THE NEURAL NETWORKS UNDERLYING TREATMENT-RESISTANT DEPRESSION

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

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Abstract

Treatment-resistant depression (TRD) presents significant clinical challenges, characterized by persistent symptoms despite multiple antidepressant treatments. Understanding the TRD's neurobiological mechanisms is essential for improving treatments. This thesis investigates functional connectivity (FC) alterations in key brain regions associated with TRD, focusing on the "default mode" network (DMN) of self-referential processing, the reward system, and the affective network.

The aim of this project was to explore how FC differences between TRD and treatment-sensitive depression (TSD) could inform the mechanisms underlying treatment resistance. Using task-based and resting-state fMRI, we examined the connectivity of the habenula, rostral anterior cingulate cortex (rACC), and subgenual ACC (sgACC) with other brain regions from the networks above in patients with TRD, TSD, and healthy controls (HC).

Results revealed that TRD patients, compared to TSD, exhibited hyperconnectivity of the habenula, part of the reward system, with the DMN, which may contribute to anhedonia, a core symptom of TRD. Altered DMN connectivity distinguished TRD from TSD and HC, reflecting self-referential and emotion regulation processes during rest. Additionally, TRD patients showed abnormal rACC connectivity during emotional processing, particularly hypoconnectivity with the hippocampus during supraliminal processing of positive emotions.

These findings advance our understanding of TRD by highlighting distinct patterns of FC, particularly within the default-mode, reward and affective networks, that differentiate TRD from TSD. These connectivity patterns suggest disruptions in self-referential processing, emotion regulation, and reward sensitivity, which may contribute to the persistence of symptoms in TRD. This research underscores the importance of a network-based approach to both diagnosis and treatment and offers insights into the neurobiological mechanisms of treatment resistance.

Statement of Original Authorship

I, Ana Rita Gadelho Tavares da Anunciacao Barreiros, declare that, to the best of my knowledge, the content presented in this thesis consists of original work carried out by the authors unless otherwise stated and acknowledged in the text. Ethical clearance was granted for this work, and no part of this thesis has been submitted in whole or in part for any other degree or diploma in any University.

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Ana Rita Barreiros

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Appendix A Barreiros, A.R., Breukelaar, I., Harris, A., Korgaonkar, M. (2024). fMRI neurofeedback for the modulation of the neural networks associated with depression. Clin Neurophysiol, 168: 34-42. doi: 10.1016/j.clinph.2024.

Appendix B Prentice, A., Barreiros, A.R., van der Vinne, N., Stuiver, S., van Dijk, H., van Waarde, J.A., Korgaonkar, M., Sack, A.T., Arns, M. (2023). Rostral Anterior Cingulate Cortex Oscillatory Power Indexes Treatment-Resistance to Multiple Therapies in Major Depressive Disorder, Neuropsychobiology, 82 (6): 373–383. doi: 10.1159/000533853.

Author Attribution Statements

Chapters 3 and 4, and Appendices A and B are published as per citation above. For manuscripts from Chapters 3, 4, 5 and Appendix A, I designed the study, analysed the data and wrote the drafts of the manuscript. Co-authors contributed either to design of the study, data collection, and/or by reviewing the manuscripts. In addition to the statements above, the manuscript from Appendix B was a collaborative work, resulting from a research visit overseas as part of this candidature. In this case, where I am not the corresponding author, permission to include the published material has been granted by the corresponding author.

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Ana Rita Barreiros

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

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Table of Contents

Structure of Thesis

This thesis has been written as a combination of two published first-author manuscripts (Chapters 3 and 4) and one first-author manuscript currently under review for publication (Chapter 5), in addition to introduction, methods, and discussion chapters. As such, there is redundancy in the introduction and methods of some chapters. Two additional published manuscripts (one first-author and one second) are included as appendices (Appendix A and B). Appendix A outlines a proposed neuromodulation study designed to serve as a foundation for future research on applying the thesis's findings to innovative treatments, while Appendix B presents a collaborative research project, tangential to the thesis, developed during an overseas research visit.

