely, decreased CST connectivity in MDD has been linked to high levels of inflammation within corticostriatal white matter tracts, making these tracts a potentially ideal target for treatments that reduce inflammation or increase dopamine transmission to relieve motivational and behavioural symptoms (Felger et al., 2015).

Recently, researchers have suggested three candidate ‘endophenotypes’ of MDD that capture different realms of neural dysfunction, including cognitive, neurotic, and anhedonic subtypes (Webb et al., 2015). These subtypes are becoming increasingly well characterized by specific functional connectivity patterns based on resting state and task-based functional neuroimaging investigations (Section 1.5 provides a more
comprehensive review of pathological activity in specific functional networks relevant to MDD). As subtypes of MDD likely respond differentially to certain treatments, the parsing of MDD into a heterogeneous disorder warrants further investigation (Mulders et al., 2015).

1.2.5 Treatment Strategies in Depression

Current treatment modalities in MDD include medications, psychotherapies, and brain stimulation treatments. While some individuals benefit from informal or periodic sessions with counsellors or therapists, about two-thirds of MDD patients are successfully treated with pharmacotherapy and structured psychotherapy (Rush et al., 2006). Pharmacotherapy is a common first-line treatment that may be implemented alone or concurrently with psychotherapy; most commonly prescribed are selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs) (John and Antai-Otong, 2016). For patients who fail a first medication trial, a second medication trial or attempt at psychotherapy has been shown to achieve response or remission in 22-35% of individuals (Schatzberg et al., 2005a; Thase et al., 2007).

Patient preference is an important consideration in choosing an appropriate treatment. In certain cases, individuals may be averse to medication and opt for a behavioural intervention. For example, the Cognitive Behavioural Analysis System of Psychotherapy (CBASP) targets patients’ maladaptive cognitions and behaviours through psychoeducation and by introducing realistic coping skills. These skills are cemented through frequent ‘homework’ assignments that require patients to realistically analyze their patterns of maladaptive thoughts, feelings, and behaviours (John and Antai-Otong, 2016). In controlled studies, CBASP has achieved response and remission rates between 22-35% (Schatzberg et al., 2005a). Another psychotherapeutic technique, mindfulness-based therapy, attempts to interrupt dysfunctional thinking patterns by training patients in meditation and present-moment awareness. This strategy has been shown to reduce ruminative thinking and increase the experience of positive mind states (van Vugt et al., 2012).
Unfortunately, medication and therapy-based techniques are not always sufficient for patients to achieve remission: mood disorders are heterogeneous, difficult to treat, and commonly return even after symptoms have fully remitted (John and Antai-Otong, 2016). For one, the accessibility and effectiveness of psychotherapeutic interventions is limited not only by the meagre supply of trained therapists, but also by a substantial proportion of unresponsive patients (Schatzberg et al., 2005b). Additionally, the largest study to date of sequential MDD treatment trials (STAR*D) found that one-third of patients remain resistant to treatment following four failed attempts at psychotherapy and/or pharmacotherapy (Rush et al., 2006). This data reflects the notion that MDD, and particularly treatment resistant depression (TRD), remains a burdensome public health issue due to ineffectiveness of available treatments (Nemeroff, 2007; Rush et al., 2006).

1.2.6 Treatment-Resistant Depression

As noted above, depressive illness that is unresponsive to traditional treatment strategies or at least two antidepressant trials—representing up to one-third of individuals with MDD—is classified as treatment-resistant depression (TRD) (Nemeroff, 2007). TRD is incredibly disabling, and until recently, patients had few viable treatment options (Mayberg et al., 2005). Failure to benefit from medications, psychotherapy, or electroconvulsive therapy (ECT) impacts patients personally, and also has larger-scale consequences by increasing hospital burden, reducing productivity, and disrupting personal relationships (Fava et al., 2007; Giacobbe et al., 2009). This impact is magnified by the prevalence of TRD: an estimated 1.6-2% of Ontarians fail to respond to standardized treatments, representing up to 274,000 individuals (Nemeroff, 2007). Attempting further medication or psychotherapy trials does not often hold promise, since each additional trial becomes more likely to fail (Rush et al., 2006). The ineffectiveness of pharmacotherapies and psychotherapies in TRD may be attenuated with the implementation of new treatment approaches, such as brain stimulation.
1.2.7 Brain Stimulation for Treatment-Resistant Depression

In cases where medications and psychotherapy fail, a potentially effective strategy involves stimulation of the brain itself. In recent years, researchers and clinicians have developed brain stimulation techniques based on the assumption that abnormal functional connectivity can be 'normalized' by increasing or decreasing local activity at a cortical or subcortical node, which exerts downstream effects on larger scale networks (Downar et al., 2016).

Several brain stimulation techniques are available in TRD. The oldest of these is electroconvulsive therapy (ECT), which applies electric current to the brain to induce a seizure under general anaesthesia. Due to its invasive and extensive nature, ECT is often sought only after other treatments have failed: one course of ECT can involve up to 18 individual treatments and extend up to six weeks (Sackeim et al., 2008; Spaans et al., 2013). Between 65-75% of patients who undergo ECT achieve remission (Sackeim et al., 1993; Spaans et al., 2013), but the treatment is associated with significant cognitive side effects (Brakemeier et al., 2011; Sackeim et al., 2007). These effects in combination with the expenses and stigma associated with ECT limit its effectiveness as a suitable treatment for the hundreds of thousands of Canadians experiencing TRD (Nemeroff, 2007).

Another emerging option is deep brain stimulation (DBS), an invasive technique that involves the surgical placement of electrodes into target subcortical regions whose hyper- or hypo-activity is linked to symptoms of MDD (Giacobbe et al., 2009). DBS has commonly targeted white matter tracts near the subgenual cingulate cortex (sgCC), a region that is metabolically overactive in TRD, with stimulation measures that aim to reduce its activity (Mayberg et al., 2005). DBS in TRD is effective, with response rates close to 50% (Bewernick et al., 2010; Lozano et al., 2008; Malone et al., 2009). Despite its success, DBS is invasive and has more serious potential side effects compared to less invasive procedures. Its cost and time requirement render it less appealing as a first-line treatment for the high proportion of individuals suffering from TRD.
Invasive brain stimulation options are generally successful, but protocols are needed that are accessible, cost-effective, and less likely to produce unpalatable side effects. A potentially more accessible option is repetitive transcranial magnetic stimulation (rTMS), a non-invasive technique that alters cortical excitability of a stimulation target with focused magnetic pulses that penetrate the skull. The following section presents an overview of the development of rTMS, its clinical utility, and current research directions for improving the technique.

1.3 rTMS

1.3.1 Historical Overview

Transcranial magnetic stimulation (TMS) was initially developed as a tool to study central and peripheral motor physiology (Barker et al., 1985; Hallet, 2007). Early devices produced single magnetic pulses, and quickly became useful tools for investigating neural motor pathways by initiating motor-evoked potentials (MEPs) in the motor cortex and observing the effects in musculature (Hallet, 2007). The ability of single pulse TMS devices to briefly activate or inhibit focal cortical regions enabled the exploration and mapping of other localized brain functions, including visual and sensory phenomena (Amassian et al., 1989), memory processes (Mull and Seyal, 2001), and specific cognitive functions (Hallet, 2007).

Later, newer TMS devices were developed that could apply repeated pulses of stimulation at trains of varying frequencies (Hallet, 2007). In contrast to the temporary effects of single pulse TMS, repetitive TMS (rTMS) produced durable changes in cortical excitability: applying rTMS at slower frequencies (< 1 Hz) decreased brain excitability (Chen et al., 1997), while faster rates (5 – 20 Hz) increased brain excitability (Pascual-Leone et al., 1994). Applying TMS in short, repetitive, high-frequency trains, known as ‘theta-burst stimulation’ (TBS) (Di Lazzaro et al., 2005; Huang et al., 2005), mirrors these patterns: intermittent TBS (iTBS) increases cortical excitability, and continuous TBS (cTBS) decreases cortical excitability (Hallet, 2007).
The finding that repeated trains of TMS pulses could have lasting facilitatory or inhibitory effects on brain activity raised the possibility of therapeutic applications. For example, in some early studies, individuals receiving TMS reported improvements in mood (Bickford et al., 1987; Grisaru et al., 1998). Around the same time, early neuroimaging studies identified specific frontal lobe regions that were underactive in MDD (Austin et al., 1992; Baxter Jr. et al., 1989a; Drevets et al., 2002), among which was the dorsolateral prefrontal cortex (dlPFC). These findings led to speculation that rTMS directed at the dlPFC might exert an antidepressant effect. As a result, some of the first clinical populations who received experimental therapeutic rTMS were patients with major depression (Geller et al., 1997; George et al., 1995; Hoflich et al., 1993; Kolbinger et al., 1995; Pascual-Leone et al., 1996). The therapeutic use of rTMS in MDD is reviewed in further detail in Section 1.3.3.

1.3.2 Neuroplastic Mechanisms of rTMS

TMS-induced increases or decreases in cortical excitability can be described in relation to neural plasticity, which may be induced by strategically stimulating neurons to durably increase (long-term potentiation, LTP) or decrease (long-term depression, LTD) the strength of synaptic connections (Hallet, 2007). The rTMS stimulator coil produces a focal, pulsed magnetic field, which induces an electric field in target brain tissue (Roth et al., 1991). Coil geometry affects focality of the pulses, with figure-of-eight coils producing the most focal pulses (Roth et al., 1991). Both local and network-level effects can be observed depending on the cortical stimulation target; however, the enduring physiological effects of rTMS on neuroplasticity depend on the pattern of stimulation (Paus et al., 1997).

The ability of rTMS to induce neuroplasticity is associated with changes in both brain structure and functional connectivity. In particular, for rTMS of cortical targets, there may be an important mechanistic role for cortico-striatal-thalamic loop circuits, and specifically for dopamine release in the basal ganglia. For example, following left dlPFC-rTMS, dopamine release is reported in the corresponding striatal region in the head of the caudate nucleus, suggesting that generally, cortical stimulation effects propagate
throughout interconnected white matter tracts and can exert effects on neurotransmitter release in subcortical structures (Strafella et al., 2001).

Regarding functional connectivity, stronger baseline rs-FC between the primary motor cortex (M1) and premotor areas is shown to correlate with responsiveness to M1-iTBS (Volz et al., 2015), as well as with increased MEP amplitudes (Nettekoven et al., 2015). These results imply that response to stimulation may rely in part on the strength of existing functional connections. Non-invasive neuroimaging techniques used in conjunction with TMS experiments reveal that stimulation can also cause changes in functional connectivity. For example, relationships between network connectivity changes in MDD and responsiveness to rTMS have been observed in both reward circuitry (Downar et al., 2014) and corticostriato-thalamic tracts (Salomons et al., 2014). Taken together, these studies suggest that the local effects of rTMS can propagate through networks of connected brain regions, and may impact long-term functioning of integrated brain networks by affecting local neurotransmitter availability.

As noted above, high-frequency stimulation is conventionally considered excitatory and low-frequency stimulation is conventionally considered inhibitory (Hallet, 2007). However, recent work has revealed considerable heterogeneity in the effects of most stimulation patterns, with a substantial proportion of individuals showing minimal or ‘reversed’ effects rather than the classically described effects of high or low frequency stimulation (Maeda et al., 2000; Nettekoven et al., 2015). For example, one study that applied iTBS to the motor cortex identified a group of ‘non-responders’ who did not demonstrate increases in either rs-FC or motor-evoked potentials (MEPs), even when stimulation dose was increased (Nettekoven et al., 2015). Others have long noted the striking degree of interindividual variability in response to rTMS, regardless of stimulation frequency (Maeda et al., 2000). Acknowledging this heterogeneity in rTMS-induced neuroplasticity, we now review the therapeutic use of rTMS in depression, followed by a summary of the effects of several rTMS stimulation protocols in common use.
1.3.3 Therapeutic Use in Depression

As described above, clinical applications of rTMS appeared in the wake of early TMS studies that produced detectable emotional changes in subjects after stimulating localized cortical regions (Geller et al., 1997; George et al., 1995; Hoflich et al., 1993; Kolbinger et al., 1995; Pascual-Leone et al., 1996). Initially, investigators reported that TMS exerted only a slight therapeutic effect on depressive symptoms (Hoflich et al., 1993), or merely improvements in mood (Bickford et al., 1987; Grisaru et al., 1998). Still, these results encouraged a number of larger trials to improve stimulation protocols and further investigate the effect of targeted brain stimulation on psychological health. Early investigators drew upon reports that the left dlPFC was hypoactive in patients with TRD, and found that high-frequency rTMS applied to this region resulted in marked symptom improvement, associated with normalization of dlPFC activity (Geller et al., 1997; George et al., 1995). Similarly, early reports of hyperactivity in the right dlPFC in TRD led to application of right-lateralized low-frequency rTMS (Fitzgerald et al., 2009; Isenberg et al., 2005). Overall, investigators reported that dlPFC-rTMS was a safe and well-tolerated technique for achieving antidepressant effects in patients with MDD (George et al., 1995; Kolbinger et al., 1995).

Subsequently, larger randomized controlled trials compared active left dlPFC-TMS to sham TMS. These studies reported significantly higher response rates and better outcomes following active TMS (O'Reardon et al., 2007), with up to 30% of subjects achieving remission in open-label follow-up (George et al., 2010). Taken together with earlier investigations, these findings indicated that dlPFC-rTMS could consistently reduce depressive symptoms (George et al., 1995, 2010; Hoflich et al., 1993; Li et al., 2010a; Lisanby et al., 2009; Pascual-Leone et al., 1996) and outperform sham stimulation in patients with MDD (Baeken et al., 2014; Berlim et al., 2013a; Loo et al., 2007). Evidence for the therapeutic benefit of dlPFC stimulation is bolstered by the more recent finding that applying stimulation to adjacent cortical regions—for example, the frontal eye fields—dramatically reduces or even eliminates antidepressant effects (Johnson et al., 2013).
Current efforts to improve the therapeutic utility of rTMS for MDD focus on illuminating its physiological mechanisms and experimenting with alternative treatment parameters, such as number of treatments per day (Baeken et al., 2014). Additionally, more recent techniques for image-guided neuronavigation have enabled more accurate and precise targeting of a specific dlPFC region, resulting in some improvements in remission and response rates (Daskalakis et al., 2008; Downar and Daskalakis, 2013; Herbsman et al., 2009). In modern studies of patients with TRD who receive rTMS, up to 35% achieve remission and over half (55%) typically demonstrate some degree of response (Fitzgerald et al., 2011; Li et al., 2010a; McDonald et al., 2011).

Nonetheless, a substantial proportion (based on the statistics reported above, up to 45%) of patients who undergo therapeutic rTMS do not demonstrate a clinically significant response (Fitzgerald et al., 2011; Li et al., 2010a; McDonald et al., 2011). Thus, results of rTMS studies in MDD mirror the bimodal ‘responder’ and ‘non-responder’ groups identified in physiological TMS investigations (Maeda et al., 2000; Nettekoven et al., 2015). In these cases, response distributions have been noted to be bimodal (Downar et al., 2014; Fitzgerald et al., 2016) or even trimodal (Bakker et al., 2015). While response may represent physiological amenability to the cortical changes induced by rTMS, it could also be possible that response relies on stimulation of an individually appropriate cortical target.

Overall, targeting different cortical sites with rTMS induces a variety of therapeutic effects that depend on the target site’s function and role in psychiatric illness. While the most common therapeutic target thus far has been the left dlPFC, other cortical targets have recently been evaluated in randomized controlled trials (Fitzgerald et al., 2011; O’Reardon et al., 2007). For example, the dmPFC was suggested as a clinical treatment target due to its integral location at the ‘crossroads’ of cognitive control, reward, and motivation. This region sits within the medial wall of the PFC and serves as a key hub of cognitive processing and emotional self-regulation (Downar and Daskalakis, 2013; Wager et al., 2008). Improvements in depressive symptomology following stimulation of this medial target site are comparable to those of the traditional
dlPFC target, though it remains to be seen if responders to each target site begin with similar patterns of neural dysfunction (Bakker et al., 2015). Alternatively, hyperactivity in the OFC has been proposed to contribute to MDD symptoms (Rolls, 2016). Inhibitory rTMS to the right OFC is associated with a reduction in bilateral OFC metabolism in obsessive-compulsive disorder (OCD) patients (Nauczyciel et al., 2014), and with reduction in functional connectivity from the OFC to the nucleus accumbens (NAcc) in one patient with MDD (Fettes et al., 2017). Randomized, sham-controlled studies using OFC as an rTMS target could provide support for new treatment strategies for MDD patients who are non-responsive to conventional stimulation targets.

1.3.3.1 Use in Treatment-Resistant Depression

As discussed in previous sections, TRD affects up to one-third of individuals with depression, and non-invasive brain stimulation techniques have been introduced as an alternative treatment option (Downar et al., 2016; Rush et al., 2006). Evidence that rTMS can modify functional activity throughout corticostriatal loops has been a driving force in developing rTMS protocols that aim to effect change in specific circuits that underlie affect, cognition, and behaviour regulation (Concerto et al., 2015). Recent studies consistently demonstrate rates of remission and response reaching up to 35% and 55%, respectively (Blumberger et al., 2012; Fitzgerald et al., 2011; Holtzheimer et al., 2010; Levkovitz et al., 2009; Li et al., 2010a; McDonald et al., 2011). These rates are higher compared to older trials of TRD-rTMS, and reflect improvements in aspects such as coil positioning, stimulation target localization, and treatment length (i.e., number of sessions) (Downar and Daskalakis, 2013).

1.3.3.2 Drawbacks to rTMS in MDD/TRD

Following years of research, implementation in clinical populations (George et al., 2010; O’Reardon et al., 2007), and meta-analyses evaluating its clinical utility (Berlim et al., 2013c; Lam et al., 2008), rTMS was approved by both Health Canada and the Food and Drug Administration (FDA) in the early 2000s. Still, access to rTMS has grown more slowly in Canada than in the U.S. The relatively slow adoption of rTMS by provincial
healthcare systems has been partially attributed to high costs, which can reach up to $5,000 CAD for a typical 20-session treatment course in private clinics. A ‘typical’ rTMS protocol requires about 40 minutes of daily stimulation over the course of 4 to 6 weeks. This length allows only around 10 patients to be treated per day per device, reducing clinical space for patients and increasing treatment cost per patient. Thus, there is a need for clinicians to reduce treatment length or equipment costs in order to improve access to the treatment.

Another concern is the relatively modest efficacy seen even in recent studies, with full remission rates under 20% in some major trials (George et al., 2010; O’Reardon et al., 2007). Additionally, conventional stimulation targets like the dlPFC are effective in some patients, but have little or no effect in others. Improving treatment outcomes thus requires a better understanding of the physiological mechanisms that underlie treatment response, and further exploration of alternative stimulation targets that may be more beneficial for certain patient populations.

In sum, extensive work must be undertaken to optimize treatment protocols, patient selection, and patient-protocol matching for rTMS. Drawbacks to existing and conventional treatments have prompted a need for exploration of alternative—and potentially more beneficial and effective—stimulation targets and parameters. In future years, improvements to rTMS could reduce treatment costs, increase the amount of patients treated per device, and optimize clinical response by tailoring individualized treatment targets and stimulation protocols based on a thorough understanding of both clinical and neurobiological predictors of outcome. These improvements can be achieved by (a) more thoroughly investigating the comparative efficacy of a variety of rTMS protocols; and (b) developing predictors of treatment response or non-response based on biological factors or functional neuroimaging.
1.3.4 rTMS Stimulation Protocols in MDD

1.3.4.1 10 Hz Excitatory Stimulation

Improving the clinical utility of rTMS involves evaluating the effectiveness and tolerability of various stimulation protocols. Early experiments that compared the size and direction of rTMS effects on motor evoked potentials reported a frequency-dependent trend of increased excitatory aftereffects when comparing 1 Hz, 10 Hz, 15 Hz, and 20 Hz stimulation (Maeda et al., 2000). Significant facilitatory effects for 10 Hz stimulation emerged only after applying 1600 pulses; for comparison, an experiment that applied 240 pulses only found 20 Hz stimulation to exert significant effects (Maeda et al., 2000). Again, it is important to note that individual subjects still show a great amount of variability with regards to cortical excitability change, despite consistent group-wide effects (Hallet, 2007).

Heterogeneity notwithstanding, 10 Hz stimulation is among the most commonly used excitatory protocol in reported trials of rTMS (Berlim et al., 2014a), and in clinical use. The standard FDA-approved protocol targets the left dlPFC with 10 Hz stimulation for 3000 pulses in 75 trains, 4 s on and 26 s off, over 37.5 min (George et al., 2010; O’Reardon et al., 2007). This protocol has been employed in many studies (for excellent reviews, see Berlim et al., 2013b, 2014). 10 Hz rTMS appears equally effective compared to primary treatment strategies for MDD, with response rates between 29% (Berlim et al., 2014b) and 43% (Berlim et al., 2013c). However, the length of each treatment session—and the number of sessions required to achieve clinically significant effects—are a barrier to this protocol’s utility.

1.3.4.2 1 Hz Inhibitory Stimulation

Early physiological investigations of low-frequency TMS indicated that stimulation close to 1 Hz could produce lasting inhibitory effects on cortical activity (Chen et al., 1997) and on MEPs compared to stimulation at higher frequencies (e.g., 10 Hz, 15 Hz, and 20 Hz) (Maeda et al., 2000). Based on these findings, 1 Hz stimulation has been used to
therapeutically modulate brain regions in which hyperactivity rather than hypoactivity is considered to be central to the pathophysiology of the illness (Eldaief et al., 2013).