List of Figures

List of Tables

Chapter 1: Introduction

1.1. A Brief History of Depression

The recognition and understanding of depression have evolved significantly through the ages, tracing back to ancient civilizations. The earliest recorded descriptions come from ancient Greece, where Hippocrates, a seminal figure in medical history, described a condition called melancholia characterized by enduring fear or sadness, aversion to food, despondency, sleeplessness, irritability, and restlessness (Horwitz et al., 2017). Hippocratic writings emphasized an equilibrium within the body, suggesting that imbalances led to diseases like melancholia.

The concept of melancholia endured through the centuries, with notable mentions like Robert Burton's "The Anatomy of Melancholy" in the 17th century, which discussed depression in terms of mood, cognition, and physical symptoms, viewing it as a common human condition (Horwitz et al., 2017). Burton posited that genuine indicators of a melancholic disorder are symptoms that manifest without any discernible cause. He distinguished this from ordinary melancholy by defining the disorder as a type of folly devoid of fever, characterized primarily by unwarranted fear and sadness, which occur without any clear reason (Horwitz et al., 2017; Burton, 1621/2001). However, it wasn't until the 19th and 20th centuries that the understanding of depression began to shift significantly. The inductive methods of 17th-century English scientists, Francis Bacon and Isaac Newton, emphasized observation and experimentation, laying the foundation for a more empirical approach to understanding diseases and shaping modern scientific inquiry. This shift laid foundational principles for later scientific inquiry into conditions like depression, emphasizing evidence-based observations over speculative theories (Horwitz et al., 2017). Melancholia was increasingly understood as a disease of the emotions, characterized by sullenness, taciturnity, deep contemplation, dread, and despair (Rowley as cited in Kendler, 2020). Von Krafft-Ebing conceptualized it as 'simply mental depression,' viewing it as psychic pain in its most fundamental form, suggesting a psychological cause as much as a physical one. Griesinger, a 19th-century German psychiatrist, noted the inability to experience pleasure as part of the syndrome of melancholia: 'The patient can no longer rejoice in anything, not even the most pleasing' (Kendler, 2020). First named as anhedonia by Ribot (Berrios, 1996), it has become

one of the basic criteria of MDD and one of the two key criteria used to describe the subtype of depression with melancholic features in DSM-5 (American Psychiatric Association, 2013).

The 20th century saw a further evolution in the understanding of depression. The introduction of the Diagnostic and Statistical Manual of Mental Disorders (DSM) marked a significant shift. The DSM-III, introduced in 1980, standardized the diagnosis of depression, moving away from a contextual approach to a criteria-based one, improving the reliability of diagnosis which was known to be poor (Helzer et al., 1977). This approach has continued to evolve, with the DSM-5 now defining major depressive disorder (MDD) entirely based on criteria linked to symptoms, duration, loss of function, and the absence of medical or substance related factors that could better explain the syndrome (American Psychiatric Association, 2013). Melancholia, in turn, has become a diagnostic subtype of this broader concept of depression, characterised by the above symptoms and physical symptoms (American Psychiatric Association, 2013). This drift of depression away from a severe disorder to one that is much more broadly defined has led to continued debates about the differentiation between normal sadness and depressive disorders (Wakefield et al., 2017) and the validity of the construct (Horowitz & Wakefield, 2007).