Early models of MDD posited that the right dlPFC was hyperactive in MDD, thus providing a rationale for applying inhibitory rTMS to this target (Austin et al., 1992; Baxter Jr. et al., 1989b; Davidson and Irwin, 1999; Drevets et al., 2008). Although subsequent studies called into question the hyperactivity of the right dlPFC in MDD (Biver et al., 1994; Mayberg et al., 2005), empirical studies have nonetheless found 1 Hz right dlPFC-rTMS to be superior to sham stimulation in treating MDD (Brunelin et al., 2014; Fitzgerald et al., 2009; Isenberg et al., 2005). Indeed, meta-analyses (Berlim et al., 2013b) indicate that this protocol generally achieves similar rates of remission and response compared to both the conventional left dlPFC protocol and alternative protocols that combine left and right excitatory and inhibitory stimulation, respectively (Feinsod et al., 1998; Fitzgerald et al., 2003, 2013; Klein et al., 1999). Once again, however, 1 Hz protocols can be hampered by long session lengths as well as heterogeneity of physiological effects (Maeda et al., 2000).

1.3.4.3 Theta-burst Stimulation

In response to the length and response heterogeneity associated with conventional rTMS protocols, Huang and colleagues (2005) developed a new protocol whose effects were consistent, durable, and more rapidly achieved. This protocol, known as theta burst stimulation (TBS), typically applies a ‘burst’ of three 50 Hz pulses at a rate of 5 Hz, and produces either excitatory (intermittent TBS) or inhibitory (continuous TBS) effects on cortical excitability, depending on the on-off cycle (Huang et al., 2005).

Some reports indicate that TBS may be superior to conventional protocols with regards to its ability to induce long-term potentiation of targeted neuronal populations. For example, 40 seconds of continuous TBS is shown to have stronger, longer-lasting inhibitory effects than 15 minutes of 1 Hz stimulation (Di Lazzaro et al., 2011). Similarly, 600 pulses of intermittent TBS (2 s on, 8 s off) produced stronger, longer-lasting excitatory effects than five other conventional protocols in a comparison study (Di
Lazzaro et al., 2011). These superior effects could be due to the similarity of theta burst stimulation to natural theta rhythms in the brain.

Individual sessions of TBS require only one to three minutes to produce comparable physiological effects to those of 10 Hz stimulation sessions, which require almost 40 minutes each (Huang et al., 2005). Implementing more or equally effective TBS-based protocols in MDD would reduce both cost and time of treatment, and recent studies have provided evidence that TBS is effective in treating MDD (Chistyakov et al., 2010; Holzer and Padberg, 2010). For example, one study found both left-lateralized iTBS and right-lateralized cTBS to improve depressive symptoms in patients, suggesting that each protocol restored interhemispheric balance of dIPFC activity (Chistyakov et al., 2010). Similarly, a smaller case series reported MDD symptom reduction following left dIPFC-iTBS (Holzer and Padberg, 2010).

There is less evidence available on the comparative efficacy of theta-burst rTMS versus conventional protocols for the treatment of major depression; however, some recent randomized controlled trials have compared theta-burst protocols to sham stimulation. For example, a recent study confirmed that active iTBS, or combined iTBS and cTBS, was more effective than sham stimulation (Li et al., 2014). In addition, one large non-randomized case series compared clinical outcomes for dmPFC-iTBS and 10 Hz rTMS, finding no significant differences between treatment groups (Bakker et al., 2015). Thus, to date, few studies have directly compared TBS to conventional rTMS protocols in MDD, and there have been no randomized controlled non-inferiority trials to compare iTBS to the conventional, FDA-approved 10 Hz protocol.

1.3.5 Predicting rTMS Treatment Outcome

Aside from optimizing the stimulation site and stimulation protocol, another strategy to improve the clinical utility of rTMS is to develop reliable ways to predict which patients will successfully respond to treatment. As noted earlier, discernible subpopulations likely exist within populations of MDD patients who undergo rTMS: distinct responder and non-responder groups have been identified across a variety of rTMS protocols (Bakker
Early prediction or identification of rTMS responders and non-responders could potentially improve outcomes by sparing non-responders from undergoing unnecessary weeks of treatment. It could be possible to distinguish these populations by using clinical or neurobiological measurements.

Treatment outcome can potentially be predicted by clinical, genetic, or physiological markers. For example, one study noted that dmPFC-rTMS non-responders were distinguished from responders by severity of anhedonia (Downar et al., 2014). Additionally, a recent systematic review identified several neurobiological factors that correlate with rTMS treatment outcome in MDD (Silverstein et al., 2015). Though a comprehensive survey of the extensive literature on non-neuroimaging predictors of rTMS response lies outside the scope of this review, in brief, some reported predictive markers include: genetic polymorphisms of serotonin genes; baseline levels of cortisol, estrogen, and progesterone; and regional cerebral blood flow.

Functional imaging techniques are also potentially useful tools for assessing predictors of treatment outcome (Crowther et al., 2015; Grammer et al., 2015; Silverstein et al., 2015), and for evaluating potential functional subtypes of MDD that may be characterized by unique activity differences in cognitive networks, affective circuits, and frontostriatal loops (Downar et al., 2014; Lener and Iosifescu, 2015; Webb et al., 2015). Changes in network function following targeted therapeutic interventions can also be visualized using various neuroimaging techniques, and emerging evidence points to the importance of specific functional connections in predicting response. Here, we briefly review functional imaging predictors as outlined by Silverstein et al. (2015).

rTMS response is associated with pre-treatment global brain metabolism. For example, Kimbrell et al. (1999) found that response to high-frequency rTMS is significantly associated with lower baseline global metabolism; specifically, response is correlated with lower baseline metabolism in the left parahippocampal gyri and left fusiform gyri (Li et al., 2010b). Similarly, Speer et al. (2000) found that response to low-frequency rTMS
is associated (albeit not significantly) with higher baseline global metabolism. More specifically, response is correlated with higher baseline metabolism in the medial prefrontal cortex, rostral anterior cingulum (Li et al., 2010b), dIPFC, and anterior cingulate cortex (ACC) (Baeken et al., 2009).

Interestingly, findings involving the dIPFC as a response predictor are mixed. Increased post-treatment regional cerebral blood flow in the left inferior frontal lobe is associated with response to left dIPFC-rTMS (Kito et al., 2008b; Mottaghy et al., 2002; Teneback et al., 1999). Conversely, Kito et al. (2012) found no correlation between dIPFC activity and treatment response, Paillere Martinot et al. (2011) found no relationship between treatment response and dIPFC baseline hypoactivity, and Weiduschat and Dubin (2013) found that treatment responders showed hyperactivity in the left dIPFC. This region may be more useful as a predictor when evaluated in conjunction with activity of the ventromedial PFC (vmPFC), a node of the reward network. For example, Kito et al. (2012b) found that treatment response was associated with a lower ratio of dIPFC/vmPFC activity. Alone, baseline activity in the vmPFC correlates with subsequent treatment response (Kito et al., 2012a); additionally, Downar et al. (2014) found that non-responders to dmPFC-rTMS were characterized by abnormal baseline resting-state functional connectivity to the vmPFC. This pattern has also been observed in the vlPFC based on whole brain SPECT (Kito et al., 2008a).

There have also been mixed findings with regards to the insular and orbitofrontal cortex (OFC) as a predictor of rTMS response. Kito et al. (2012a) found that left OFC activity was positively correlated to subsequent treatment response; the same study found that decreased blood flow in the anterior insula, near the OFC, was associated with symptom improvement following high-frequency left dIPFC-rTMS (Kito et al., 2012a).

Functional predictors of response have also emerged in limbic and subcortical regions. For example, Furtado et al. (2013) and Kito et al. (2008b) found that improvement following either high-frequency left, or bilateral left and right, rTMS, was predicted by lower baseline volume, but higher baseline activity, of the left hippocampus. Nadeau et
al. (2002) demonstrated that responders showed decreased baseline blood flow in the left amygdala. Finally, Mottaghy et al. (2002) and Richieri et al. (2011) found that non-responders to high-frequency left dlPFC-rTMS were characterized by hypoperfusion in the thalamus, with outcome negatively correlated to regional cerebral blood flow.

1.3.6 Summary

Randomized controlled trials, meta-analyses, and treatment guidelines support rTMS as a safe and effective treatment for individuals with TRD. Still, conventional rTMS protocols are expensive, lengthy, and achieve non-normal clinical outcomes that include a large proportion of non-responders. The comparative efficacy of newer theta-burst protocols, which could increase the cost-effectiveness of non-invasive brain stimulation treatments for TRD, is promising but has yet to be adequately tested in large-scale, randomized controlled trials. However, even when shorter rTMS protocols are adopted, a proportion of patients still fail to achieve response or remission. Surmounting this obstacle requires further investigation of clinical and neurobiological factors that predict treatment response. Existing evidence points to the use of functional imaging modalities to distinguish and predict treatment outcomes; however, to date, no major trials have incorporated fMRI data collection for the purpose of identifying and comparing predictors and correlates of treatment response across different stimulation protocols.

Functional network connectivity holds promise as a potential biomarker for predicting treatment response. The following sections evaluate functional networks that are relevant to MDD, and review methods for transforming raw functional data into clinically meaningful markers of treatment response.

1.4 Functional Neuroimaging and Functional Brain Networks

1.4.1 Overview

Recent advances in brain imaging methodology have encouraged detailed exploration of the structural and functional basis of psychiatric illness in terms of network-level brain dysfunction. Our understanding of psychiatric illness has benefited from such
exploration, because neuroimaging enables psychiatric disorders to be visualized as abnormalities in the architecture of structural or functional networks. In brief, such networks of brain regions can be conceptualized as specific anatomical regions, or 'nodes,' linked by structural/functional connections or 'edges' that may not only connect functionally related nodes within a network, but may also serve as 'interstates' conveying information between functionally distinct brain nodes or networks (Sporns and Betzel, 2016). Dysfunction in any node or edge is thought to disrupt the overall functional architecture of the brain, either locally within the aberrant network itself or more diffusely across interconnection between networks throughout the brain (Menon, 2011). It is through such node or edge dysfunction that behavioural or psychiatric abnormalities are proposed to arise (Menon, 2011).

1.4.2 Functional and Effective Connectivity

Unlike structural connectivity, which refers to the anatomical links between neurons and brain regions, functional connectivity refers to the relationships of temporal activity among neurophysiological events (Friston, 1994). Two regions of the brain are considered to be functionally connected if statistical dependencies exist between their activity (Friston, 2011); for the purposes of functional neuroimaging, this is often operationalized in terms of whether the time courses of a pair of voxels or regions have high cross-correlation coefficients (Cordes et al., 2001). Effective connectivity takes this concept a step further by describing the causal influence that networks exert on other networks within a larger model of interactions (Friston, 2011). This can be explored using hypothetical models—for example, structural or dynamic causal models—that represent a possible set of influential relationships among brain networks (Friston, 2011).

1.4.3 Summary of Techniques for Identifying Functional Networks

Neuroscientists aim to map the brain’s functional and anatomical connections—the ‘human connectome’—to understand in detail the neural mechanisms that underlie complex cognition, emotion, and behaviour (Craddock et al., 2012; Sporns et al., 2005).
Functional neuroimaging can localize patterns of neural activity via a variety of markers of neuronal firing, ranging from indirect markers such as glucose metabolism to more directly recorded electrical field potentials or action potentials. Resting state brain networks can be studied non-invasively via several modalities, including positron emission tomography (PET), electroencephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI), which permit varying degrees of spatial and temporal resolution (Haber and Knutson, 2009).

PET imaging measures brain activity indirectly via localized changes in blood flow or metabolism. Tracer substances that emit gamma rays are first injected into the bloodstream, and then visualized upon uptake into the brain. Common tracers for functional neuroimaging with PET include H$_2$O$^{15}$-PET, for indexing blood flow, and $^{18}$fluorodeoxyglucose (FDG), for indexing metabolic activity. Blood flow is increased transiently and locally in association with increases in neural activity (Logothetis and Wandell, 2004a), so higher H$_2$O$^{15}$-PET emissions detected in various brain regions are associated with transiently higher neural activity. Metabolic activity can be measured via FDG-PET, over longer timescales of around one hour.

MEG and EEG both take advantage of electrical signals produced by coordinated neuronal activity. EEG electrodes, placed in a montage of 64-256 sensors arranged over the scalp and head area, can detect the electrical fields generated by large ensembles of neurons with synchronized activity. Since the electrical fields propagate through the tissues of the brain, cerebrospinal fluid, scalp, and head in complex ways, it can be challenging to localize the EEG sources with accuracy. However, the temporal resolution of these modalities is in the millisecond range, allowing characterization of networks linked by high-frequency (>40-100 Hz) oscillations. Spatial localization can be somewhat improved using MEG, which employs an array of magnetic field detectors of high sensitivity rather than scalp electrodes. Since the tissues of the brain, skull, and scalp are transparent to magnetic fields, source localization is more straightforward and temporal resolution of network activity remains high.
fMRI takes advantage of the physiological properties of deoxyhemoglobin as an endogenous tracer, sensitive to a combination of blood flow and blood oxygenation. This technique offers spatial resolution equal or superior to PET, without exposure to radiotracers, and is suitable for studying functional networks whose activity is most apparent in low-frequency (>10 s) fluctuations. fMRI is explored in greater detail in the next sections.

1.4.4 fMRI and Resting-State Functional Connectivity

Historically, investigators who observed oxygen consumption in brain tissues during tasks and at rest noticed little difference between these states, implying that even the 'resting' brain maintained a high level of neuronal activity (Sokoloff et al., 1955). The last 20 years have produced a substantial literature on the nature of this resting brain activity, as observed through a variety of techniques, of which one of the most commonly used is functional magnetic resonance imaging (fMRI).

Resting state functional magnetic resonance imaging (rs-fMRI) allows for the visualization of widespread brain activation in the absence of tasks or directed activities. This technique, which is described in greater detail below, is ideal for large samples of individuals, or for patients or other populations for whom task performance may be challenging, because it is a relatively straightforward and time-effective procedure that generates useful data with a high degree of spatial resolution (Fox and Greicius, 2010b). In particular, given recent observations that the functional architecture of the brain at rest closely resembles that of the brain during a variety of different tasks (Cole et al., 2014, 2016), resting-state fMRI may serve as a logistically straightforward yet informative tool for studying the functional architecture of the human brain in health and disease.

1.4.5 fMRI and the BOLD Signal

Magnetic resonance imaging (MRI) relies on the physical principles of radio frequency waves that interact with the spin properties of hydrogen nuclei in biological tissues in a
magnetic field. MRI devices generate a high strength magnetic field, which first aligns and harmonizes the spin axes of hydrogen nuclei that are present within water molecules in the brain. Next, a radio frequency wave pulse is generated toward the subject, which pushes the aligned nuclei into a higher energy state. Following the pulse, the nuclei re-emit radio signals as they relax into their initial, lower-energy alignment, in a manner that depends on the magnetic field properties of brain tissue itself.

Of particular significance is the measurement of transverse relaxation, T2, referred to as T2* in the presence of local field inhomogeneities (for example, in brain tissue) (Logothetis and Wandell, 2004a). A key insight in the development of functional MRI is that deoxyhemoglobin is a paramagnetic substance, acquiring magnetization when placed in a magnetic field (Ogawa et al., 1990). As such, tissues with greater concentrations of deoxyhemoglobin have less local homogeneity of the magnetic field, and thus a lower T2* value. Deoxyhemoglobin can thus act as an endogenous contrast agent, sensitive to blood flow and blood oxygenation, on MRI images acquired using a sequence sensitive to T2* (Ogawa et al., 1990). This property is known as the blood oxygenation level dependent (BOLD) effect (Logothetis and Wandell, 2004b).

Functional magnetic resonance imaging (fMRI) takes advantage of the natural BOLD signal by detecting fluctuations of oxygen concentration in brain regions. The BOLD signal reflects the magnetic properties of blood and represents the ratio of oxygenated, diamagnetic blood to deoxygenated, paramagnetic blood. When a brain region becomes active, it receives an oversupply of oxygen through an increase in cerebral blood flow, which locally increases the proportion of diamagnetic blood molecules and changes the magnetic properties of the active region in relation to surrounding regions (Logothetis and Wandell, 2004b). Since neural activity is closely coupled to blood flow and oxygenation at the local level via the mechanisms of cerebral blood flow autoregulation (Paulson et al., 1990), the BOLD effect may be used as an indirect marker of neural activity (Logothetis, 2007). Unlike earlier imaging methods such as H2O15-PET, BOLD functional MRI does not require exposure to a radioactive tracer.
agent, and whole-brain image volumes may be acquired in much shorter timeframes, on the order of two seconds.

1.4.6 Acquisition

fMRI is acquired in an MRI scanner with a field strength ranging from 1.5 to 7 Tesla (Logothetis and Wandell, 2004b). In contrast to task-based paradigms, which require subjects to engage in a directed activity while in the scanner, resting state fMRI (rs-fMRI) paradigms require that subjects are awake, still, and engaged in natural, ongoing thought. Sessions typically require five to ten minutes, and eyes may be open or closed (Cole et al., 2010).

Acquiring fMRI data involves the selection of a series of parameters that impact data quality, including number of volumes, voxel size, and image resolution (Smith, 2004). ‘Volumes’ represent the number of whole-brain images recorded during a scan. Each volume is comprised of ‘voxels,’ or volume pixels, which capture functional information from a particular spatial location, and are often only a few millimeters in width, height, and depth. Thus, for example, acquisition of 100 volumes results in the collection of 100 functional ‘snapshots’ of the brain over the course of the scan, and a particular voxel \((x, y, z)\) in each volume represents the time series of spatial location \((x, y, z)\) in the brain over the course of 100 snapshots. Other important parameters include the number of slices per volume, thickness of slices, and time between volumes (Smith, 2004). Temporal and spatial resolution of fMRI data will vary depending on the values of these parameters.

1.4.7 Analyzing fMRI Data

Resting-state functional connectivity analysis involves subjecting the raw T2* image slices acquired on the scanner to a ‘pipeline’ of information processing steps, with the pipeline varying somewhat depending on the goal of analysis. Making raw fMRI data suitable for any analyses first requires preprocessing; ordinarily, this is followed by the computation of single-subject functional connectivity maps based on the brain activity
acquired for each individual at each session. This is followed by a second-level analysis comparing these functional connectivity maps across subjects (Cole et al., 2010).

1.4.7.1 Preprocessing

The T2* images acquired during functional MRI contain not only the neural activation signals of interest, but also a variety of noise sources. Thus, a set of preprocessing steps must be implemented in order to remove or minimize the effects of sources of noise in the data, which can include physiological artefacts (e.g., respiration, effects of cardiac activity), movement (e.g., head rotation or translation), or signal distortion (e.g., from an implanted medical or orthodontic device). These ‘noise’ signals can confound data analysis and interpretation by introducing spurious correlations that amplify estimates of functional connectivity among various brain regions (Birn et al., 2006, 2008). Notably, spurious correlations can be generated due to ‘regionally-specific noise’ introduced by signals from white matter (WM) and cerebrospinal fluid (CSF) (Cole et al., 2010; Fox et al., 2005). Thus, readying fMRI data for analysis requires preprocessing to separate nuisance sources of signal from true brain activity.

Preprocessing can be conducted with a variety of open-source software toolboxes available to the neuroimaging community, including FSL (Jenkinson et al., 2012), CONN (Whitfield-Gabrieli and Nieto-Castanon, 2012), and AFNI (Cox, 1996). Shared among these tools are specific steps that are generally accepted as necessary for fMRI data preprocessing, including frequency filtering and motion correction. Each of these steps aims to make adjustments to the raw functional data to render a more accurate representation of intrinsically generated BOLD signal fluctuations. Data is often filtered to exclude signal frequencies above 0.1 Hz, which represent signals generated from respiration (0.1 – 0.5 Hz) and cardiac activity (0.6 – 1.2 Hz) (Cordes et al., 2001). Signal frequencies below 0.01 Hz may also be excluded, because functionally relevant BOLD fluctuations are generally characterized by inclusion within the 0.01 – 0.1 Hz frequency range (Cole et al., 2010).
Head motion can also introduce error into fMRI time series, by two mechanisms: first, by relocating the structures of the brain such that a given voxel does not correspond to the same region of the brain through the entire time series; and second, by generating signal fluctuations as the excited spins move in or out of the slice of tissue from which the image is being acquired. Correcting for motion involves transforming each image volume into a common space and making necessary adjustments to account for motion-induced signal fluctuations (Friston et al., 1996). To account for variation in head motion, individual scans must be examined for excessive movement in any plane. Subjects whose head motion significantly exceeds the mean of a group of subjects, or whose excessive head motion generates observable image artefacts, may be excluded from further analysis. In addition, because some psychiatric disorders are associated with psychomotor retardation (e.g., MDD) or agitation (e.g., anxiety disorders, attention deficit hyperactivity disorder), it is in some cases advisable to examine whether there are differences in head motion between individuals being compared at the second level; such differences could potentially introduce spurious correlations into the group-wide analyses.