The evolution of the DSM and its classification of Major Depression has not been without controversy. There was a notable lack of agreement regarding the specific symptoms critical for defining nonpsychotic depressive states, as well as ongoing debates about whether depression should be categorised based on its symptoms, origins, or treatment responses. By the early 21st century, Major Depression accounted for a significant 38% of all outpatient diagnoses, highlighting its prevalence amidst numerous potential diagnoses (Horwitz et al., 2017). The possibility of subtyping depression into biologically mediated illnesses, marked by physical symptoms and biological markers, was supported by differential responses to early treatments like electroconvulsive therapy (Parker & Hadzi-Pavlovic, 1996). This subtype was distinguished from neurotic depression, which was thought to be caused by psychological and environmental factors. This was rejected by other researchers who were unable to find a clear differentiation between melancholia and reactive or neurotic constructs of depression through other means (Kendell, 1976). Nonetheless, medication was increasingly used for the treatment of a broadly defined depression, initially tricyclic antidepressants or monoamine oxidase inhibitors, but these were followed by the hugely popular selective serotonin uptake inhibitors (SSRIs) - introduced in the late 1980s and now some of the most prescribed medication worldwide (Australian Prescriber, 2023). This

prominence of Major Depression in clinical settings and pharmaceutical research led to questions about the distinctiveness of melancholic depression, once considered the principal depressive condition, raising inquiries into whether it represents a unique disorder or just an intense form of Major Depression (Taylor & Fink, 2006).

Adding a layer of complexity, Andrews and Durisko's work provides an intriguing evolutionary perspective on melancholia. They suggest that the cognitive aspects of melancholia, such as enhanced ruminative thinking, may be a result of natural selection. This adaptation for advanced cognitive processing could have evolved from the body's mechanism to reallocate resources in response to persistent threats or challenges (Andrews & Durisko, 2017). This theory suggests that some depressive responses may be adaptive reactions to environmental stressors, thus blurring the line between disorder and normal emotional responses. This view aligns with historical observations that melancholia was once considered a disorder of intellect or judgment, often associated with sadness but not exclusively so (Kendler, 2020).

The history of depression is a testament to the evolving understanding of mental health, reflecting broader shifts in medical thought and societal attitudes. The journey from ancient Greek notions of melancholia to the modern DSM classifications illustrates the complexity and nuance involved in understanding and diagnosing depression and how much work there still is to be done in understanding this disorder.

1.2. Prevalence and Heterogeneity

The realm of mood disorders, as outlined in the DSM-5, is bifurcated into unipolar depressive disorders and bipolar and related disorders, encompassing conditions like MDD and bipolar I and II disorders, among others (Strunk & Sasso, 2017).

The heterogeneity within mood disorders is profound, with variability manifesting across different disorders and even among individuals diagnosed with the same disorder. This variability extends to the disorder's course—some experience episodic occurrences while others face chronic challenges. The DSM-5 has endeavoured to categorize meaningful subtypes of mood disorders, considering the diverse symptom presentations and courses of depression (Strunk & Sasso, 2017).

Further complicating the landscape is the comorbidity often observed with mood disorders, especially between depression and anxiety or substance use disorders, raising questions about diagnostic validity and the interplay of common risk factors (Strunk & Sasso, 2017).

According to Paykel (2008), depression is broadly recognized as a clinical syndrome, characterized by a set of symptoms that tend to co-occur, suggesting a shared underlying pathophysiology but this remains to be elucidated. The symptomatic profile of this syndrome includes enduring sadness, a lack of pleasure or interest in almost all activities (anhedonia), significant weight loss or gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive guilt, diminished ability to think or concentrate, and recurrent thoughts of death or suicide. These symptoms reflect a complex interplay of psychological and biological factors, indicative of the syndromal nature of depression, which aligns with medical syndromes in other fields, highlighting the potential for diverse aetiologies behind a common set of symptoms (Paykel, 2008).

The concept of chronicity in depression, particularly as defined by “persistent depressive disorder” (PDD) in DSM-5, identifies a group of people for whom depression persists over extended periods, often with significant functional impairment and comorbid conditions, distinguishing it from episodic forms of depression (DSM-5). PDD underscores the chronic aspect of depression, where individuals experience a pervasive low mood for most days over a span of at least two years, accompanied by additional symptoms such as fatigue, sleep disturbances, low self-esteem, poor concentration, and feelings of hopelessness. This long-

term persistence significantly impairs daily functioning and quality of life. Moreover, PDD is often accompanied by comorbid conditions, including anxiety disorders, substance use disorders, and particularly personality disorders such as avoidant, borderline, and dependent personality disorders. This prevalence of comorbidities in PDD emphasizes the intricate relationship between chronic depression and other mental health disorders, suggesting shared risk factors or the potential for chronic depression to predispose individuals to additional psychological challenges (Klein & Black, 2017).