Additionally, because signal fluctuations are often seen in white matter and cerebrospinal fluid during fMRI acquisition, and because these fluctuations may also introduce noise into the signals acquired from tissues of interest (i.e., the cortical gray matter), steps must be taken to remove nuisance signals originating from WM and CSF. One way to accomplish this is by applying anatomical components-based noise correction (aCompCor) (Behzadi et al., 2007). This approach identifies WM and CSF voxels and extracts their time courses, then applies principal components analysis (PCA) to generate a set of the five principal components accounting for the largest amount of variance within the WM and within the CSF. Finally, aCompCor uses a general linear model to regress the time courses of these components from the acquired data across the whole brain (Behzadi et al., 2007).
1.4.7.2 Seed-Based Correlation Analysis

After preprocessing is complete, rs-fMRI functional connectivity maps can be generated. One approach to measuring single-subject rs-FC is with seed-based correlation analysis (SCA). SCA requires \textit{a priori} selection of a region of interest (ROI), which may be a voxel, sphere, or predetermined region drawn from a structural or functional atlas (Cole et al., 2010). Generally, the time course of the ROI—which, in the case of a multi-voxel region, is calculated as the mean time course across all voxels in the ROI—is extracted and included as a regressor in a general linear model, often incorporating the 'nuisance' or noise regressors above. The resultant whole-brain correlation maps visualize voxels whose time courses of activity are correlated—and therefore inferred to be functionally connected—to the ROI (Cole et al., 2010).

1.4.7.3 Independent Component Analysis

Independent component analysis (ICA) was introduced to neuroimaging research in the late 1990s as a tool for separating functional components of resting-state data (McKeown et al., 1998). This technique statistically separates functional data into a set of spatially and temporally independent and uncorrelated components (Beckmann and Smith, 2004). ICA is not only useful for identifying sources of artefacts and noise that can be subsequently removed from the data, but also for isolating task-activated or resting-state functional networks of interest that can be explored in additional analyses (Greicius et al., 2007; McKeown et al., 1998).

When applied to fMRI data, ICA generates spatially—or temporally—independent components from a series of functional image volumes across a single subject or across a group of subjects, temporally concatenated. Each of the resultant ‘independent components’ represents an independent source of variance within the 4-dimensional whole-brain time series (Jenkinson et al., 2012). These sources of variance are not necessarily attributable to brain activity; based on their spatial distribution, some components can be attributed to motion, nuisance sources of noise such as those described above, eye movements, or other contributors to the patterns of change in T2*
signal over time across the brain. However, other components correspond well to the anatomical distribution of the DMN, central executive network (CEN), salience network (SN), ventromedial network (VMN), and other established RSNs within the brain. These components can then be studied at the individual level to assess their activity over time, or at the group level to determine how their temporal activity or spatial distribution differs across individuals.

ICA is a data-driven approach, as it does not require *a priori* selection of regions of interest; additionally, the default ICA settings also allow the number of output components to be driven entirely based on the data itself. However, this number can also be input manually prior to analysis, thus pre-determining the number of unique components detected (Uddin et al., 2008). Using ICA to compare different groups (e.g., two treatment groups) can introduce some subjectivity. Comparing component output between groups may be difficult, since the voxels included per component depend on unique variability within each group (Uddin et al., 2008). Temporal concatenation of the time series for all individuals of interest can ensure that the components identified by ICA are derived from all of the data in the population under study.

1.4.8 Functional Network Parcellations

Functional neuroimaging techniques such as fMRI are powerful tools that can be used to unravel the intricate relationships among brain regions, and deduce links between functional activity and behaviours. This is especially meaningful in the context of psychiatric illnesses, in which maladaptive and dysfunctional modes of behaviour, cognition, and affect can be linked to specific neurobiological substrates, which can in turn help to guide treatment strategies that target the relevant dysfunctional regions or circuits (Diekhof et al., 2008).

The notion that complex behaviour and functions are coordinated by spatially distributed brain regions is not new; however, early postulations of this idea faced technical limitations to gathering supportive evidence (Bressler, 1995). Now, in the contemporary context of neuroimaging, spatially distributed brain areas are considered functionally
connected when activity fluctuations between single voxels are correlated. By examining patterns of correlation among brain regions over time, the ongoing activity of the brain as a whole can be parsed into consistent, replicable functional networks; if these are detected when the brain is in a resting state, the networks are referred to as ‘resting state networks’ (RSNs), or ‘intrinsic connectivity networks’ (ICNs) (Seeley et al., 2007; Greicius et al., 2003). However, recent work suggests that a similar set of networks may also be recovered from ongoing brain activity during performance of a wide range of active tasks (Cole et al., 2014). The brain regions comprising any given RSN are thought to coordinate their activity because they operate in synchrony to serve a particular function (Cole et al., 2010).

Advances in data-driven fMRI modeling have led to the identification of an increasingly stable set of functional networks that can be identified consistently across individuals (Beckmann et al., 2005; Cole et al., 2010; Damoiseaux et al., 2006; Fox and Raichle, 2007). One of the earliest described resting state networks was in the motor cortex (Biswal et al., 1995). This landmark discovery has been followed by the identification of several other major resting state networks, including the default mode network (Raichle et al., 2001) and executive and cognitive control networks (Bressler and Menon, 2010).

To characterize whole-brain activity based on RSNs, large-scale analyses of cortical activity have generated reliably reproducible divisions of functional networks and subnetworks (Choi et al., 2012; Yeo et al., 2011). One influential study used functional brain activity to generate a functional cortical atlas (Craddock et al., 2012); another reported a stable division of whole-brain connectivity into seven or 17-network parcellations (Yeo et al., 2011). Certain networks, including the visual, motor, and somatosensory networks, primarily serve a specific function and are confined to associated brain regions (i.e., the visual, motor, and somatosensory cortices). Other networks that support cognitive, behavioural, and affective processes are more widely distributed across the cortex, but can nonetheless be reliably identified in individuals. For example, the ‘attentional network’ can be separated into discrete dorsal and ventral
attention networks, comprising the dorsolateral and mediolateral frontal and temporal regions, respectively (Vossel et al., 2014).

Reproducible parcellations of RSNs in combination with anatomical maps provide a framework for understanding the functional architecture of the brain, and offer a roadmap for selecting functional networks of interest to psychiatric illness. Before exploring functional networks relevant to MDD, we will first review the anatomical context in which RSNs operate.

1.4.9 Cortico-Striatal-Thalamo-Cortical Loop Circuits

Cortico-striatal-thalamo-cortical (CSTC) circuits span the cortex, striatal nuclei, and thalamus, and underlie complex functions and behaviour. These functional circuits were classically described by Alexander et al. (1986), who identified five parallel, functionally and anatomically segregated circuits based on observations in non-human primates. These CSTC loops included: a motor circuit through the supplementary motor area, putamen, ventrolateral globus pallidus internal (GPi), and ventrolateral thalamus; an oculomotor loop through the frontal eye field, body of the caudate nucleus, caudomedial GPi, and lateral ventral anterior thalamus; a lateral orbitofrontal loop through the lateral orbitofrontal cortex (IOFC), ventromedial caudate, mediodorsal GPi, and medial ventral anterior thalamus; a dorsolateral prefrontal loop through the dlPFC, dorsolateral caudate, lateral dorsomedial GPi, and parvocellular mediodorsal thalamus; and an anterior cingulate loop through the dorsal anterior cingulate cortex (dACC), ventral striatum, ventral pallidum, and posteriomedial mediodorsal thalamus.

Though informative, the CSTC loops described by Alexander et al. (1986) are likely not an exhaustive set. Other CSTC circuits probably exist, and are being explored in detail using functional neuroimaging techniques. For example, a recent 1000-subject functional parcellation of the human striatum (Choi et al., 2012) revealed that additional, distinct subregions of the striatum were uniquely associated with resting-state cortical networks as described by Yeo et al. (2011). Importantly, these findings demonstrate that RSN parcellations can also capture subcortical activity associated with cortical network
hubs (Choi et al., 2012). With this information, studies can delve further into understanding cortico-striatal network interactions (Cole et al., 2010).

The following section explores the specific anatomy and function of several consistently identified resting-state CSTC networks, and examines their relevance to psychiatric disease.

1.5 Networks Implicated in Major Depression

1.5.1 Overview

Advances in both anatomical and functional neuroimaging techniques have contributed to our understanding of the role of network dynamics in healthy brain function. Dysfunction in certain networks is linked to psychiatric illness. Conventionally defined diagnostic categories of psychiatric disorders, such as depression and schizophrenia, are characterized by distinct constellations of symptoms. However, structural and functional abnormalities in similar sets of nodes and networks may appear transdiagnostically across a variety of conventional categories of psychiatric disorders (Downar et al., 2016). For example, the anterior cingulo-opercular network, also known as the salience network (SN), shows consistent abnormalities in structure and function across a variety of psychiatric illnesses including MDD, schizophrenia, obsessive-compulsive disorder, anxiety disorders, and substance use disorders (Goodkind et al., 2015).

It is likely that a spectrum of functional network aberrations, ranging from hypo- to hyperactivity through CST loops, can trigger a broad range of cognitive and behavioural symptoms that contribute to psychiatric symptomatology. As such, it is worth reviewing what is presently known about the contributions of the major functional networks to healthy brain activity, as well as how these networks are thought to contribute to psychiatric pathology.
This section provides an overview of several functional networks relevant to MDD. First, the default mode network (DMN) and central executive network (CEN) are briefly reviewed. Next, the anatomy and function of the salience network (SN) and ventromedial network (VMN), the two primary networks of interest in this study, are explored in depth. To address the relevance of these networks to MDD, two final subsections summarize (a) network abnormalities that are observed in MDD, and (b) the effects of left dlPFC-rTMS on resting-state functional networks.

1.5.2 Default Mode Network

The default mode network (DMN), alternatively deemed the ‘task-negative network,’ is the most metabolically active of the brain’s RSNs, and was among the first resting-state network to be identified using functional neuroimaging techniques (Fox et al., 2005; Greicius et al., 2003; Raichle et al., 2001). The DMN was identified based on the observation that activity in a set of spatially distributed cortical regions, notably regions of the prefrontal cortex, was low during external task engagement, and high in the absence of an external task (Mazoyer et al., 2001; Raichle et al., 2001; Shulman et al., 1997). Further investigation revealed that the DMN is also relevant to internally-directed and self-referential thought processes, such as autobiographical memory, prospection, and theory of mind (Buckner et al., 2008).

Anatomically, the DMN spans the posterior cingulate cortex (PCC), precuneus, inferior parietal lobule, and ventromedial prefrontal regions (Dutta et al., 2014). Evidence from ICA-driven analyses suggests that the DMN can be divided into anterior (centered on the mPFC) and posterior (centered on the PCC) sub-networks, which activate synchronously but may have different functional roles (Andrews-Hanna et al., 2010; Buckner et al., 2008). While the anterior DMN has dense projections to the limbic system and is implicated in self-referential and emotional processing, the posterior DMN has connections to the hippocampus, which tie it to memory processes (Andrews-Hanna et al., 2014; Cavanna & Trimble, 2006; Leech & Sharp, 2014). Together, the sub-networks of the DMN underlie spontaneous, internal, self-referential cognitive processes (Mulders et al., 2015). Though speculation of the role of the PCC is ongoing
(Leech et al., 2012), prevailing theories characterize it as a hub of internally directed cognition (Raichle et al., 2001). Within the context of other functional networks, smaller dorsal subregions of the PCC have been shown to correlate functionally with cognitive control networks (Leech et al., 2011). Of note, the DMN’s relationship to other RSNs places it in a peripheral, rather than central, location within the overall functional architecture of the brain (Power et al., 2011). Graph theoretical analyses of resting-state connectivity suggest that the DMN acts as a ‘processing system,’ analogous to sensory and motor systems, and receives ‘input’ from ventromedial prefrontal regions; though it is classically described as a ‘hub’ RSN, it in fact appears to function in relative segregation from most other RSNs (Power et al., 2011).

The DMN activates most strongly at rest and underlies self-reflection, introspection, and internal monitoring (Buckner et al., 2008). Based on observations in control subjects without psychiatric illness, the DMN activates during periods of introspection and mind-wandering (Andrews-Hanna, 2012), and while incorporating memories and associations into self-referential reflections on goals, motivation, and future events (Buckner et al., 2008; Schacter et al., 2007).

1.5.3 Central Executive Network

The central executive network (CEN), also referred to as the ‘task-positive network’ and the frontoparietal control network (Petrican et al., 2015; Sheline et al., 2010), is integral to goal-directed behaviour (Fox et al., 2005). The CEN is characterized by activity during effortful and externally-directed tasks that require attention or executive function (Bressler and Menon, 2010); thus, the CEN tends to anti-correlate with the DMN, the ‘task-negative’ network (Fox et al., 2005). This opposing relationship with the DMN reflects the CEN’s involvement in externally—rather than internally—focused cognition and behaviour (Fox et al., 2005; Greicius et al., 2003).

Anatomically, the CEN includes regions in the frontal (dIPFC, precuneus) and parietal (intraparietal sulcus, inferior parietal lobule) lobes. It is implicated in top-down regulation of externally focused executive functions, including attention, working memory, and
decision making (Sheline et al., 2010). Specifically, connections between the lateral PFC, anterior inferior parietal lobule, medial superior PFC, and anterior insula allow flexible control of goal-oriented behaviour by coupling executive control with attentional and ‘task-negative’ networks (Petrican et al., 2015). The CEN engages to switch and initiate tasks, mediate error responses, and adjust behaviour in response to feedback (Fair et al., 2007).

The CEN has strong functional integrity—that is, high levels of functional connectivity—among its nodes, but weaker cross-connectivity with other networks involved in cognitive control (e.g., the salience network, described in detail below) (Fair et al., 2007). This suggests that executive and cognitive control networks may have distinct functional and temporal properties that work synchronously during tasks that require top-down control. Thus, CEN and related frontoparietal networks comprise a ‘task-positive network’ (TPN) that is involved in externally directed thoughts and behaviour. In contrast to the internally focused default mode network, the TPN activates during goal-directed behaviour and externally focused cognitive activity, and represents a system of networks devoted to task control (Downar et al., 2016; Fair et al., 2007).

1.5.4 Salience Network

Another brain network of particular psychiatric significance is the anterior cingulo-insular network (aCIN), or salience network (SN). Early event-related fMRI studies identified a set of cortical regions that activated in response to the presentation of novel stimuli, indicating the existence of a functional network responsible for evaluating salient sensory stimuli (Downar et al., 2001, 2003). Later, this salience-detection network was explicitly distinguished from other executive control networks (Seeley et al., 2007), and further reports implicated the network in task-switching, cognitive control, and redirection of thought (Menon, 2011). The SN primarily serves to integrate and filter salient internal and external stimuli (Menon, 2011; Seeley et al., 2007); more specifically, the SN may coordinate appropriate functional brain networks to guide behavioural responses to motivationally salient stimuli (Downar et al., 2016; Yeo et al., 2011).
The SN extends across brain regions with a diversity of functions, ranging from emotional responses in the limbic circuitry to reward and motivational responses in dopaminergic brainstem regions (Menon, 2011). Nodes of the SN are implicated in cognitive control processes both individually and synchronously: for example, the dlPFC is a classical hub of executive functioning networks (Kuo and Nitsche, 2012), but also has extensive projections to striatal nuclei and is involved in top-down modulation of goal-directed behaviour (Furman et al., 2011). The SN likely plays a transdiagnostic role in the pathophysiology of MDD and several other psychiatric illnesses due to its key role in modulating cognitive control and goal-directed behaviour (McTeague et al., 2016); notably, pathophysiology in certain cortical nodes of the SN is consistently implicated across a variety of psychiatric disorders (Goodkind et al., 2015). The next two sections review the anatomy and function of this network in greater detail.

1.5.4.1. SN Anatomy

The SN’s primary cortical nodes include the dorsal anterior cingulate cortex (dACC) and the bilateral anterior insula (AI) (Downar et al., 2016). Keyword searches in the meta-analytic neuroimaging tool Neurosynth reveal that these regions overlap with areas integral to cognitive control, response selection, and response inhibition (Downar et al., 2016; Yarkoni et al., 2011). Subcortical SN components include regions of the dorsal striatum, mediodorsal nucleus, and dopaminergic brainstem nuclei (Menon, 2011). These structures complete a discrete cortico-striatal-thalamic (CST) loop that in concert coordinates associated attention to motivationally salient stimuli and engages cognitive control.

Subcortically, the dACC projects primarily to the caudate nucleus and putamen, overlapping with frontostriatal projections from the dlPFC (Haber, 2016). Strengthened resting state functional connectivity between the AI and dACC has also been associated with enhanced cue reactivity in other brain areas including the putamen, suggesting that functional connections throughout the loop allow incoming information to exert downstream effects on modulatory striatal areas (Janes et al., 2015).
Dopaminergic regions also play a significant modulatory role in the SN cortico-striato-thalamo-cortical loop. Dopamine is integral to SN function: for example, the ventral tegmental area (VTA) and substantia nigra play important roles in SN activity and modulation. These midbrain dopaminergic neurons are integral to CST loops that encompass integrated learning, executive function, and motor control. Importantly, studies of the mesolimbic and nigrostriatal dopaminergic pathways has implicated both of them in the encoding of ‘saliency prediction errors’ (Haber, 2016). Dopamine projections throughout the SN also have a function in reward-oriented learning, goal-directed behaviour, and processing motivationally salient stimuli by directing attention to positive, adaptive, or rewarding environmental stimuli (Berridge, 1996; Koob and Volkow, 2010; Kroemer et al., 2014).

As a complete regulatory circuit, the SN CST loop links cortical and subcortical regions involved in cognition, attentional control, motivation, and motor control, and has been implicated as a cortical input ‘filter’ that selectively processes stimuli on which to base cognitive and behavioural responses (Choi et al., 2012; Furman et al., 2011). The dIPFC primarily projects to the dorsal caudate to serve executive functioning (Furman et al., 2011), while the MDN, a thalamic nucleus that supports cortico-cortical information transfer, modulates cortical activity through extensive connections with the PFC and dopaminergic regions (Mitchell, 2015). Dopaminergic nuclei in the midbrain and brainstem, which are often associated with limbic circuitry, demonstrate looser organization than cortical regions, both structurally and functionally. For example, the substantia nigra has extensive connections to the striatum, cortex, thalamus, and neighbouring brainstem regions, allowing dopaminergic outputs to exert far-reaching effects on the flow of CST information (Haber, 2016). In summary, subcortical nuclei within the SN CST loop communicate extensively with cortical areas to select motivationally relevant stimuli, thus guiding cognition and responsive behaviours.
1.5.4.2 SN Function

Functional imbalances within the SN CST loop appear to impair cognitive control, and specifically may impair the self-regulation of cognition, behaviour, and emotion, thereby leading to symptoms of psychiatric illness (Peters et al., 2016). Dysfunction within the SN CST loop, particularly within frontal cortical areas and associated striatal regions, has been suggested to underlie psychiatric symptoms involving impaired regulatory control of negative thoughts and behaviours (Mink, 1996). Recent meta-analyses have identified SN cortical regions with consistent structural and functional abnormalities in a wide range of neurobiological and psychiatric disorders (Downar et al., 2016; Goodkind et al., 2015). In particular, abnormal structure and function in the dorsal anterior cingulate cortex (dACC) and anterior insula (AI) have been observed as a common neurobiological substrate across a spectrum of psychiatric disorders encompassing depression, bipolar disorder, schizophrenia, post-traumatic stress disorder, substance use disorders, obsessive-compulsive disorders, and anxiety disorders (Crossley et al., 2015; Goodkind et al., 2015). Therefore, it appears that SN functionality plays a central role in the etiology of a wide range of psychiatric disorders.

Cortical nodes of the SN have separately been linked to self-awareness, body perception (Craig, 2009), and fundamental cognition (Delevich et al., 2015). The AI plays an integral role in the response to, and experience of, emotional states (Craig, 2003), and level of activity has been shown to correlate with stimulus valence (Anders et al., 2004). In addition to emotional perception, the AI estimates changing environmental demands to modulate flexible cognitive control (Jiang et al., 2015). The AI also responds during action selection during decision-making (Paulus and Stein, 2006), and is therefore an integral component of cognitive control and the SN. Further, the dACC has separately been implicated in cognitive regulation (Delevich et al., 2015), divergent thinking, error detection, and response selection (Abraham et al., 2012; Sun et al., 2016). The dlPFC is also a key hub of cognitive control, as it is implicated in executive function (Kuo and Nitsche, 2012) and has historically been identified as a dominant cortical area within the brain’s central cognitive control network (Downar and Daskalakis, 2013).
The MDN is a thalamic nucleus with reciprocal connections to regions of the medial prefrontal cortex (mPFC) that assists in flexible action selection by integrating information from cortical, limbic, and basal ganglia regions (Delevich et al., 2015). Loss of functional communication between the MDN and mPFC due to physical or chemical lesions has shown to interrupt behavioural flexibility (Parnaudeau et al., 2013), learning, and decision-making in both humans and animals (Mitchell, 2015). The MDN is integral to quick associative learning and other executive tasks that involve complex cognition, though its precise role in integration and cognition is not well understood (Mitchell, 2015).

Co-activation among cortical regions of the SN is also associated with cognitive and behavioural phenomena related to decision-making and cognitive control. For one, the AI, dACC, and dlPFC activate synchronously in response to uncertainty in the environment; these regions overlap with areas implicated in the experience of negative mood states (Davis and Hasson, 2016; Feinstein et al., 2006; Naqvi and Bechara, 2009). The dACC and AI activate together during decision-making; co-activation has been shown to increase with task difficulty and stimulus ambiguity. This finding suggests that the 'difficulty dependent functional architecture' between the dACC and AI plays a role in cognition by filtering and integrating internal and external stimuli during cognitive tasks (Lamichhane et al., 2016).

In addition to being critical nodes of the SN, both the dACC and dlPFC have also been identified as key regions in the central executive network (CEN), which is responsible for cognitive control (Downar et al., 2016; Sun et al., 2016). A possible reason that the dACC and dlPFC are coactive with multiple networks is that these SN nodes play a role in mediating between the CEN, DMN, and other integral functional networks in order to direct and support neural responses (Sun et al., 2016). In addition to coactivity with separate functional networks, the dACC also possesses extensive cortico-cortical connections within the prefrontal cortex, including the cognitive dlPFC hub and premotor regions, placing it at the crossroads of learning and behaviour systems (Haber, 2016).
1.5.5 Ventromedial Network

The ventromedial network (VMN) corresponds fairly closely to the classically described reward pathways of the brain, which were originally identified by electrical self-stimulation studies in rats (Olds and Milner, 1954). Later, studies of the neural underpinnings of substance use and abuse led to the more concrete identification of reward circuitry (Carlezon and Wise, 1996; Haber and Knutson, 2009; Phillips et al., 1989). VMN circuitry involves cortico-thalamo-striatal projections from medial prefrontal areas to ventral striatal and other dopaminergic subcortical regions. The VMN regulates response to positively and negatively reinforcing stimuli to guide reward-mediated learning and behaviour (Knutson and Cooper, 2005).

1.5.5.1 VMN Anatomy

Cortical components of the VMN include the anterior cingulate cortex (ACC), ventromedial prefrontal cortex (vmPFC), and orbitofrontal cortex (OFC) (Haber and Knutson, 2009). Subcortical structures of the VMN include the ventral striatum, ventral tegmental area (VTA), and substantia nigra, the latter of which are midbrain dopaminergic nuclei (Haber and Knutson, 2009). The medial PFC and OFC project to the amygdala (Ongur and Price, 2000) and the ventral striatum, which projects to dopaminergic regions of the midbrain (Haber et al., 1995). These deep nuclei complete the VMN cortico-striatal-thalamo-cortical loop by relaying to the PFC via the medial dorsal nucleus (MDN) of the thalamus (Haber and Knutson, 2009). This discrete regulatory loop underlies the brain’s ‘reward system’ along with other contributing structures, including the hippocampus and associated nuclei in the brainstem (Haber and Knutson, 2009).

Reciprocal cortico-striatal circuits within the VMN synchronize reward processing. For example, a recent animal study demonstrated that the mPFC exerts top-down inhibitory control over certain populations of midbrain dopaminergic neurons, preventing excitatory activity from being projected to the striatum (Ferenczi et al., 2016).
Dopaminergic neurons originating in the VTA project to the nucleus accumbens (NAcc)—which makes up a portion of the ventral striatum along with the olfactory tubercle—and are involved in processes underlying motivation and reinforcement (Horvitz, 2000). Comparatively, dopaminergic projections from the substantia nigra terminate in the dorsal striatum to assist in the regulation of motor activity (Horvitz, 2000). The NAcc is strongly functionally connected to specific areas of the vmPFC and frontal pole, as reported in both functional imaging and deep brain stimulation studies in humans (Bewernick et al., 2010; Dunlop et al., 2015a). The NAcc activates in response to valenced, salient motivational stimuli, and is in fact comprised of intermingled neuronal populations that respond differentially to stimuli of either negative or positive valence (Xiu et al., 2014).

1.5.5.2 VMN Function

The VMN activates in response to affective information, including positively valenced, negatively valenced, or rewarding stimuli (Price, 1999). It is implicated in driving reward-based learning (Adcock et al., 2006), goal-directed behaviour, and adaptive responses to environmental stimuli (Haber and Knutson, 2009). Specifically, activation of midbrain dopaminergic nuclei signals reward prediction error by propagating signals to the ventral striatum and medial PFC (Diekhof et al., 2008). In addition to driving motivation and urges, regions of the VMN also play a role in directing attention. For example, the NAcc is theorized to activate in response to stimuli that are not necessarily rewarding, but rather, salient because they could be relevant to an impending behavioural decision, such as approach or withdrawal (Knutson and Cooper, 2005). This function represents a fundamental role in signalling motivational salience in response to affective stimuli (Diekhof et al., 2008).

Inclusion of the OFC in the VMN cortico-striatal circuit implicates the reward network in processes that are more complex than fundamental reward-based learning. Individually, the OFC is involved in reward-based decision-making (Rogers et al., 1999), inhibition of adverse emotions (Berthoz et al., 2002), and behavioural inhibition (Del-Ben et al., 2005; Völlm et al., 2006). In summary, the OFC is integral to stimulus valuation,
especially in the context of comparing outcomes for the purpose of selecting a
behavioural response (Diekhof et al., 2008; O’Doherty, 2004).

1.5.6 The SN and VMN

With regard to the fundamental regulation of behaviour, the SN and VMN likely function in opposition: while the VMN underlies drive and motivation, the SN has been described as a ‘gatekeeper network’ that exerts inhibitory control over cognition and behaviour, in terms of response selection or inhibition (Hanlon et al., 2013). However, healthy brain function (and thus, adaptive behaviour) requires integration of reward-related valuation, attentional direction, and action selection (Diekhof et al., 2008; O’Doherty, 2004). Furthermore, cortical cognitive control processes—such as consideration of the future, evaluation of goals, and reasoning—are required for effective decision-making. Such processes serve to balance VMN activity, which is guided by emotional salience and lower-level reward prediction (Diekhof et al., 2008). Imbalance between these functions could result in decreased control over prepotent urges, cravings, impulses, or thoughts.

Dopaminergic nuclei involved in the reward system are intrinsically linked to the function of higher-order cognitive control networks, such as the SN (Alexander et al., 1986; Peters et al., 2016). These neurons preferentially respond to indicate detection of environmental stimuli that could require behavioural modification or response, necessitating executive processes such as working memory that enable continued evaluation (Horvitz, 2000). Activity of these neurons modulates levels of dopamine in striatal VMN nodes, which propagate the signal to appropriate cortical regions to activate or inhibit a response (Everitt et al., 1989; Horvitz, 2000; Salamone et al., 1997).

Integration of circuits underlying reward, cognition, and motor control is necessary for adaptive behaviours that rely on both immediate and long-term stimulus evaluation and response (Haber and Knutson, 2009). A unique subcortical region that could link salience and affective networks is the dorsal striatum, and specifically, the ventral part of the head of the caudate nucleus. While the ventral striatum—the NAcc—is involved in reward-prediction error and signalling motivational salience, the dorsal striatum plays a
role in indicating stimulus-reward contingency (Diekhof et al., 2008; O’Doherty, 2004; Wrase et al., 2007). Taken together, the ventral and dorsal striatum may function collaboratively to compute reward expectations and select the most appropriate and beneficial responsive actions, respectively (Haber and Knutson, 2009).

Functional imaging studies suggest that healthy individuals exhibit top-down regulatory control over ventral and subcortical regions involved in affect, via cortico-striatal circuits from the left lateral PFC. In similar studies involving negative stimulus reappraisal tasks, healthy individuals demonstrate lower activity in the amygdala and higher activity in lateral PFC areas (Ochsner et al., 2002; Phan et al., 2006). These results imply that an inhibitory cortico-striatal circuit spans from the dorsal and ventral lateral PFC to the amygdala and associated ventral affective regions (Morris and Dolan, 2004; Rolls, 1996). Specifically, the lateral orbitofrontal cortex (lOFC) is linked to higher-order evaluations of stimulus valence and reversal of previously learned reward values (Morris and Dolan, 2004; Rolls, 1996).

Although a general topographic organization exists between the frontal cortex and striatal/subcortical targets, there exists a complex convergence of pathways across CST loops that originate in prefrontal areas including the ventromedial PFC and orbitofrontal cortex. This overlap suggests that CST circuit integration is structural as well as functional, and provides modulation between and across reward, prediction, and saliency circuits (Averbeck et al., 2014; Haber, 2016). Despite a wealth of insight into the role of the SN and VMN cortico-striato-thalamic loops in the pathophysiology and treatment of psychiatric disorders, the full extent of dysfunction within these networks—and how to best remedy their dysfunction through the application of therapeutic brain stimulation—remain a critical target of further investigation.

1.5.7 Resting-State Networks in Major Depression

Healthy and adaptive brain function relies on integrity within and between nodes of major functional brain networks. Neuroimaging studies consistently implicate the DMN, CEN, SN, and VMN in depression (Hamilton et al., 2013; Menon, 2011; Raichle et al.,
Each of these networks are capable of independent activity, but also possess convergent circuits that allow for inter-network communication, and accordingly, flexibility in behavioural generation and response (Haber, 2016). Consequently, dysfunction in a central node or connection among any of these major networks can have a broad impact on cognitive and emotional processes, potentially leading to a variety of depressive symptoms (Drevets et al., 2008; Hamilton et al., 2013; Mayberg, 1997; Mulders et al., 2015).

1.5.7.1 Internally- and Externally-Directed Cognition

The DMN is of primary interest in MDD due to its role in self-referential, internally directed, and ruminative thinking (Hamilton et al., 2015), and nodes of this network consistently demonstrate hyperactivity in individuals with MDD. Particularly, depression is characterized by increased activity in, and connectivity between, nodes of the anterior DMN, including the vmPFC (Mulders et al., 2015). Compared to control subjects without MDD, these regions are hyperactive during the resting-state (Drevets et al., 2008; Mayberg et al., 2005) and during emotional stimulus presentation tasks (Gotlib et al., 2005; Sheline et al., 2009).

One medial prefrontal region is uniquely involved with the DMN in MDD: the subgenual cingulate cortex (sgCC). In MDD, the sgCC is hyperactive and becomes functionally coupled to the DMN (Greicius et al., 2007); further, increased activity in the sgCC is suggested to predict treatment response (Pizzagalli, 2011). The sgCC is often activated during states of illness and inflammation, and in ‘sickness behaviour’ (Harrison, 2016). As such, coupling of the sgCC to the DMN could reflect the symptoms of increased negative rumination coupled with ‘sickness-mode’ behaviours that commonly occur in MDD patients (Krishnadas & Harrison, 2016). Additionally, depression is linked to abnormal connectivity between the anterior and posterior sub-networks of the DMN; however, findings of both dissociation and increased connectivity between these subnetworks have been reported (Mulders et al., 2015).
Disruption of externally directed executive control is also linked to MDD symptoms. Dysfunction in CEN nodes or circuits has been linked to loss of cognitive flexibility, apathy, and irritability (Rogers et al., 2004); specifically, the left dlPFC tends to show reduced metabolic activity (Mayberg et al., 1994) that has been linked to severity of cognitive disturbance in MDD (Bench et al., 1992, 1993). Furthermore, depressed individuals typically show worse performance on tasks requiring executive function such as set-shifting (Konishi et al., 1999), planning (Elliott et al., 1996), and attentional control (Rogers et al., 2004). Hypoconnectivity within the entire CEN has been observed in MDD, and is suggested to contribute to depressive symptoms involving memory, attentional, and cognitive deficits (Liston et al., 2014).

Depression may involve maladaptive interactions between the DMN and CEN. Depressed individuals show decreased connectivity between nodes of the DMN and CEN (Mulders et al., 2015), including between the PCC and the caudate nucleus (Bluhm et al., 2009). Similarly, large-scale models of effective connectivity in MDD suggest that impaired executive function is related to decreased functional activity of prefrontal nodes and networks (Davidson et al., 2002; Mayberg, 1997), indicating that effective inter-network connectivity from the DMN may be negatively impacted in depression. As such, functional disturbance in the CEN—or imbalance between the CEN and the opposing DMN—could reflect the symptoms of impaired concentration and decision-making that commonly occur in patients with MDD.

1.5.7.2 Interactions Between Salience, Valence, and Response

Abnormalities in the activity of the SN and VMN may also contribute to distinct aspects of depressive symptoms. The SN has been described as a ‘paralimbic emotional processing’ system that relies on extensive connections to the DMN, CEN, and limbic structures to mediate emotional control (Mulders et al., 2015). MDD is associated with abnormal functional connectivity between the SN and medial frontal regions, including both the anterior DMN (Fang et al., 2012) and the VMN. Dysfunction within or among VMN nodes is also linked to maladaptive behaviours and psychiatric illness; mood disorders are commonly reported to involve disturbances of motivation and emotion that
are associated with altered structure or function of VMN nodes (Diekhof, Falkai, & Gruber, 2008). For example, mPFC hyperactivity is linked to psychiatric disease states (Hanlon et al., 2013), and abnormal VMN activation in response to negative or drug-related stimuli is observed in MDD and substance use disorders, respectively (Dunlop, Hanlon, & Downar, 2015).

A region that may have a role in linking the SN and VMN is the amygdala. In MDD, the amygdala shows opposing patterns of functional connectivity to the SN and VMN (Mulders et al., 2015; Northoff et al., 2011). Hyperactivation of the amygdala—and increased connectivity between the amygdala and VMN—is thought to reflect maladaptive perception of (or increased attention to) negative cues, which may contribute to symptoms such as anhedonia (Dunlop et al., 2015; Price & Drevets, 2012; Tremblay et al., 2005). The notion of the VMN as an engine driving pathological incentive salience is further supported by an analysis that revealed VMN activation in response to negative, but not positive, stimuli in MDD (Knutson & Cooper, 2005). In contrast, other studies have observed decreased connectivity between the amygdala and emotional control areas (Mulders et al., 2015). For example, the amygdala has lower connectivity to SN nodes—such as the insula and lateral PFC—in depression, representing a potentially pathological imbalance between limbic and salience networks (Northoff et al., 2011). Interestingly, some investigators note that such patterns of amygdalar connectivity are comparable to those observed in anxiety disorders, suggesting that they may reflect generalized internalizing pathology (Treadway & Pizzagalli, 2014).

Imbalance or dysfunction in the interactions between the SN and VMN may be linked to specific constellations of MDD symptoms. Current models of network dysfunction in MDD posit abnormal interactions among a variety of regions, including the dlPFC, dACC, vLPFC, amygdala, and hippocampus (Greicius et al., 2007; Johnstone et al., 2007; Price & Drevets, 2012; Seminowicz et al., 2004). For example, the limbic-cortical dysregulation model (Mayberg, 1997) proposed that depression could be characterized as an inability of “dorsal limbic” areas, including nodes in the SN and posterior DMN, to
regulate hyperactive “ventral limbic” areas, including nodes in the VMN and anterior DMN (Mulders et al., 2015). Thus, some individuals with MDD may be unable to effectively regulate negative affective states (Johnstone et al., 2007); however, it remains unclear whether depressive symptoms are perpetuated by a failure to exert top-down modulation of excessive limbic activity, or by hyperactive limbic and reward regions exerting bottom-up interference in cognitive processes (Mulders et al., 2015).

Network dysfunction may also affect the subcortical components of these resting-state networks in MDD. It is commonly observed that abnormal connectivity between cortical and ventral striatal regions affects perception and valuation of rewarding stimuli (Treadway & Pizzagalli, 2014). Specifically, individuals with MDD show decreased recruitment of subcortical regions involved in reward salience and learning, which could reflect abnormalities in dopaminergic projections to the striatum (Treadway & Pizzagalli, 2014). It is necessary to further characterize such abnormalities of cortical-subcortical activity, because functional subtypes of MDD may be characterized by unique disruptions in inter-network interactions. Future investigations should strive to determine how changes in cortico-striatal connectivity throughout circuits underlying emotional responses are uniquely related to depression, or to internalizing pathology in general (Treadway & Pizzagalli, 2014). Identifying functional subtypes could aid in the development of targeted brain stimulation treatments that influence network activity by altering excitability of a principal node (Downar & Daskalakis, 2013).

1.5.8 Effects of left dlPFC-rTMS on Resting-State Networks

rTMS has widespread effects on network connectivity. Post-treatment functional connectivity changes between nodes of different networks likely reflect changes in inter-network communication and interaction (Mulders et al., 2015). Specifically, rTMS of the dlPFC in MDD appears to normalize dysfunctional connectivity within and between the DMN, CEN, and SN (Anderson et al., 2016). In control subjects, left dlPFC-rTMS has been shown to affect dopamine transmission in the caudate nucleus (Strafella et al., 2001), sgCC, rostral ACC, and medial OFC (Cho & Strafella, 2009), reflecting the potential for rTMS to exert widespread functional alterations.
TMS for MDD likely ‘normalizes’ some, but not all, network interactions. For example, response to left dlPFC-rTMS in MDD has been linked to normalization of functional connectivity both within the DMN, and between the DMN and CEN, effectively improving interactions between these networks (Liston et al., 2014). However, in the same study, abnormal CEN activity was not impacted by treatment (Liston et al., 2014). In line with this evidence, a recent meta-analysis of dlPFC-rTMS for patients with a variety of neuropsychiatric illnesses failed to identify treatment-related improvements across a number of cognitive domains (e.g., attention, working memory, and processing speed) (Martin et al., 2016). This could suggest that left dlPFC-rTMS does not simply enhance executive control, but rather impacts effective interactions between regulatory and emotional networks.

rTMS delivered to the left dlPFC consistently results in functional connectivity changes between the stimulation site and remote regions, which have been measured using a variety of neuroimaging modalities, and which provide insight into functional circuits related to rTMS response. A recent perfusion SPECT study observed that rTMS responders showed decreased post-treatment functional connectivity from the left dlPFC to the temporal lobe and interconnected limbic regions (Richieri et al., 2017). In contrast, an EEG study demonstrated that high-frequency left dlPFC-rTMS induced more synchronized activity between the left dlPFC and ventral limbic regions, including the sgCC and parahippocampal gyrus (Kito et al., 2017). Left dlPFC-rTMS has also been shown to reduce functional connectivity between the left dlPFC and the caudate, based on fMRI analysis (Kang et al., 2016).

Examination of regional functional connectivity changes sheds some light on the therapeutic mechanisms of rTMS, and has provided hints of broader scale change in networks such as the DMN and CEN (e.g., Liston et al., 2014; Salomons et al., 2014). Surprisingly, though, few studies have assessed the impact of left dlPFC-rTMS on global network dynamics in MDD or TRD (Anderson et al., 2016). Additionally, inconsistencies in the available evidence warrant more thorough investigations of how
left dIPFC-rTMS affects resting-state networks in MDD, and in particular, its effects on the SN and VMN.

1.6 Summary

Major depression is disabling and does not always respond to front-line therapies, leaving hundreds of thousands of Canadians with few cost- and time-effective treatment options. New strategies are needed that are successful even after multiple treatment attempts have failed. One potential approach is non-invasive brain stimulation—specifically, rTMS—which can achieve favourable response rates in otherwise treatment-resistant patients. Nonetheless, a large proportion of TRD patients remain as rTMS non-responders. Patients with TRD would benefit from the identification of functional neuroimaging predictors of rTMS response, which could improve treatment outcomes and avoid the cost and burden of futile courses of treatment for non-responders.

Advances in structural and functional neuroimaging techniques have enabled the identification of networks of brain regions that underlie behaviour, emotion, and complex cognition. Investigations of these functional networks in populations with MDD have revealed that depression may involve network-level dysfunction in the salience and ventromedial networks, as well as subcortical counterparts in the striatum. Dysfunction throughout and between these CSTC loop circuits may be manifested by pathology in attentional control, incentive salience, and behavioural control via response selection and inhibition. It could be possible to alleviate these symptoms by using rTMS to normalize function in these circuits.

To improve rTMS treatment outcomes in TRD, resting-state fMRI could be used to characterize distinctive predictor patterns of network activity in rTMS responders. Neuroimaging analyses may also be helpful for characterizing the mechanisms, or correlates, by which rTMS affects resting-state network activity to exert a therapeutic effect in MDD. In sum, the need for effective brain stimulation treatments for MDD, our present understanding of functional networks that are relevant to psychiatric illness, and
the accessibility of functional neuroimaging techniques all provide a rationale for the investigation of functional predictors and correlates of rTMS treatment response.
2.0 Research Aims and Hypotheses

2.1 Aims

Two primary neuroimaging aims were defined for this project:

1. To identify functional MRI predictors of response in MDD patients receiving left dlPFC-rTMS with iTBS and 10 Hz stimulation protocols. This will be achieved by identifying regions of the brain where the baseline (pre-treatment) resting state functional connectivity to a pre-specified seed region of interest is significantly correlated to the degree of clinical improvement over the course of treatment.

2. To identify changes in functional connectivity from pre- to post-treatment that correlate with degree of improvement in MDD patients receiving left dlPFC-rTMS with iTBS or 10 Hz stimulation protocols. This will be achieved by identifying regions of the brain where the pre- to post-treatment changes in functional connectivity to a pre-specified seed region of interest are significantly correlated to the degree of clinical improvement over the course of treatment.

A secondary clinical aim was defined for this project:

3. To evaluate whether iTBS of the left dlPFC produces comparable outcomes to those of 10 Hz stimulation of the left dlPFC. This will be assessed by comparing both clinical outcomes, and functional MRI predictors and correlates of response, as described above, for both iTBS and 10 Hz stimulation.

2.2 Hypotheses

With regards to these aims, three neuroimaging hypotheses, and one secondary clinical hypothesis, were proposed:

1. Lower baseline functional connectivity through the salience network corticostriatal loop will predict a higher degree of improvement following left dlPFC-rTMS for both iTBS and 10 Hz treatment protocols.

2. Symptom improvement will be associated with increased pre- to post-treatment rs-FC throughout the salience network corticostriatal loop, and increased pre-
post-treatment rs-FC between the salience network and the ventromedial network, for both iTBS and 10 Hz treatment protocols.

3. Brain regions identified as predictors and correlates of treatment response, based on baseline resting state functional connectivity and pre- to post-treatment changes in functional connectivity, will be the same for both iTBS and 10 Hz treatment protocols.

4. iTBS and 10 Hz rTMS of the dIPFC will produce comparable clinical outcomes, with weekly symptom measurements and final outcomes not differing significantly between groups.

2.3 Summary of Rationale

The salience network plays a key role in cognitive control, response inhibition, and response selection. The left dIPFC is a core node of the salience network, and projects to subcortical nodes including the dorsal caudate nucleus, mediodorsal thalamus, and midbrain dopaminergic nuclei. This cortico-striatal-thalamo-cortical loop circuit modulates cognitive control and behavioural selection (Downar et al., 2016; Seeley et al., 2007). Hypoactivity of the dIPFC has been widely observed in MDD (Geller et al., 1997; George et al., 1995), and dIPFC hypofunction may impair self-regulation of cognition, behaviour, and emotion in psychiatric disorders including MDD. Furthermore, other salience network nodes including the anterior insula and dorsal anterior cingulate cortex exhibit functional abnormalities across several psychiatric disorders, including MDD (Goodkind et al., 2015). Integrity of the salience network loop circuit, incorporating the dIPFC, may be necessary for cognitive control and for normal regulation of mood (Peters et al., 2016).

Cognitive control may also require integrity of corticostriatal connections through the salience network. rTMS administered to the dIPFC is shown to increase endogenous dopamine release in the ipsilateral caudate nucleus, indicating that stimulation of corticostriatal fibers affects neurotransmission in the striatum (Strafella et al., 2001). This effect is achieved through direct modulation of cortico-striatal fibers, or through indirect activation of dopaminergic midbrain neurons that enhance the activation of
downstream cortical and subcortical targets (Paus et al., 2001). Either, or both, of these outcomes likely enhance the integrity of cortico-cortical and cortico-striatal functional connections within the salience network.

In this context, individuals who respond to left dlPFC-rTMS, which will theoretically increase left dlPFC excitability, should represent a subgroup of patients whose symptoms (a) were associated in part with low connectivity between salience network nodes, including the dlPFC; and (b) improve due to normalized left dlPFC activity, which will be accompanied by normalized functional connectivity to downstream cortical and subcortical components of the salience network corticostriatal loop. Restored functional connectivity within this loop should manifest in improved ability to respond to salient environmental cues and coordinate adaptive behavioural responses, and thus in a reduction in clinical symptom severity.

Dysfunction through the ventromedial network is also hypothesized to underlie certain symptoms of MDD. In MDD, the VMN may show ‘paradoxical’ activation in response to negatively valenced stimuli, thereby generating pathological signals of incentive salience (Tremblay et al., 2005). It has been proposed that the VMN and SN work in opposition by mediating either motivation and urge, or response selection and inhibition (Hanlon et al., 2013). Healthy and adaptive behavioural control necessitates coordination of networks that enable realistic evaluation of, and response to, valenced motivational stimuli. Thus, post-treatment improvement should be accompanied by increased functional connectivity between the VMN and SN (cortical and subcortical nodes), as a neural correlate of normalized adaptive reward and salience valuation, and by extension, normalized behavioural selection and modulation.

Regarding clinical outcomes, in previous literature iTBS has achieved similar symptom improvements in patients with TRD when compared to conventional high-frequency protocols, including in a case series directly comparing these two protocols for rTMS targeting the dmPFC (Bakker et al., 2015). For this reason, in this study of rTMS targeting the dlPFC, we anticipate that iTBS will achieve non-inferior clinical outcomes.
compared to conventional 10 Hz stimulation, as indicated by scores on the Hamilton Rating Scale for Depression.

Finally, the identified neuroimaging predictors and correlates are expected to be similar for both treatments. Both iTBS and 10 Hz stimulation are shown to have long-lasting neural effects mediated by changes in the balance between excitatory and inhibitory cortico-striatal interactions (Iwabuchi et al., 2016). There are presently no studies that directly compare neuroimaging outcomes between iTBS and 10 Hz stimulation, but the similarity of clinical outcomes observed in these protocols gives us reason to hypothesize that predictors and correlates of treatment response on functional neuroimaging will also be similar across the two groups.
3.0 Methods

3.1 Study Overview

The clinical and neuroimaging data for this project were drawn from a larger study of rTMS in MDD. For context, the larger study was a three-site (rTMS clinics at the Centre for Addition and Mental Health, CAMH; University Health Network, UHN; and the University of British Columbia, UBC) randomized controlled trial (RCT) comparing iTBS to conventional 10 Hz stimulation of the left dlPFC for medication-resistant MDD, using a non-inferiority design. Enrolled outpatients were randomized to receive dlPFC-rTMS according to one of two protocols: 1) iTBS (50 Hz triplet bursts 5 times per second, 2 s on 8 s off, 20 trains, 600 pulses, 120% resting motor threshold; 3 min); or 2) conventional 10 Hz stimulation using the standard FDA-approved protocol (10 Hz, 4 s on 26 s off, 75 trains, 3000 pulses, 120% resting motor threshold; 37.5 min).

3.2 Randomization and Blinding

Treatment group allocation was stratified with respect to the severity of treatment resistance (score on the Antidepressant Treatment History Form > 4), since degree of treatment resistance has previously been identified as a predictor of response to rTMS (George et al., 2010; O’Reardon et al., 2007). A computer algorithm (sealedenvelope.com) generated randomization sequences, and sealed envelopes containing treatment assignment chits were created for treatment allocation of enrolled patients. Treatment technicians and patients were of necessity aware of treatment allocation; however, they were instructed not to discuss their treatment allocations with other patients or staff, and all symptom raters were blinded with respect to randomization.
Figure 1. Timeline of events. 303 patients with TRD were randomized to receive either iTBS or 10 Hz rTMS of the left dlPFC. Subjects underwent one structural and functional MRI scan before beginning treatment, and one scan after completing treatment. All scans (303 pre-treatment, 303 post-treatment) were acquired on the same scanner (Toronto Western Hospital, UHN). Subjects received daily treatment sessions for 4 weeks, and were extended by 2 weeks if their symptoms improved >30% by Week 4. Clinician-rated HRSD assessments were conducted at baseline, weekly during treatment, and at three follow-up points.
3.3 Subject Selection

Study inclusion criteria required that the patients: were outpatients; were competent to consent to voluntary treatment; were diagnosed with MDD based on the Mini-International Neuropsychiatric Interview (MINI); were between the ages of 18 and 65; were unable to achieve clinical response to an antidepressant at adequate dosage based on Antidepressant Treatment History Form (ATHF) criteria, or unable to tolerate at least two separate antidepressants at adequate dosage or duration; had a score greater than or equal to 18 on the Hamilton Rating Scale for Depression (HRSD) at baseline; had no changes to psychotropic medication regimen in the four weeks before treatment; were able to adhere to treatment schedules; were able to pass the TMS safety screening questionnaire; and had normal thyroid function as indicated by blood testing.

Study exclusion criteria applied to patients who: had a history of substance abuse or dependence within the previous three months; had a concomitant, major, unstable medical illness, cardiac pacemaker, or implanted medication pump; demonstrated active suicidal intent; were pregnant; were diagnosed with bipolar I or II, schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, or psychotic symptoms based on the MINI; were diagnosed with obsessive-compulsive disorder, post-traumatic stress disorder, anxiety disorder, dysthymia, or personality disorder that was assessed to cause greater impairment than MDD; had failed a course of ECT; had previously received rTMS; had a neurological disorder or injury, such as cerebral aneurysm, Parkinson’s disease, Huntington’s chorea, multiple sclerosis, or significant head trauma; had any intracranial implant or other metal object that could not be safely removed; if undergoing psychotherapy, were not in stable treatment for at least three months prior to enrolment; showed a clinically significant laboratory abnormality; at time of enrolment or in previous four weeks, took more than 2 mg lorazepam (or equivalent) daily, or any dose of an anticonvulsant; had a non-correctable, clinically significant sensory impairment; or failed more than three adequate medication trials during the current depressive episode.
Regarding withdrawal criteria, patients were withdrawn from the study if their depressive symptoms became worse (as defined by a >25% increase in HRSD score compared to baseline), if they developed active suicidal intent, or if they attempted suicide. Patients were also withdrawn from the study if they missed four or more consecutive days of treatment.

For the present project, subjects from the RCT were selected for inclusion if they underwent both pre-treatment and post-treatment structural MRI and fMRI scans on the University Health Network MRI scanner (all patients from both the CAMH and UHN sites were scanned on the UHN scanner to avoid inter-scanner sources of variability in the neuroimaging data). Patients were excluded if their scans contained anatomical anomalies or artefacts that affected data quality (as assessed via the methodology below). Thus, subjects were 303 patients (179 female, 124 male, mean age 42.76 ± SD 11.57 years) who provided consent to participate in the RCT following a referral to the University Health Network (UHN) or the Centre for Addiction and Mental Health (CAMH) for treatment-resistant depression (TRD).

3.4 rTMS Technique

All treatments were administered using a MagPro X100/R30 stimulator equipped with a B70 fluid-cooled coil (MagVenture, Farum, Denmark). Patients underwent daily treatment sessions, five days per week, for four to six weeks, targeting the left dIPFC under MRI-guided neuronavigation. Non-remitter patients who experienced symptom improvement of at least 30% by week four (session 20) received two additional weeks of treatment. Patients who did not improve to this extent concluded participation after four weeks.

3.4.1 Neuronavigation

The stimulation target site for each individual was identified based on registration from the standard Montreal Neurological Institute (MNI) template brain onto patients’ structural MRI. The Visor 2.0 system (ANT Neuro, Madison, WI) was used for
neuronavigation in coil positioning over the left dlPFC target. MRI-guided neuronavigation was employed during every session of stimulation throughout the course of treatment. The stimulation site was at MNI coordinates (X -38, Y +44, Z +26), selected based on a study that identified this region as an optimal rTMS target based on both clinical outcomes and functional connectivity to the subgenual cingulate cortex (Fox et al., 2012).

3.4.2 Motor Threshold

Before beginning treatment, patients’ resting motor thresholds (RMT) were determined through TMS of the primary motor cortex. The coil was oriented at 45 degrees to the midline, over the left and right primary motor cortices, and RMT was established based on visual inspection of contralateral abductor pollicis brevis contraction, as per standard published methods for dlPFC-rTMS (Schutter and van Honk, 2006). The RMT was defined as the stimulation intensity required to elicit a contraction on 5/10 trials. The target stimulation intensity for treatment sessions was set at 120% of RMT.

3.4.3 iTBS Protocol

Patients assigned to this treatment group received left dlPFC stimulation at 120% RMT, using the iTBS protocol as originally defined by Huang et al., (2005), i.e., 50 Hz triplet bursts repeated at 5 bursts per second, with 2 s trains and an intertrain interval of 8 s, for 20 trains, totalling 600 pulses per session over 3 minutes and 9 seconds.

3.4.4 10 Hz Protocol

Patients assigned to this treatment group received left dlPFC stimulation at 120% RMT, according to the standard FDA-approved protocol of O'Reardon et al., (2007), i.e., at 10 Hz, with 4 s trains and an intertrain interval of 26 s, for 75 trains, totalling 3000 pulses per session over 37.5 minutes.
3.5 Clinical Assessments

3.5.1 Clinical Data Collection

Patients were assessed at baseline in the week before treatment, weekly during treatment, and at one, four, and 12 weeks post-treatment, by trained raters at each site (UHN and CAMH) using the clinician-rated 17-item Hamilton Rating Scale for Depression (HRSD). The HRSD is a standard assessment tool used in MDD treatment trials that range from medications to rTMS, and has been widely used in previous major rTMS trials (George et al., 2010; O’Reardon et al., 2007). Raters were blinded with respect to treatment allocation and were not involved in treatment provision. Patients were instructed not to reveal their treatment allocation to raters.

3.5.2 Clinical Data Analysis

Baseline, weekly, and follow-up HRSD scores were evaluated separately for iTBS and 10 Hz treatment groups. The variable “degree of improvement” was calculated based on percent change in HRSD score from baseline to follow-up one (one week post-treatment) for each patient. A Shapiro-Wilk test and kernel density estimations were performed to compare and examine the distribution of improvement in each group. Cumulative frequency distributions were created for each group and compared using the non-parametric Kolmogorov-Smirnov test. Based on these outcomes (as described in detail in Section 4.2, Clinical Outcomes), for the purposes of analysis, clinical outcomes were defined according to tertiles of percent improvement on the HRSD, with the upper and lower tertiles representing strong responders and non-responders, and the middle tertile representing patients of ambiguous response (neither clear responders nor clear non-responders). Since the tertiles contained different numbers of individuals, unpaired, two-tailed Student’s T-tests, Fisher’s Exact Tests, and a 2-way analysis of variance (ANOVA) were performed to evaluate differences in age, proportion of females to males, degree of improvement, and effect of treatment group on degree of improvement.
3.6 Neuroimaging

3.6.1 Neuroimaging Acquisition

Participants underwent an anatomical and functional MRI scan one week before beginning treatment, and in the week after the final session of treatment. All subjects’ scans were acquired on the same 3T GE Signa HDx scanner at Toronto Western Hospital, University Health Network. Anatomical MRI data acquisition involved an 8-minute high-resolution T1-weighted scan (TE 12 ms, TI 300 ms, flip angle 20°, 116 sagittal slices, 1.5 mm thickness, no gap, 256 x 256 matrix, 240 mm FOV). Functional MRI data acquisition involved a 10-minute, eyes-closed, T2* BOLD sequence (TE 30 ms, TR 2000 ms, flip angle 85°, 32 axial slices, 5 mm thickness, no gap, 64 x 64 matrix, 220 mm FOV, 300 frames).

3.6.2 Preprocessing

Data preprocessing and analysis employed the FSL neuroimaging analysis software suite (Jenkinson et al., 2012). First, the first ten seconds of functional resting state MRI data (5 of 300 volumes) were removed to allow for T2* signal equilibration effects. Next, motion correction (FSL MCFLIRT) and slice-timing correction (FSL FEAT) were conducted (Jenkinson et al., 2012) before continuing preprocessing using anatomical components-based noise correction (aCompCor) (Behzadi et al., 2007). The aCompCor pipeline performed the following functions: first, FSL BET extracted brains from anatomical MRI (Jenkinson et al., 2012); next, FSL FAST segmented anatomical data into grey matter, white matter, and cerebrospinal fluid, and FSL FLIRT registered these components to functional data (Jenkinson et al., 2012); finally, principal components analysis (PCA) performed in MATLAB identified the top five principal components of WM and CSF, which were regressed out of the functional data. The previously identified six motion parameters were also regressed out of the functional data. Finally, spatial smoothing (6 mm) and temporal bandpass filtering (0.01 - 0.1 Hz) were applied to the resulting image series.
3.6.3 Neuroimaging Analysis

3.6.3.1 Data Quality Inspection

Due to the large volume of data, a series of quality control steps were implemented at several steps of processing and analysis. Checkpoints included: verifying coded IDs for subjects’ scans; verifying subject numbers; verifying subject to scan matching; visually inspecting structural and functional scans for abnormalities prior to preprocessing steps; visually inspecting registration of preprocessed files; and inspecting all preprocessing output reports for errors.

3.6.3.2 Defining Regions of Interest

3.6.3.2.1 Network-Based ROIs

Network-based seed ROIs were defined for specific networks of interest: the salience network (SN) and ventromedial network (VMN). To localize these networks, group-wide independent component analysis (ICA) was performed to generate network-level ROIs. First, subjects from both treatment groups were compiled into one group \( n = 303 \). Next, FSL MELODIC performed subject-level registration of functional data to standard space before performing multi-session temporal concatenation and generating a data-driven number of components (Jenkinson et al., 2012). 27 components were generated. Each of these was visually inspected, and two were identified as the SN and VMN based on the regional locations of the peak voxels (anterior cingulate, insula and dIPFC for the SN, and ventral striatum and vmPFC for the VMN). The SN and VMN components were then transformed into 2 mm MNI standard space using FSL FLIRT, thresholded at \( Z > 8 \) (to constrain the ROI mask to the peak voxels of the nodes of the SN and VMN, respectively), and binarized to create the final ROI mask.
<table>
<thead>
<tr>
<th>Regions of Interest</th>
<th><img src="image" alt="Salience Network ROIs" /></th>
<th><img src="image" alt="Salience Network ROIs" /></th>
<th><img src="image" alt="Salience Network ROIs" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Salience Network</td>
<td><img src="image" alt="Salience Network ROIs" /></td>
<td><img src="image" alt="Salience Network ROIs" /></td>
<td><img src="image" alt="Salience Network ROIs" /></td>
</tr>
<tr>
<td>Ventromedial Network</td>
<td><img src="image" alt="Ventromedial Network ROIs" /></td>
<td><img src="image" alt="Ventromedial Network ROIs" /></td>
<td><img src="image" alt="Ventromedial Network ROIs" /></td>
</tr>
</tbody>
</table>

**Figure 2. Network-level regions of interest.** Salience network (SN) and ventromedial network (VMN) ROIs were generated using two component outputs of group-wide independent component analysis. Each component was thresholded at $Z > 8$ and binarized to create network seeds.
3.6.3.2.2 Regional ROIs

Two cortical and three subcortical regions of interest (ROIs) were defined \textit{a priori}. One cortical and two subcortical ROIs were hypothesis-driven; the remaining cortical and subcortical ROIs were exploratory.

The left dIPFC was a hypothesis-driven ROI selected based on its role as (a) the primary treatment stimulation target; and (b) its role as a node of the salience network responsible for coordinating cognitive control, response selection, and response inhibition. This ROI was a 6 mm sphere centered on the MNI coordinates of the stimulation site, \((X -38, Y +44, Z +26)\). The anterior mid-cingulate cortex (aMCC) was defined as an exploratory ROI due to its proximity to and overlap with the dACC, an integral hub of the salience network. Previous investigations have identified functional connectivity changes from this region that are associated with rTMS response in patients with MDD (Dunlop et al., 2015b, 2016). This ROI was retrieved from Craddock et al.’s (2012) whole-brain ROI atlas, and has previously been used for investigations of dACC functional connectivity (Dunlop et al., 2015b).

Three unilateral, striatal \textit{a priori} ROIs were generated based on bilateral striatal seed regions previously employed in a functional mapping of the human striatum (Di Martino et al., 2008). Two of these seeds were hypothesis-driven and represent well-established subcortical nodes of the SN (dorsal caudate) and VMN (nucleus accumbens). They were selected to investigate fluctuations in striato-cortical connectivity associated with symptom improvement following rTMS. These ROIs were left lateralized 3 mm cubes centered on the MNI coordinates \((X -13, Y +15, Z +9)\) (dorsal caudate nucleus), and \((X -9, Y +9, Z -8)\) (nucleus accumbens). The third striatal ROI, the ventral caudate nucleus, was defined as an exploratory ROI \((X -10, Y +15, Z 0)\) since it has been shown to have projections to both dorsal and ventral cortical regions.
**Figure 3. Regions of interest.** Coordinates are in MNI standardized space. The L dIPFC seed was a 6 mm sphere; the aMCC seed was a predetermined ROI retrieved from a whole-brain ROI atlas (Craddock et al., 2012); and the NAcc, VC, and DC seeds were left lateralized 3 mm cubes recreated based on a previous study that mapped functional connectivity of the human striatum (Di Martino et al., 2008). L dIPFC, left dorsolateral prefrontal cortex; aMCC, anterior mid-cingulate cortex; NAcc, nucleus accumbens; VC, ventral caudate nucleus; DC, dorsal caudate nucleus.
3.6.3.3 First-level Analyses

First-level analyses were conducted by generating subject-level, whole-brain resting state functional connectivity maps for each network and regional ROI. These analyses were performed on all pre-treatment and post-treatment scans \((n = 303 \text{ pre-}, 303 \text{ post-})\). First, FSL FLIRT registered each ROI into the space of each subject’s functional data time series using the inverse of the transform for that subject into the standard MNI space of the source ROIs (Jenkinson et al., 2012). Next, these individual-subject-registered ROIs were binarized and used to mask preprocessed functional data from the corresponding subject to generate a mean time series, representing the mean intensity of ROI voxels over time in that subject. Next, for each subject, a general linear model was constructed with FSL FEAT, using each ROI mean time series as a regressor (Jenkinson et al., 2012). The resulting first-level outputs for each subject represented functional maps of voxels whose time courses were correlated or anticorrelated to the time course associated with each ROI.

3.6.3.4 Second-level analyses

For each ROI, the maps of functional connectivity across all individuals were entered into second-level multivariate regression analyses, accounting for effects of treatment type, patient age, and patient sex, to identify predictors and correlates of treatment. The goal of these analyses was to identify first predictors, and then correlates, of treatment response. The structure of the general linear model for each of these second-level analyses is described below.

3.5.3.5.1 Predictors of Response

To identify group-level fMRI predictors of response, resting state functional connectivity maps for each ROI in all pre-treatment scans were entered into a second-level analysis using a general linear model that incorporated a set of six explanatory variables. The first explanatory variable modelled mean group functional connectivity to each ROI by assigning each subject a value of +1. The second explanatory variable modelled the degree of improvement, which was calculated as percent change in HRSD score from
baseline to the first follow-up visit. As the distribution of outcomes on this measure was non-unimodal (see Section 4.2), a non-parametric approach was used, in which each subject’s percentage improvement was converted into a rank order from lowest to highest improvement across all the individuals in the sample. These ranks were then centered (de-meaned) so as to be orthogonal with respect to the group mean. The third explanatory variable modelled treatment group (iTBS or 10 Hz), with arbitrary units of +1 for all subjects in the iTBS treatment group and -1 for all subjects in the 10 Hz treatment group. The fourth explanatory variable modelled the interaction between response and treatment group. This value was calculated by multiplying each subject’s de-meaned ranked improvement (i.e., improvement) by his or her group indicator (i.e., treatment group; value of 1 or -1) from the second predictor variable. The fifth and sixth explanatory variables modelled each subject’s age and sex (both de-meaned; sex itemized as values of 1 for females and 0 for males), as covariates of no interest, to account for differences in baseline ROI functional connectivity associated with the demographic characteristics of the individuals in the sample.

The linear model was fit using a mixed effects model (FLAME 1, FMRIB’s Local Analysis of Mixed Effects) (Jenkinson et al., 2012). This process uses Bayesian modelling to estimate mixed effects variance (the sum of fixed effects and random effects variance) and fit the model, identify voxels near a specified threshold, perform further processing on near-threshold voxels to estimate parameters using Metropolis-Hastings Markov Chain Monte Carlo (MH MCMC) sampling, and finally fit a general t-distribution on which hypothesis testing (i.e., higher-level contrasts) can be performed (Jenkinson et al., 2012). For the whole-brain maps resulting from each of the planned statistical contrasts described below, a cluster Z threshold of 2.3, and a cluster-wise P threshold of 0.05, were used.

After the fitting of the model above for the second-level analysis, the following set of planned statistical contrasts were performed in FEAT: 1) “Mean correlated functional connectivity,” entered as the vector [1 0 0 0 0 0] across the six explanatory variables described above; 2) “Positive correlation between baseline functional connectivity and
improvement," entered as the vector [0 1 0 0 0 0]; 3) “Negative correlation between baseline functional connectivity and improvement,” entered as the vector [0 -1 0 0 0 0]; 4) “Baseline functional connectivity differentially associated with iTBS,” entered as the vector [0 0 1 0 0 0]; 5) “Baseline functional connectivity differentially associated with 10 Hz,” entered as the vector [0 0 -1 0 0 0]; 6) “Baseline functional connectivity differentially associated with greater improvement after iTBS,” entered as the vector [0 0 0 1 0 0]; 7) “Baseline functional connectivity differentially associated with greater improvement after 10 Hz,” entered as the vector [0 0 0 -1 0 0]; additionally, for the explanatory variables “age” and “sex,” two contrasts each were designed to account for functional connectivity differences resulting from demographic characteristics.

Finally, for peak regions emerging in selected whole-brain maps resulting from these contrasts, parameter estimates were extracted for each individual in each of the tertiles, in order to compare the resting state connectivity in each of these groups in absolute terms. Parameter estimates of each individual subject's functional connectivity from each seed ROI to peak regions (mean z-score) were extracted by first visually inspecting the whole-brain maps from the contrasts above. Voxels with peak intensity in each region were selected as the centers of 6 mm spheres. Spheres were registered from standard space to subjects' functional data based on subject-specific transformation matrices. Finally, the “fslmaths” tool (Jenkinson et al., 2012) was used to extract mean Z-scores for each sphere from individual subjects' pre-treatment ROI connectivity maps. These values were extracted by masking image outputs containing Z-statistic data for significant voxels with all peak cluster ROIs in subjects' functional space. Bar graphs of the means and standard error of the parameter estimates in each group were plotted using Microsoft Excel.

3.5.3.5.2 Correlates of Response

Next, to identify group-level fMRI correlates of response (i.e., changes from the pre- to the post-treatment MRI relating to treatment response), a second set of multivariate regression analyses were conducted. This set of analyses paralleled the predictors-of-response analyses above, except that the inputs were not the pre-treatment functional
connectivity maps but rather the pre-versus-post treatment maps of change in functional connectivity, for each ROI in each subject. To generate the change maps, first, subject-level “subtraction maps” were acquired by performing a within-subjects, fixed-effects general linear model analysis in each subject. This produced a set of individual-subject contrast maps of post-treatment minus pre-treatment resting-state functional connectivity to the ROI in question. These subject-level “subtraction maps” were then input into the second-level, group-level multivariate regression analyses as described in the previous section. The set of explanatory variables in the general linear model for this analysis was the same as in the previously described analysis above, and after model fitting, the same set of eleven statistical contrasts were performed.

Likewise, for parameter estimation and comparison of the absolute values of regional change across groups, estimates of subjects’ functional connectivity from each ROI to peak regions (mean z-score) were extracted according to the same method described above (Section 3.5.3.5.1). For parameter estimation in this case, subject-level mean Z-scores were extracted from both the pre-treatment and post-treatment ROI connectivity maps in order to assess resting-state functional connectivity before and after treatment in absolute terms across groups.
4.0 Results

4.1 Demographic Characteristics

A total of 303 patients underwent left dlPFC-rTMS. The group that received iTBS included 149 subjects (92 female, 57 male; mean age 42.10 ± SD 10.57). The group that received 10 Hz rTMS included 154 subjects (87 female, 67 male; mean age 43.39 ± SD 12.46). Overall, the proportion of females and males did not differ between groups (Fisher’s exact test, p = 0.413). The groups did not significantly differ in age (unpaired, two-tailed t-test, t_{301} = 0.96, p = 0.333) (Table 1).

<table>
<thead>
<tr>
<th>Overall</th>
<th>Group A (iTBS)</th>
<th>Group B (10 Hz)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td># Patients</td>
<td>303</td>
<td>149</td>
<td>154</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>179</td>
<td>92</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>42.76 ± 11.57</td>
<td>42.10 ± 10.57</td>
<td>43.39 ± 12.46</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Table 1. Demographic characteristics. There were no differences in proportion of females or age between treatment groups. P-values are for Fisher’s exact test for proportion comparisons and for two-sample t-statistics for continuous variable comparisons. Values for age represent group means, plus or minus standard deviation.
4.2 Clinical Outcomes

We next compared HRSD scores, pre-treatment and post-treatment, between iTBS and 10 Hz groups. These results are summarized in Table 2 and Figure 4. There was no significant difference in HRSD score between treatment groups prior to treatment (iTBS, 23.55 ± SD 4.32; 10 Hz, 23.44 ± SD 4.55; t = 0.20, p = 0.841), or following treatment (iTBS, 13.97 ± SD 7.75; 10 Hz, 14.11 ± SD 8.01; t = 0.15, p = 0.874). There was no significant difference in overall percent improvement (iTBS, 40.82% ± SD 32.56%; 10 Hz, 39.61% ± SD 32.65%; t = 0.32, p = 0.748). A two-way ANOVA revealed a significant main effect of time ($F(1,301) = 463.931$, $p < 0.0001$), but not of group ($F(1,301) = 0.001$, df = 1, p = 0.972) or interaction between time and group ($F(1,301) = 0.079$, p < 0.779). Plotted week-by-week, the trajectories of improvement in the two groups appeared strikingly similar across the entire course of treatment and follow-up (Figure 4).
Clinical Outcomes in Treatment Groups

<table>
<thead>
<tr>
<th>HRSD Score</th>
<th>Overall</th>
<th>Group A (iTBS)</th>
<th>Group B (10 Hz)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>23.49 ± 4.43</td>
<td>23.55 ± 4.32</td>
<td>23.44 ± 4.55</td>
<td>0.20</td>
<td>0.841</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>14.04 ± 7.87</td>
<td>13.97 ± 7.75</td>
<td>14.11 ± 8.01</td>
<td>0.15</td>
<td>0.874</td>
</tr>
<tr>
<td>% Improvement</td>
<td>40.21 ± 32.55</td>
<td>40.82 ± 32.56</td>
<td>39.61 ± 32.65</td>
<td>0.32</td>
<td>0.748</td>
</tr>
</tbody>
</table>

Table 2. HRSD scores in iTBS and 10 Hz groups. Table comparing pre-treatment and post-treatment HRSD scores, and percent improvement from pre-treatment to post-treatment, separately in treatment groups. P-values are based on two-tailed, unpaired t-tests that compared HRSD pre-treatment and post-treatment scores, and percent improvement, between treatment groups. HRSD values represent the group mean plus or minus standard deviation.

Figure 4. Depression severity during treatment in groups A and B. HRSD scores are illustrated at each measurement point, from baseline (pre-treatment) to follow-up (1 month post-treatment). Comparing treatment groups, there was no significant difference in HRSD score at any timepoint. Error bars represent standard error of the mean.
A Shapiro-Wilk test revealed a significantly non-normal distribution of outcomes in both the iTBS ($W = 0.980, p = 0.03$) and 10 Hz ($W = 0.972, p = 0.003$) groups. Further inspection of kernel density estimates for each group revealed a seemingly trimodal distribution of outcome, with distinct non-responder and responder subgroups, in addition to an intermediate, partial-responder subgroup (Figure 5). In light of these findings, we performed a non-parametric (two-sample Kolmogorov-Smirnov) comparison of the cumulative distribution functions for the degree of improvement (calculated as percent improvement from baseline to first follow-up) across all subjects in each group (Figure 6). Once again, there was no significant difference in the distribution of clinical outcomes between the two treatment groups ($D = 0.059, p = 0.954$).
Outcome Distribution in Treatment Groups

Figure 5. Kernel density estimates of outcomes. Kernel density estimates of the distribution of outcomes (expressed as percent improvement from pre-treatment to first post-treatment follow-up) in patients receiving iTBS or 10 Hz rTMS on HRSD measures. HRSD, Hamilton Rating Scale for Depression. iTBS, intermittent theta burst stimulation.

Figure 6. Empirical cumulative distribution function of outcomes. Empirical cumulative distribution function plot comparing the outcome (expressed as percent improvement from pre-treatment to first post-treatment follow-up) in patients receiving iTBS or 10 Hz rTMS on HRSD measures. A Kolmogorov-Smirnov test revealed no significant difference in these distributions. HRSD, Hamilton Rating Scale for Depression. iTBS, intermittent theta burst stimulation.
In light of the trimodal distribution noted above, for subsequent analyses, each group was divided into tertiles representing the top, middle, and bottom thirds of response, hereafter termed ‘upper tertile,’ ‘middle tertile,’ and ‘lower tertile.’ Since treatment groups did not have an exactly equal number of subjects, response segmentation occurred at marginally different levels between iTBS and 10 Hz groups (iTBS: upper tertile > 58.6% improvement; lower tertile < 23.8% improvement; 10 Hz: upper tertile > 59.0% improvement; lower tertile < 24.1% improvement). However, neither pre-treatment nor post-treatment HRSD scores were significantly different between groups for either the ‘lower tertile’ or ‘upper tertile’ outcome categories (Table 3). In the lower tertiles from iTBS and 10 Hz groups, there were no significant differences in average pre-treatment HRSD score (t = 0.386, p = 0.700), post-treatment HRSD score (t = 0.445, p = 0.656), or overall percent improvement (t = 0.618, p = 0.537). In the upper tertiles from iTBS and 10 Hz groups, there were no significant differences in average pre-treatment HRSD score (t = 0.132, p = 0.894), post-treatment HRSD score (t = 0.146, p = 0.883), or overall percent improvement (t = 0.490, p = 0.624).

<table>
<thead>
<tr>
<th>HRSD Score</th>
<th>Lower Tertile</th>
<th>Upper Tertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Pre-Treatment</td>
<td>23.54 ± 3.62</td>
<td>23.23 ± 4.27</td>
</tr>
<tr>
<td>Post-Treatment</td>
<td>22.29 ± 3.65</td>
<td>22.71 ± 4.98</td>
</tr>
<tr>
<td>% Improvement</td>
<td>3.76 ± 17.75</td>
<td>1.61 ± 17.18</td>
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</tbody>
</table>

**Table 3. Clinical outcomes in lower and upper tertiles.** This table compares pre-treatment and post-treatment HRSD scores, and percent improvement from pre-treatment to post-treatment, separately in ‘lower’ and ‘upper’ tertiles, each of which comprise one-third of the group sample size, as described in **Section 4.2.** T-values and P-values were calculated from two-tailed, unpaired T-tests.
4.3 Neuroimaging Results

4.3.1 Predictors of Response

Pre-treatment resting state fMRI revealed regions in which higher baseline functional connectivity was associated with poorer clinical outcome for the seed ROIs from the SN, DC, VMN, NAcc, aMCC, and VC. No significant results were identified from the left dIPFC seed ROI. There were no regions for which higher baseline functional connectivity was associated with better clinical outcome for any of the seed ROIs examined (SN, VMN, dIPFC, aMCC, DC, VC, or NAcc). There were also no regions for which baseline connectivity was differentially associated with clinical outcome in either treatment group, for any of the seed ROIs examined (SN, VMN, dIPFC, aMCC, DC, VC, or NAcc).

From the SN seed, poorer outcomes were associated with higher pre-treatment connectivity to the right lateral orbitofrontal cortex (IOFC) \((X +32, Y +16, Z -20; Z = 4.50, p < 0.00001)\), right hippocampus \((X +24, Y -24, Z -14; Z = 3.99, p < 0.0001)\), right posterior superior temporal gyrus (STG) \((X +48, Y -24, Z -2; Z = 3.49, p < 0.001)\), right amygdala \((X +20, Y -2, Z -20; Z = 3.46, p < 0.001)\), and right intracalcarine cortex \((X +6, Y -82, Z 0; Z = 3.30, p < 0.001)\) (Table 4; Figures 7, 8, 9). Parameter estimates revealed that pre-treatment functional connectivity to the IOFC and STG was positive in all subjects, with highest values in lower tertile subjects, and lowest values in upper tertile subjects. Pre-treatment functional connectivity to the right hippocampus was negative in all subjects, with lowest values in upper tertile subjects. Pre-treatment functional connectivity to the right amygdala was positive in lower tertile subjects, and negative in upper tertile subjects.

From the DC seed, poorer outcomes were associated with higher pre-treatment connectivity to the left middle temporal gyrus \((X -60, Y -18, Z -8; Z = 3.70, p < 0.001)\) and left opercular cortex \((X -58, Y -22, Z +14; Z = 3.54, p < 0.001)\) (Table 4).
From the VMN seed, poorer outcomes were associated with higher pre-treatment connectivity to the left lateral occipital cortex (X -14, Y -80, Z +48; Z = 4.38, p < 0.0001), right superior parietal lobule (X +32, Y -48, Z +54; Z = 4.12, p < 0.0001), left middle temporal gyrus (X -58, Y -52, Z -2; Z = 4.10, p < 0.0001), left temporal pole (X -54, Y +6, Z -10; Z = 3.58, p < 0.001), and posterior cingulate cortex (X -12, Y -24, Z +42; Z = 3.41, p < 0.001) (Table 4).

From the NAcc seed, poorer outcomes were associated with higher pre-treatment connectivity to the left middle frontal gyrus (MFG) (X -42, Y +16, Z +48; Z = 4.23, p < 0.0001) and left middle temporal gyrus (MTG) (X -60, Y -18, Z -10; Z = 4.10, p < 0.0001) (Table 4; Figures 10, 11, 12). Parameter estimates revealed that pre-treatment functional connectivity to the left MFG and MTG was positive in all subjects, with lowest values in upper tertile subjects. For the MFG, values were highest in lower tertile subjects in both treatment groups. For the MTG, values were highest in middle tertile subjects (iTBS) and lower tertile subjects (10 Hz).

Regarding the exploratory ROIs: from the aMCC seed, poorer outcomes were associated with higher pre-treatment connectivity to the left middle temporal gyrus (X -48, Y -34, Z -2; Z = 4.48, p < 0.00001), right posterior superior temporal gyrus (X +64, Y -24, Z 0; Z = 4.45, p < 0.00001), and left opercular cortex (X -54, Y -2, Z +8; Z = 3.55, p < 0.001) (Table 4). From the VC seed, poorer outcomes were associated with higher pre-treatment connectivity to the left frontal pole (X -8, Y +62, Z +20; Z = 3.82, p < 0.001), left middle frontal gyrus (X -36, Y +14, Z +46; Z = 3.78, p < 0.001), and precuneus cortex (X -10, Y -58, Z +46; Z = 3.59, p < 0.001) (Table 4).
**Figure 7. Predictors of response: baseline salience network connectivity is negatively associated with improvement.** Baseline rs-FC from the cortical SN nodes to the regions in red was negatively associated with improvement. These results imply that responders exhibited lower baseline connectivity between these regions, and non-responders exhibited higher baseline connectivity between these regions. Images are thresholded at $3 < Z < 6$. 

<table>
<thead>
<tr>
<th>Predictors of Response</th>
<th>Anatomical Views</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Intracalcarine Cortex</td>
<td><img src="image16" alt="Image" /></td>
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Baseline Connectivity from SN Compared to HRSD Improvement

Figure 8. Baseline connectivity from the salience network compared to HRSD improvement. There was a negative correlation between baseline connectivity from the SN to several regions, and depressive symptom improvement. This indicates that subjects who improved to a greater degree began treatment with lower functional connectivity between the SN and these regions, and subjects who improved to a lesser degree began treatment with higher functional connectivity between the SN and these regions. Percent change from baseline to follow-up one (1-week post-treatment) was calculated to represent HRSD improvement. Here, these scores are compared to Z-scores that represent functional connectivity from the salience network. SN, salience network; HRSD, Hamilton Rating Scale for Depression; IOFC, lateral orbitofrontal cortex; STG, superior temporal gyrus.
Parameter estimates revealed a trend of higher baseline functional connectivity in lower tertiles, and lower baseline functional connectivity in higher tertiles, from the SN ROI to the lateral orbitofrontal cortex (IOFC), hippocampus (HC), superior temporal gyrus (STG) and amygdala (Amyg). Error bars represent standard error of the mean.

**Figure 9a.** Baseline connectivity from the salience network in response tertiles. Parameter estimates revealed a trend of higher baseline functional connectivity in lower tertiles, and lower baseline functional connectivity in higher tertiles, from the SN ROI to the lateral orbitofrontal cortex (IOFC), hippocampus (HC), superior temporal gyrus (STG) and amygdala (Amyg). Error bars represent standard error of the mean.
Parameter estimates revealed a trend of higher baseline functional connectivity in lower tertiles, and lower baseline functional connectivity in higher tertiles, from the SN ROI to the lateral orbitofrontal cortex (lOFC), hippocampus (HC), superior temporal gyrus (STG) and amygdala (Amyg). Error bars represent standard error of the mean.

**Figure 9b. Baseline connectivity from the salience network in response tertiles.**
Figure 10. Predictors of response: baseline nucleus accumbens connectivity is negatively associated with improvement. Baseline rs-FC from the left nucleus accumbens to the regions in red was negatively associated with improvement. These results imply that responders exhibited lower baseline connectivity between these regions, and non-responders exhibited higher baseline connectivity between these regions. Images are thresholded at $3 < Z < 6$. 
Figure 11: Baseline connectivity from the left nucleus accumbens compared to HRSD improvement. There was a negative correlation between baseline connectivity from the L NAcc to several regions, and depressive symptom improvement. This indicates that subjects who improved to a greater degree began treatment with lower functional connectivity between the L NAcc and these regions, and subjects who improved to a lesser degree began treatment with higher functional connectivity between the L NAcc and these regions. Percent change from baseline to follow-up one (1-week post-treatment) was calculated to represent HRSD improvement. Here, these scores are compared to Z-scores that represent degree of functional connectivity from the left nucleus accumbens. L NAcc, left nucleus accumbens; HRSD, Hamilton Rating Scale for Depression.
Figure 12. Baseline connectivity from the left nucleus accumbens in response tertiles. Parameter estimates revealed a trend of higher baseline functional connectivity in lower tertiles, and lower baseline functional connectivity in higher tertiles, from the NAcc ROI to the middle frontal gyrus (MFG) and middle temporal gyrus (MTG). Error bars represent standard error of the mean.
Table 4. Predictors of response. Significant voxels of local maximum intensity are represented by anatomical name, Brodmann area, and coordinate in MNI standard space. Connectivity between seed regions and local maxima were negatively associated with symptom improvement.
4.3.2 Correlates of Response

Resting state fMRI revealed regions in which pre- to post-treatment increases in functional connectivity were associated with better clinical outcome for the seed ROIs from the DC, VMN, NAcc, and VC; and regions in which pre- to post-treatment increases in functional connectivity were associated with better clinical outcomes in the iTBS group only, for the aMCC seed ROI. No significant regions of functional connectivity change associated with treatment response were identified from the left dIPFC, aMCC, or SN seed ROIs. There were no regions for which pre- to post-treatment decreases in functional connectivity was associated with better clinical outcome for any of the seed ROIs examined (SN, VMN, dIPFC, aMCC, DC, VC, or NAcc). For the interaction contrasts, there were no regions aside from the aMCC (described above) for which baseline connectivity was differentially associated with clinical outcome in either direction, for any of the seed ROIs examined (SN, VMN, dIPFC, DC, VC, or NAcc).

From the DC seed, better outcomes were associated with pre- to post-treatment increases in functional connectivity to the posterior cingulate cortex (X +8, Y -26, Z +44; Z = 4.38, p < 0.0001), right parietal opercular cortex (X +46, Y -28, Z +20; Z = 4.21, p < 0.0001), anterior cingulate cortex (X +6, Y +44, Z +6; Z = 3.67, p < 0.001), right medial orbitofrontal cortex (X +18, Y +12, Z -14; Z = 3.27, p < 0.01), dorsal anterior cingulate cortex (X +8, Y +30, Z +32; Z = 3.05, p < 0.01), and subcallosal cortex (X +4, Y +14, Z -12; Z = 2.80, p < 0.01) (Table 5; Figure 13). Inspection of parameter estimates revealed an overall trend of decreased pre- to post-treatment functional connectivity in lower tertiles, increased pre- to post-treatment functional connectivity in upper tertiles, and intermediate functional connectivity change in the middle tertile (Figure 14).

Parameter estimates for pre-treatment rs-FC to the PCC were near zero in lower tertiles, and negative in upper tertiles; post-treatment, these values became negative in lower tertiles, and near zero, or slightly above zero, in upper tertiles. Parameter estimates for pre-treatment rs-FC to the right parietal opercular cortex were negative in all subjects; post-treatment, these values became more negative in lower tertiles, and less negative in upper tertiles. Parameter estimates for pre-treatment rs-FC to the ACC,
right mOFC, dACC, and subcallosal cortex were positive in all subjects; post-treatment, these values became less positive in lower tertiles, and more positive in upper tertiles.

From the VMN seed, better outcomes were associated with pre- to post-treatment increases in functional connectivity to the posterior cingulate cortex ($X = -10, Y = -42, Z = +40; Z = 4.44, p < 0.00001$), right lateral prefrontal cortex ($X = +44, Y = +12, Z = +26; Z = 3.88, p < 0.001$), right frontal pole ($X = +24, Y = +56, Z = +12; Z = 3.64, p < 0.001$), and right lateral orbitofrontal cortex ($X = +46, Y = +28, Z = -8; Z = 3.45, p < 0.001$) (Table 5; Figure 17).

Inspection of parameter estimates revealed an overall trend of decreased pre- to post-treatment functional connectivity in lower tertiles, increased pre- to post-treatment functional connectivity in upper tertiles, and intermediate functional connectivity change in the middle tertile (Figure 18). Parameter estimates for pre-treatment rs-FC to the right IOFC, right insular cortex, PCC, and right lateral PFC were positive in all subjects; post-treatment, these values decreased in lower tertiles, and increased in upper tertiles. One exception was in rs-FC to the right lateral prefrontal cortex, where group A’s middle tertile showed a greater post-treatment increase in parameter estimates than the upper tertile.

From the NAcc seed, better outcomes were associated with pre- to post-treatment increases in functional connectivity to the right middle frontal gyrus ($X = +52, Y = +26, Z = +28; Z = 3.76, p < 0.001$), left lateral occipital cortex ($X = -50, Y = -74, Z = +20; Z = 3.74, p < 0.001$), and right frontal pole ($X = +44, Y = +54, Z = +2; Z = 3.50, p < 0.001$) (Table 5).

Regarding the exploratory ROIs: from the VC seed, better outcomes were associated with pre- to post-treatment increases in functional connectivity to the right thalamus ($X = +12, Y = -12, Z = +4; Z = 4.05, p < 0.0001$), posterior cingulate cortex ($X = +4, Y = -24, Z = +30; Z = 3.86, p < 0.001$), right lateral orbitofrontal cortex ($X = +42, Y = +28, Z = -14; Z = 3.58, p < 0.001$), and right insular cortex ($X = +28, Y = +14, Z = -18; Z = 3.41, p < 0.001$) (Table 5; Figure 15). Inspection of parameter estimates revealed an overall trend of decreased pre- to post-treatment functional connectivity in lower tertiles, increased pre- to post-treatment functional connectivity in upper tertiles, and intermediate functional
connectivity change in the middle tertile (Figure 16). Parameter estimates for pre-treatment rs-FC to the right thalamus, PCC, right IOFC, and right insular cortex were positive in all subjects; post-treatment, these values became less positive in lower tertiles, and more positive in upper tertiles.

From the aMCC seed, better outcomes were associated with pre- to post-treatment increases in functional connectivity in the iTBS group only (interaction effect) to the left lingual gyrus (X -22, Y -68, Z -4; Z = 4.97, p < 0.00001) and right lingual gyrus (X +16, Y -76, Z -2; Z = 4.47, p < 0.00001). This was the only finding that indicated an interaction effect between treatment group and response.
Figure 13. Correlates of response: post-treatment increase in dorsal caudate connectivity is positively associated with improvement. Increased post-treatment rs-FC from the DC to the regions in red was positively associated with improvement. These results imply that responders demonstrated increases in rs-FC between these regions compared to non-responders. Images are thresholded at $2.5 < Z < 6$. 
Figure 14a. Functional connectivity change from the dorsal caudate is associated with treatment response. Subjects across both treatment groups were split into response categories characterizing lower, middle, and upper tertiles of response. Average Z-scores in response categories represent functional connectivity from the dorsal caudate nucleus at times Pre (column 1) and Post (column 2) treatment. Column 3 represents overall post-treatment change. Among all subjects, parameter estimates revealed an overall trend of decreased post-treatment functional connectivity in the lower tertile, and increased post-treatment functional connectivity in the upper tertile, from the DC ROI to the posterior cingulate cortex, right parietal opercular cortex, and anterior cingulate cortex. These trends were preserved when evaluating treatment groups independently. Error bars represent standard error of the mean. Significance tests between response groups were unpaired, 2-tailed Student’s T-tests. Significance tests within response groups were paired, 2-tailed Student’s T-tests.
Figure 14b. Functional connectivity change from the dorsal caudate is associated with treatment response. Subjects across both treatment groups were split into response categories characterizing lower, middle, and upper tertiles of response. Average Z-scores in response categories represent functional connectivity from the dorsal caudate nucleus at times Pre (column 1) and Post (column 2) treatment. Column 3 represents overall post-treatment change. Among all subjects, parameter estimates revealed an overall trend of decreased post-treatment functional connectivity in the lower tertile, and increased post-treatment functional connectivity in the upper tertile, from the DC ROI to the right medial orbitofrontal cortex, dorsal anterior cingulate cortex, and subcallosal cortex. These trends were preserved when evaluating treatment groups independently. Error bars represent standard error of the mean. Significance tests between response groups were unpaired, 2-tailed Student’s T-tests. Significance tests within response groups were paired, 2-tailed Student’s T-tests.
Figure 15. Correlates of response: post-treatment increase in ventral caudate connectivity is positively associated with improvement. Increased post-treatment rs-FC from the VC to the regions in red was positively associated with improvement. These results imply that responders demonstrated increases in rs-FC between these regions compared to non-responders. Images are thresholded at $3 < Z < 6$. 

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<tr>
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Figure 16a. Functional connectivity change from the ventral caudate is associated with treatment response. Subjects across both treatment groups were split into response categories characterizing lower, middle, and upper tertiles of response. Average Z-scores in response categories represent functional connectivity from the ventral caudate nucleus at times Pre (column 1) and Post (column 2) treatment. Column 3 represents overall post-treatment change. Among all subjects, parameter estimates revealed an overall trend of decreased post-treatment functional connectivity in the lower tertile, and increased post-treatment functional connectivity in the upper tertile, from the VC ROI to the right thalamus and posterior cingulate cortex. These trends were preserved when evaluating treatment groups independently. Error bars represent standard error of the mean. Significance tests between response groups were unpaired, 2-tailed Student’s T-tests. Significance tests within response groups were paired, 2-tailed Student’s T-tests.
**Connectivity Change from Ventral Caudate Associated with Treatment Response**

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**Figure 16b. Functional connectivity change from the ventral caudate is associated with treatment response.** Subjects across both treatment groups were split into response categories characterizing lower, middle, and upper tertiles of response. Average Z-scores in response categories represent functional connectivity from the ventral caudate nucleus at times Pre (column 1) and Post (column 2) treatment. Column 3 represents overall post-treatment change. Among all subjects, parameter estimates revealed an overall trend of decreased post-treatment functional connectivity in the lower tertile, and increased post-treatment functional connectivity in the upper tertile, from the VC ROI to the right lateral orbitofrontal cortex and right insular cortex. These trends were preserved when evaluating treatment groups independently. Error bars represent standard error of the mean. Significance tests between response groups were unpaired, 2-tailed Student’s T-tests. Significance tests within response groups were paired, 2-tailed Student’s T-tests.
Figure 17. Correlates of response: post-treatment increase in ventromedial network connectivity is positively associated with improvement. Increased post-treatment rs-FC from the VMN to the regions in red was positively associated with improvement. These results imply that responders demonstrated increases in rs-FC between these regions compared to non-responders. Images are thresholded at $3 < Z < 6$. 

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Figure 18a. Functional connectivity change from the ventromedial network is associated with treatment response. Subjects across both treatment groups were split into response categories characterizing lower, middle, and upper tertiles of response. Average Z-scores in response categories represent functional connectivity from the VMN at times Pre (column 1) and Post (column 2) treatment. Column 3 represents overall post-treatment change. Among all subjects, parameter estimates revealed an overall trend of decreased post-treatment functional connectivity in the lower tertile, and increased post-treatment functional connectivity in the upper tertile, from the VMN ROI to the posterior cingulate cortex and right lateral prefrontal cortex. These trends were preserved when evaluating treatment groups independently. Error bars represent standard error of the mean. Significance tests between response groups were unpaired, 2-tailed Student’s T-tests. Significance tests within response groups were paired, 2-tailed Student’s T-tests.
**Figure 18b: Functional connectivity change from the ventromedial network is associated with treatment response.** Subjects across both treatment groups were split into response categories characterizing lower, middle, and upper tertiles of response. Average Z-scores in response categories represent functional connectivity from the VMN at times Pre (column 1) and Post (column 2) treatment. Column 3 represents overall post-treatment change. Among all subjects, parameter estimates revealed an overall trend of decreased post-treatment functional connectivity in the lower tertile, and increased post-treatment functional connectivity in the upper tertile, from the VMN ROI to the right frontal pole and right lateral orbitofrontal cortex. These trends were preserved when evaluating treatment groups independently. Error bars represent standard error of the mean. Significance tests between response groups were unpaired, 2-tailed Student’s T-tests. Significance tests within response groups were paired, 2-tailed Student’s T-tests.
### Table 5a. Correlates of response.

Significant areas of local maximum intensity are represented by anatomical name, Brodmann area, and coordinate in standard space. Increased post-treatment connectivity between seed regions and local maxima were positively associated with symptom improvement.
### Table 5b. Correlates of response

Significant areas of local maximum intensity are represented by anatomical name, Brodmann area, and coordinate in standard space. Increased connectivity between seed regions and local maxima were positively associated with symptom improvement in the iTBS group only. aMCC, anterior mid-cingulate cortex.

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**Increased Functional Connectivity in Group A Responders > Non-Responders**
4.4 Summary

Our findings fall into three categories: clinical and neural outcomes across treatment groups, neural predictors of response, and neural correlates of response.

We hypothesized that clinical outcomes would be similar in iTBS and 10 Hz treatment groups. The distribution of clinical outcomes (measured by percent symptom improvement from pre-treatment to 1-week follow-up) was not significantly different in treatment groups. Both groups showed non-unimodal distributions, and comparison of clinical outcomes between the lower, middle, and upper tertiles of response did not reveal significant differences in pre-treatment HRSD score, post-treatment HRSD score, or percent improvement between treatment groups. Our observation that clinical outcomes were not significantly different between treatment groups is supportive of this hypothesis.

We also hypothesized that neural predictors and correlates of treatment response would not differ between treatment groups. Only one effect of interaction between treatment group and clinical outcome was observed. In the iTBS group, better clinical outcomes were associated with increased pre- to post-treatment functional connectivity between the aMCC and the left and right lingual gyri. Because only one significant interaction effect emerged across all seed ROIs, and because clinical outcomes were comparable between iTBS and 10 Hz treatment groups, our observations are predominantly supportive of this hypothesis.

We hypothesized that better clinical outcomes would be predicted by lower baseline functional connectivity through the salience network corticostriatal loop. Analysis of pre-treatment fMRI scans in all subjects revealed several functional predictors of treatment response. There was a negative correlation between clinical outcomes and baseline functional connectivity between:

- The SN and the right IOFC, right hippocampus, right posterior STG, and right amygdala;
- The DC and the left MTG and left opercular cortex;
• The **VMN** and the left lateral occipital cortex, right superior parietal lobule, left MTG, left temporal pole, and PCC;
• The **NAcc** and the left MFG and left MTG;
• The **aMCC** and the left MTG, right posterior STG, and left opercular cortex;
• The **VC** and the left frontal pole, left MFG, and precuneus.

Our predictor findings from the SN ROI, in addition to the lack of findings from the left dIPFC ROI, are not supportive of hypothesis.

Finally, we hypothesized that symptom improvement would be associated with increased pre- to post-treatment functional connectivity (a) throughout the SN corticostriatal loop, and (b) between the SN and VMN. Analysis of the pre- to post-treatment change in rs-FC across all subjects revealed several correlates of treatment response. There was a positive correlation between clinical outcomes and increased pre- to post-treatment functional connectivity between:

• The **DC** and the PCC, right parietal opercular cortex, ACC, right mOFC, dACC, and subcallosal cortex;
• The **VMN** and the PCC, right lateral PFC, right frontal pole, and right IOFC;
• The **NAcc** and the right thalamus, PCC, right IOFC, and right insular cortex.

Our observations that better clinical outcome was associated with increases in rs-FC between the DC and dACC (part A), and between the DC and ACC, and DC and right mOFC (part B), are supportive of this hypothesis.

With these principal observations summarized, we may now turn to a general discussion of the key findings in the context of the original aims of the study.
5.0 General Discussion

5.1 Significance of the Project

The key aims for this project were to identify functional MRI predictors and correlates of response in MDD patients receiving left dlPFC-rTMS with iTBS and 10 Hz stimulation protocols, and to evaluate whether the two protocols produced comparable clinical and functional outcomes.

Our findings represent a step toward understanding three factors that are necessary for improving dlPFC-rTMS for patients with TRD. First, the findings demonstrate that iTBS and 10 Hz stimulation are equally effective in achieving symptom improvement. This encouraging result indicates that patients with TRD can be treated with shorter rTMS protocols, allowing more patients to be treated at reduced overall costs. Additionally, these findings provide insight into characteristics of baseline functional connectivity, and pre- to post-treatment functional connectivity changes, that are associated with symptom improvement. Essentially, such findings shed light on which patients are most likely to respond to rTMS, and the mechanisms by which rTMS exerts a therapeutic effect.

5.2 Evaluation of Clinical Outcomes

Improving rTMS treatment for patients with TRD requires refining and streamlining stimulation protocols. Thus, this project was conducted in the context of a large-scale, multi-site RCT comparing the effectiveness of two rTMS protocols: iTBS and 10 Hz rTMS. A previous report demonstrated that clinical outcomes following dmPFC-iTBS, a faster rTMS protocol with different stimulation parameters, were comparable to those of dmPFC-10 Hz rTMS (Bakker et al., 2015); 10 Hz rTMS is a well-accepted and widely utilized rTMS protocol for treatment of MDD (George et al., 2010; O'Reardon et al., 2007). However, no previous studies have directly compared functional MRI predictors or correlates of these rTMS protocols. On this basis, we hypothesized that both iTBS
and 10 Hz stimulation would achieve comparable clinical and neural outcomes in patients with TRD.

Both treatments appeared equally tolerable. There were no patients who exited treatment due to extreme, unpleasant side effects. Other reported side effects were minimal and infrequently presented as transient headaches or pain at the stimulation site, which was self-treated with over-the-counter pain medication.

We found that the distribution of response (represented by percent reduction in symptom severity from pre-treatment to 1-week follow-up) between iTBS and 10 Hz treatment groups was comparable, and there were no significant differences in HRSD score at any time points between baseline and follow-up one (one week post-treatment). Additionally, HRSD score was not significantly different between treatment groups at two other follow-up points (one month, three months). With regards to differences in functional predictors and correlates of outcome, we observed only one significant interaction effect between treatment group and degree of improvement, indicating that combining both treatment groups for analysis did not obscure any other treatment-related effects (neural predictors and correlates are discussed in depth in Section 5.3 below).

These findings support the conclusions of the previous case series described above (Bakker et al., 2015). Further, the similarity in long-term outcomes, as measured by follow-up HRSD scores, suggests that clinical improvement observed with iTBS is as robust as the conventional stimulation protocol, and does not decrease over time. The effectiveness of iTBS is encouraging: if clinics continue to operate at maximum capacity, a reduction in treatment length from ~38 minutes (i.e., 10 Hz stimulation) to ~3 minutes (i.e., iTBS) would lead to a several-fold increase in the number of patients who could be treated with each rTMS device. With over 200,000 adults suffering from TRD in Ontario alone, this represents a much-needed improvement in the potential accessibility of effective brain stimulation treatments (Downar and Daskalakis, 2013). Accordingly, implementing iTBS in wider practice would reduce treatment costs per patient, thereby
making rTMS treatments more cost-effective. Broader and more detailed evaluations of these outcomes will be explored in upcoming publications by the primary investigators of the RCT. With the acknowledgement that clinical outcomes of iTBS and 10 Hz showed no significant differences, we may now turn to discussion of the primary neuroimaging aims and outcomes.
5.3 Evaluation of Neuroimaging Outcomes

5.3.1 Predicting Response with Baseline Functional Connectivity

Of particular interest to this project were functional predictors of response to left dIPFC-rTMS in the cortico-striatal-thalamo-cortical loop circuit of the salience network. Functional integrity of the SN circuit, which includes nodes in the left dIPFC, dACC, and bilateral anterior insula, may be integral to cognitive control and mood regulation (Peters et al., 2016), and dysfunction in SN nodes has been proposed to underlie the pathophysiology of MDD (Geller et al., 1997; George et al., 1995; Goodkind et al., 2015). On this basis, we hypothesized that better clinical outcomes would be predicted by lower baseline functional connectivity through nodes of the SN corticostriatal loop in both treatment groups. The observed lack of differences in clinical outcomes between treatment groups gave us justification to perform neuroimaging analyses using the full dataset (n = 303) as opposed to separately in each treatment group. Still, we included explanatory variables in the GLM to model patterns of functional connectivity, and its relation to symptom improvement, that could be differentially explained by iTBS or 10 Hz treatment allocations.

Interestingly, all neural predictors of response were identified on one contrast of interest: negative correlation between rs-FC and clinical improvement. Thus, predictors of rTMS treatment response were identified in regions where low pre-treatment resting state functional connectivity to the seed ROIs was correlated to better clinical outcome. No significant regions were identified in the contrasts that modeled (a) positive correlation between baseline rs-FC and clinical outcome, or (b) interaction between treatment group and clinical outcome.

Overall, these findings support the notion that both iTBS and 10 Hz stimulation have similar baseline rs-FC predictors of response. However, contrary to our hypothesis, treatment response was not predicted by baseline functional connectivity within the SN, but rather between the SN and ventral cortical regions involved in affective processing. This suggests that a specific pattern of SN rs-FC to other functional networks may be
more predictive of response to left dlPFC-rTMS than functional connectivity within the SN alone. Better clinical outcomes were also predicted by lower baseline functional connectivity from a subcortical component of the VMN—the left NAcc—to a region of the left lateral PFC involved in executive planning and control. Similarly, it could be suggested that patients who responded to left dlPFC-rTMS were characterized by deficient interactions between attentional and affective functional networks, instead of deficient functional connectivity within the SN corticostriatal loop. This provides support for the postulation that treatment responders exhibit dysfunctional connectivity between reward and modulatory systems (Dunlop et al., 2015a, 2015b; Lantrip et al., 2017).

We observed that connectivity from the entire SN ROI—rather than its individual cortical nodes in the dlPFC and aMCC—was predictive of response. These findings contrast with previous findings that have identified baseline activity in the ACC and dlPFC as positive predictors of rTMS response (Baeken et al., 2009). Specifically, we observed that lower baseline rs-FC from the SN to the right hippocampus, right amygdala, and right lOFC was associated with better outcomes following treatment. The hippocampus, amygdala, and OFC comprise a functional affective circuit which consistently shows reduced gray matter volume in MDD (Bora et al., 2012). The hippocampus is well established as a center of episodic memory function, but is also associated with regulation of emotional stress (Sinha et al., 2004), informed decision-making (Ernst et al., 2002), and craving (Pelchat et al., 2004); the amygdala has a primary role in emotional reactivity and fear. The right lOFC plays a role in a variety of functions, including inhibition of behaviour (Del-Ben et al., 2005; Völlm et al., 2006) and adverse emotion (Berthoz et al., 2002), and conflict or reward-based decision-making (Rogers et al., 1999). The overlap of affective and executive function among these functionally connected regions places them in an interesting juncture that seems to require input from networks involved in both behavioural regulation and appetitive stimulus valuation.

In some individuals with MDD, hyperactivity throughout the OFC-amygdala circuit is thought to represent compensatory activation that functions to reduce negative emotional reactivity (Fettes et al., 2017). On this basis, low regulation of the circuit’s
activity by the SN could play a role in MDD; consequently, left dIPFC-rTMS may beneficially impact cortical SN regulation over this affective circuit. Such treatment effects may not be therapeutically useful for non-responders, who presumably represent a proportion of individuals with already-high baseline connectivity between the SN and OFC-amygdala circuit. Of course, our observations are limited such that we did not examine rs-FC from seed ROIs in the IOFC, hippocampus, or amygdala alone; thus, we cannot say with certainty that this circuit was hyperactive in treatment responders. In light of more recent probes into new rTMS stimulation targets, non-responders to left-dIPFC rTMS may benefit more from dmPFC- or OFC-rTMS, both of which have been suggested to selectively treat particular ‘functional subtypes’ of MDD (Downar et al., 2014; Downar and Daskalakis, 2013; Fettes et al., 2017).

Our initial prediction that responders to left dIPFC-rTMS would be characterized by reduced integrity throughout the SN’s nodes was not supported; instead, we observed that responders showed reduced rs-FC from the SN to a ventral and orbitofrontal affective circuit. As such, it is imperative to investigate the relationship not only between treatment response and functional connectivity within the SN, but also to evaluate the SN’s effective role in modulating the activity of other functional networks based on its assessment of motivationally salient stimuli. The path from motivation and urge to response selection requires a ‘gatekeeper’ network to choose adaptive behaviours, but this network must also interact effectively with upstream affective valuation circuits to avoid pathological valuation of rewarding stimuli.

5.3.2 Pre- to Post-Treatment Changes in Functional Connectivity

Also of interest to this project were correlates of treatment response—i.e., pre- to post-treatment changes in functional connectivity within the cortico-striatal-thalamo-cortical loop circuit of the salience network, and between SN and the VMN, that were associated with clinical outcome. Interactions between the SN and VMN may be important for appropriate coordination of attention toward valenced motivational stimuli; for example, abnormal VMN activation during negative stimulus evaluation has been noted in individuals with MDD (Tremblay et al., 2005). On this basis, we hypothesized
that better clinical outcomes would be associated with increases in pre- to post-
treatment functional connectivity within the SN cortico-striatal loop, and between nodes
of the SN and VMN. Using the same explanatory variables and contrasts of interest as
the ‘predictors’ analysis, we performed neuroimaging analyses in the full dataset (n =
303) to identify ‘correlates’ of treatment response.

Interestingly, the majority of neural correlates of response were identified in one
contrast of interest: increased rs-FC correlated with better treatment outcomes. Thus,
increases, but not decreases, in functional connectivity to specific ROIs were associated
with better outcome. This suggests that, overall, response to both stimulation protocols
involved increased cortico-cortical and cortico-striatal rs-FC; conversely, non-response
to both stimulation protocols appeared to be associated with decreased rs-FC.

We found that treatment response was correlated with increased pre- to post-treatment
functional connectivity among cortico-striatal nodes of the SN. Specifically, positive
treatment response was associated with increased rs-FC from a subcortical component
of the SN (DC) to a cortical component of the SN (dACC). rTMS is shown to increase
endogenous dopamine release in the left DC (Strafella et al., 2001), which has direct
and indirect effects on the functional activity of downstream interconnected regions; in
this case, increased neurotransmission in the DC may have resulted in increased
functional connectivity to the dACC, which is involved in executive behavioural control
(Burton et al., 2001; Kübler et al., 2006; Sarazin et al., 1998) and planning (Crozier et
al., 1999; De Waele et al., 2001). rTMS-induced increases in striato-cortical SN
connectivity could represent improved capacity for attentional and behavioural control.
Although interpretation of this finding is limited by a lack of data reflecting cognitive
function (e.g., response inhibition), other studies have similarly reported that left dlPFC-
rTMS improved depressive symptoms and also improved executive function, reflected
by better performance on a Stroop task (Concerto et al., 2015).

We also found that treatment response was correlated with increased pre- to post-
treatment functional connectivity between nodes of the SN and VMN. Specifically,
positive treatment response was associated with increased rs-FC from a subcortical component of the SN (DC) to a cortical component of the VMN (right mOFC). In light of the previous finding, the DC’s role as a subcortical hub for cognitive control could indicate that left dlPFC-rTMS consequentially affects cognitive and attentional regulation of dysfunctional appetitive processes. The right mOFC has direct connections to both the amygdala and dorsolateral PFC (Johnstone et al., 2007); further, the mOFC is shown to coactivate with a variety of affective regions, including (but not limited to) the subgenual cingulate cortex, PCC, and ventral striatum (Zald et al., 2014). Response to left dlPFC-rTMS could be mediated by a strengthened functional relationship between cognitive regions and affective areas, linked via the DC. This is in line with previous findings that individuals with MDD show abnormal physiological coupling between the mOFC and caudate nucleus (Gao et al., 2016), and with suggestions that rTMS response necessitates enhanced cognitive control over the VMN to extinguish pathological incentive salience (Dunlop et al., 2015a).

Additionally, improvement was associated with increased rs-FC from the VMN to the right IOFC, a region of the VMN itself. The IOFC is functionally coupled with cognitive control regions (Zald et al., 2014) including the dlPFC and dmPFC. In MDD, OFC dysfunction may reflect decreased or maladaptive valuation of otherwise rewarding stimuli (Fettes et al., 2017). On this basis, our observation of increased functional connectivity within the VMN may reflect an improved ability to assign appropriate valence to normally rewarding stimuli, as well as to reduce pathological salience of aversive stimuli (Chaudhury et al., 2015).

Although not explicitly an SN-VMN interaction, improvement was also associated with increased rs-FC from the exploratory VC ROI to the right IOFC. The ventral caudate nucleus is located in an anatomically intermediate position between the DC and NAcc; its role mirrors its anatomy, as it has reciprocal connections with both dorsal cognitive and ventral affective regions (Haber and Knutson, 2009). Since improvement was predicted by low baseline connectivity from the SN to the right IOFC, it is possible that this particular finding represents restored function of a ‘line of communication’ between
dorsal modulatory regions and an orbitofrontal area involved in conflict and reward-based decision-making.

Interestingly, based on evaluation of parameter estimates, the neural correlates of improvement described above showed decreased pre- to post-treatment functional connectivity in subjects in lower response tertiles. Other studies have similarly detected opposite patterns of rs-FC change in non-responders compared to responders (Salomons et al., 2014), but the meaning of this observation is unclear and warrants further examination.

We also hypothesized that neural correlates of response would not differ between treatment groups. To account for any effects of treatment group on (a) functional connectivity change and (b) degree of improvement, we included an interaction explanatory variable, as described above. Only one significant result emerged: in the iTBS group, increases in rs-FC from the aMCC to the bilateral lingual gyri were associated with better outcome. A recent voxel-based morphometry (VBM) study identified a positive association between lingual gyrus volume and early antidepressant response; these individuals also showed superior performance on a set of cognitive tasks (Jung et al., 2014). On the assumption that these subjects were not treatment-resistant, and that early treatment response was associated with higher lingual gyrus volume, our observation may suggest that more treatment-resistance is associated with lower lingual gyrus volume. The role of bordering occipital regions in the pathophysiology of MDD has also been noted: for example, individuals with MDD are more likely to demonstrate 'occipital bending' or lobe asymmetry (Maller et al., 2014), and abnormal lingual gyrus structure and function have been reported in individuals with MDD (Dutta et al., 2014). Aside from these findings, major reports on lingual gyrus involvement in the SN or VMN are limited. On this basis, and because the distribution of response in the groups were not significantly different, our findings still overwhelmingly suggest that iTBS and 10 Hz stimulation share common mechanisms that lead to symptom reduction in a proportion of patients. The involvement of the lingual gyrus in relation to rTMS response in MDD could be an avenue for future investigations.
5.3.3 Incidental Findings

Several findings emerged that were outside the scope of our hypotheses. Specifically, connectivity to cortical regions in the temporal and parietal lobes was identified as both predictors and correlates of treatment response.

Improvement was predicted by lower baseline connectivity from the SN to the posterior superior temporal gyrus and right IOFC, which are both implicated in speech: in healthy individuals, these regions demonstrate functional coactivation during the comprehension of emotional prosody in spoken language (Wildgruber et al., 2005). Since orbitofrontal dysfunction can in part drive negative self-thoughts and self-perception in MDD (Fettes et al., 2017), it could be possible that responders to left dlPFC-rTMS also have lower attentional and behavioural control of ruminative processes, language interpretation, and internal dialogues that underlie and exacerbate depressive symptoms.

Additionally, our hypotheses did not consider that the DMN would play a significant role in treatment response. Surprisingly, all three ROIs that exhibited correlates of treatment response (DC, VC, and VMN) also demonstrated increased rs-FC to the PCC, a well-established paralimbic node of the DMN that is involved in internally-directed thought and cognition (Craig et al., 2015; Raichle et al., 2001). This could be interpreted in several ways. First, these findings align with a previous report that individuals with MDD show decreased rs-FC between the PCC and caudate nucleus (Bluhm et al., 2009), and suggests that left dlPFC-rTMS can impact DMN function. There is ample evidence to suggest that recovery from MDD involves increased top-down regulatory control of the DMN, possibly representing an ability to reduce ruminative thought processes that underlie certain depressive symptoms (Lantrip et al., 2017; Liston et al., 2014). This notion is supported by our observations that better clinical outcomes were associated with increased functional connectivity between the DC and PCC, since the DC is a subcortical component of the SN; however, on this basis, it seems unusual that none of the dlPFC, aMCC, or SN ROIs exhibited a similar pattern. On the other hand, worse depressive symptoms have been linked to increased functional connectivity between...
the dACC and PCC during affective interference, representing a maladaptive, not adaptive, interaction between cognitive control and internal attention networks (Kaiser et al., 2014).

In light of improved depressive symptoms, stronger linking of the PCC to the VMN could also reflect a strengthened ability to appropriately tag motivationally salient internal stimuli (e.g., self-generated thought, internal dialogues, memories of past events, or consideration of the future) with positive valence, in contrast to the tendency of depressed individuals to tag normally rewarding stimuli with negative valence (Fettes et al., 2017). This can be further supported by the finding that response was also associated with increased rs-FC from the VMN to the frontal pole—a region heavily involved in consideration of the future (prospection). Taken together, response appears to have been associated with increased rs-FC from the reward system to cortical regions that play a role in self-referential evaluation of motivations and goals (Buckner et al., 2008; Schacter et al., 2007).

In the context of inter-network interactions, increased rs-FC to the PCC from the DC, a cognitive subcortical region; the VMN, a reward network; and the VC, a nucleus that receives and projects information from both dorsal cognitive and ventral affective regions; suggests that response to left dIPFC-rTMS involves functional integration of networks that underlie a wide variety of functions. The PCC is also suggested to play a role in maintaining vigilance in response to environmental change (Hahn et al., 2007; Leech and Sharp, 2014). Thus, increased rs-FC between the PCC, subcortical cognition, and cortical reward nodes could indicate that responders experience improved inter-network communication, resulting in more effective (and realistically-motivated) behavioural control in the context of valenced stimuli. Following the suggestion that the dorsal PCC possesses intricate connections to frontal cognitive areas (Leech and Sharp, 2014), increased connectivity to this region could indicate improvement in both attentional control and stability of network interactions.
5.4 Limitations

This study had several limitations that must be acknowledged. One important limitation in the design of the RCT involved the absence of sham stimulation. Although the non-inferiority design was meant to compare iTBS to the standard of care (i.e., 10 Hz) rather than sham, such a design cannot rule out non-specific effects of the treatments on clinical symptoms and on brain function; instead, only the relative effects of iTBS compared to 10 Hz stimulation can be assessed. Including a sham stimulation control group would have provided a second benchmark for assessing clinical efficacy, and enabled the assessment of non-specific effects (e.g., placebo effects, effectiveness of follow-up treatment, etc.) on brain function. Although sham dlPFC-rTMS has been shown to achieve meagre response and remission rates of 10% and 5%, respectively (Berlim et al., 2014a), the impact of this RCT would have been strengthened by clearly demonstrating that both iTBS and 10 Hz stimulation are equally superior to sham stimulation.

Another significant limitation of this project involves the identification of neural predictors and correlates of treatment outcome without a healthy control group. In comparing parameter estimates between upper, middle, and lower tertiles across treatment groups, an absence of ‘normal’ benchmark values prevents us from claiming that treatment is in fact ‘normalizing’ brain activity. Instead, we are limited to making comparisons between response groups. Unfortunately, the design of this project—in which each group contained ~150 individuals—warrants a massive set of healthy control data to allow for adequately powered and balanced comparisons. Although such datasets can be accessed through the use of large, collaborative networks of data from institutions around the globe (e.g., the Human Connectome project, etc.), a strength of this project was that all pre- and post-treatment scans (n = 303 pre-, 303 post-) were collected using the same MRI scanner with identical acquisition parameters. Until a comparable amount of control data can be collected on the same scanner in the same manner, it will be difficult to establish a closely matched comparator group for the patient groups examined here.
A third limitation is that our set of a priori ROIs was somewhat selective, and the network-level ROIs were not comprehensive. Many other ROIs are known to be important in MDD and rTMS treatment response, but were not examined. Some of these other regions that have shown relevance to rTMS outcome prediction include the ventrolateral PFC (Kito et al., 2008a), rectal gyrus (Kito et al., 2012a), precentral cortex (Mottaghy et al., 2002), temporal cortex (Mottaghy et al., 2002), insula (Kito et al., 2008a), amygdala (Furtado et al., 2013), subgenual cingulate cortex (Fox et al., 2012), hippocampus (Furtado et al., 2012), and putamen (Hernández-Ribas et al., 2013), among others (Silverstein et al., 2015). However, examining functional connectivity to all of these ROIs would have exacerbated the problem of multiple comparisons, in which each additional factor of interest increases the likelihood of identifying false positives. Additionally, interpretation of findings from our network-level ROIs may be limited such that both the SN and VMN ROIs included cortical, but not subcortical, regions of each network. Given the amount of predictors and correlates that emerged from our selected subcortical ROIs, and given the integral roles played by these subcortical nodes, it is possible that our network-level analyses did not capture the most critical or most central features among the potential set of neural predictors and correlates in this dataset.

Finally, understanding the therapeutic mechanisms of brain stimulation relies almost entirely on functional brain imaging. As a tool, resting-state and task-based brain imaging enables visualization of healthy and abnormal functional activity, task-specific executive networks, and diagnostically useful information. However, in clinical populations, functional imaging data may be limited by excessive motion artefacts, or by the existence and interpretation of false positives. Projects such as these, which use functional brain imaging in conjunction with brain stimulation techniques to delineate neurophysiological changes within the SN and VMN, are further limited by the fact that pre- to post-treatment functional connectivity changes and symptom improvement cannot be directly demonstrated to be causally connected, due to the correlational nature of the fMRI technique in assessing brain-behaviour relationships (Parnaudeau et al., 2013).
6.0 Conclusions

The SN and VMN demonstrate abnormalities of structure and function across a wide variety of psychiatric illnesses, including both structural changes within singular cortical nodes and functional aberration within CST loops. SN dysfunction appears to result in deficits regulating appropriate functional response to motivational stimuli, exemplified by impaired cognitive control and inability to regulate behaviour, emotion, and cognition. VMN dysfunction may impair stimulus valuation in MDD, biasing individuals toward negative external or internal stimuli. Adaptive goal-directed behaviours require complex processing power that begins with motivation and ends with action selection; this process is greatly influenced by attention, salience, and executive systems that are mediated by the SN and VMN (Dunlop et al., 2015a; Haber, 2016). Impairments in these pathways could play a role in the pathophysiology of MDD. These network-level dysfunctions create a unique opportunity to treat psychiatric illness with therapeutic brain stimulation, which has been shown to exert network-level effects by modulating singular cortical or subcortical nodes.

This study found that iTBS shows equal clinical effectiveness compared to 10 Hz rTMS. We also identified fMRI predictors and correlates of response that applied to subjects receiving either type of left dlPFC-rTMS. With regards to functional network interactions, better clinical outcomes were predicted by lower baseline rs-FC between dorsal attentional and behavioural control networks to ventral affective and reward valuation networks. Left dlPFC-rTMS appeared to result in increased pre- to post-treatment functional connectivity both within, and between, the SN and VMN, in treatment responders; on the contrary, left dlPFC-rTMS appeared to decrease functional connectivity between these regions in treatment non-responders. To our surprise, integral nodes of the DMN also demonstrated increases in connectivity with subcortical and network-level ROIs that underlie cognitive control and motivation.

In summary, responders to left dlPFC-rTMS were characterized by an apparent deficit in SN-VMN interactions, which was improved with treatment; however, the application of
these results is limited by the absence of healthy control data. This work suggests that functional networks apart from the SN, including the VMN and DMN, are likely involved in response to left dIPFC-rTMS. Thus, response is not solely based on deficient functional connectivity within the SN, but rather on the ability of the SN to interact with other networks that support the spectrum of behavioural control from motivation and valuation to response selection and inhibition.

These findings provide a stepping-stone for several avenues of continued investigation. For one, confirmation that iTBS and 10 Hz stimulation produce comparable clinical outcomes should encourage wider use of iTBS in clinical settings. Implementing iTBS as a primary protocol choice would greatly increase the amount of patients that could be treated per device per year, creating space for almost 500 additional patients. Shorter treatment sessions would additionally reduce treatment costs for patients and practitioners alike.

In addition, these findings provide insight into (a) functional connectivity patterns that correlate with response to left dIPFC-rTMS, and (b) potential mechanisms of the therapeutic effects of left dIPFC-rTMS. It must be noted that the identified correlations between rs-FC and symptom improvement were weak to moderate in terms of effect size; nevertheless, the consistent relationships observed between baseline rs-FC, pre-to post-treatment rs-FC change, and response suggest that left dIPFC-rTMS may work by increasing functional connectivity between and within the SN and VMN. It is our hope that other investigators can build upon this research to further delineate fMRI factors that are predictive of response, so that fMRI—in addition to symptoms that map onto specific, observable dysfunction in nodes or networks—can be used as diagnostic tools that aid in the selection of appropriate rTMS stimulation targets. Such advances will prevent individual patients from undergoing useless treatments, will promote exploration of alternative cortical and subcortical brain stimulation targets for patients experiencing unique constellations of symptoms, and will ultimately lend to progress in personalized psychiatric medicine.
7.0 Future Directions

Several avenues of further investigation can be pursued to (a) critically evaluate and replicate these results, and (b) explore significant questions that emerge from this work. With respect to evaluation and replication, we highlight three important directions:

1. Is the therapeutic effect of left dlPFC-rTMS ‘normalizing’ patterns of functional connectivity? This question emerges from the lack of a healthy control group, which would provide ‘benchmark’ values of functional connectivity, and would enable observation of pre-treatment rs-FC (i.e., do non-responders demonstrate similar rs-FC compared to healthy controls?) as well as observation of post-treatment rs-FC (i.e., do responders really experience ‘normalized’ rs-FC, and, do non-responders still look similar to healthy controls?).

2. Are findings the same when SN and VMN ROIs account for subcortical regions? Here, neither the SN nor VMN ICA-generated network ROI included subcortical network nodes, such as the dorsal caudate or nucleus accumbens. Findings that emerged from these ROIs only represent connectivity from cortical regions of each network. It would be useful to investigate whether findings remain similar when the activity of all cortical and subcortical network nodes are taken into account, especially since our subcortical ROIs demonstrated the most functional correlates of treatment response.

3. Are individual differences in neurophysiology impactful on treatment response? Although our findings provide a basis for predicting response based on functional connectivity, treatment responders could also represent individuals with a fundamentally higher propensity for the cortical excitability changes induced by rTMS. Alternatively, it could be possible that responders to iTBS or 10 Hz stimulation represent different patient groups who are more amenable to cortical excitability changes following either of these protocols. Further studies of individual cortical excitability change at the stimulation site could shed light on this question.
With respect to emerging questions that evoke advancement of the field, we highlight three compelling directions:

1. Is stimulation of subcortical SN and VMN nodes feasible and effective?
   Experimental treatment targets should continue to be explored, since certain stimulation techniques, such as deep-rTMS (Nordenskjöld et al., 2016), may be able to reach deeper SN nodes. Ability to affect neural excitability using non-invasive procedures, in contrast to other stimulation techniques such as deep brain stimulation (DBS), would benefit patients by achieving similar functional connectivity changes at a lower monetary—and physical—cost.

2. Can we link neurocognition to symptoms and diagnostic criteria? Neurocognition is executive functioning in relation to physical aspects of the brain. Investigations of rs-FC in psychiatric disorders should include cognitive and behavioural measures in order to link task performance to functional connectivity in and between SN nodes, or nodes of other relevant brain networks. These types of measures would enable investigators to link functional activity to tangible and observable behaviours, making interpretations of these findings more insightful and clinically applicable. Future studies should also evaluate clinical factors that are associated with the SN and link such factors to diagnostic criteria; this would contribute to a growing standardized set of mental disorder classifications that are based on a wealth of emerging behavioural and neurobiological research (Craig et al., 2015; Downar et al., 2016).

3. Can response be predicted using machine-learning mechanisms? An earlier study comparing 10 Hz rTMS and iTBS generated a machine learning mechanism that predicted treatment outcome with up to 90% accuracy (Bakker et al., 2014). Using the current dataset, it would be interesting to explore whether this accuracy could be further improved by preferentially selecting patients who exhibited the patterns of baseline rs-FC that we observed to be associated with better clinical outcome. Although the present findings emerged in the context of a massive dataset, it would be interesting to test the clinical utility of functional predictors in individual subjects.
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