Recent guidelines (e.g., Malhi et al., 2021) emphasize that depressive disorders and bipolar disorders do not exist as discrete entities but rather along a continuum. This continuum, or spectrum-based approach, spans from the nuances of individual symptoms like low mood and energy levels to the broader categorization of disorders. The spectrum approach is crucial for understanding the overlap and interplay between depressive and bipolar disorders, acknowledging that symptoms can vary widely in intensity and nature across different individuals. This dimensional view is particularly important in clinical practice, as it guides the diagnosis and management of mood disorders. For example, recognizing the presence of mixed features in a patient with depression might indicate a bipolar diathesis, necessitating careful consideration when prescribing dual acting antidepressants due to the potential risk of inducing mania. Similarly, understanding that a patient's depression includes significant anxious distress may highlight an increased risk of suicide and inform the choice of treatment strategies to address this heightened anxiety alongside depressive symptoms.

The comprehensive view of mood disorders acknowledges the intricate interplay between persistent depressive symptoms and comorbid conditions like anxiety and personality disorders (Klein and Black, 2017). Within this complex landscape, the spectrum concept provides a crucial framework for understanding and addressing the varied presentations of mood disorders. It underscores the importance of a nuanced approach to diagnosis and treatment, considering the entire spectrum of symptoms and their potential overlap between depressive and bipolar disorders. This holistic view not only enriches our understanding of mood disorders but also enhances the precision and effectiveness of treatment strategies tailored to the diverse manifestations of these conditions.

The clinical landscape of mood disorders, particularly depression, is marked by a heterogeneity in symptomatology, course, and comorbidity, challenging clinicians, and researchers to refine diagnostic and treatment approaches to accommodate this variability.

1.3. When Depression is Difficult to Treat

While many patients with depression find relief through established treatment modalities unfortunately more than 60% individuals prescribed with antidepressant medications (ADMs) fail to achieve remission and 50% further remain resistant to second and subsequent rounds of ADMs (Taylor et al., 2019). This subset that struggles to respond to established treatments are defined as having treatment-resistant depression (TRD) (Li et al., 2023).

TRD is a condition of utmost clinical relevance given the impact of residual depressive symptoms on functioning, the higher risk of recurrence, the lower chances of remission, and the risk of suicide (which is at least twice the rate of those with non-resistant depression) (Bergfeld et al., 2018). Months or years of trying various treatment regimens without finding relief results in increased emotional and financial burden to the individual, family and caregivers. TRD also represents the highest direct and indirect medical costs among MDD patients. Individuals with TRD are twice as likely to be hospitalized; the cost of this hospitalization is more than six times the mean total cost for depressed patients who are not treatment-resistant (Greenberg et al., 2015).

Another common way of classifying non-response to treatment is difficult to treat depression (DTD). DTD is defined as depression that continues to impose a significant burden despite standard treatment efforts (Paganin et al., 2023). This condition represents a clinical challenge where standard interventions fail to achieve sufficient therapeutic outcomes, necessitating a re-evaluation of treatment paradigms.

Distinguishing between DTD and TRD is critical for clinicians to apply targeted and effective treatment strategies. The concept of DTD aligns with a broader understanding of mental health, where the goal is to enable patients to regain their roles and responsibilities in society, enhancing their sense of well-being and fulfillment. In clinical practice, addressing DTD involves a dynamic and patient-centred methodology, where treatment plans are continuously adapted based on ongoing assessments of patient progress and response.

In research, the use of a more precise definition like TRD can facilitate a more homogeneous study population, enhancing the comparability and replicability of findings. This precision is crucial for identifying biological markers, understanding the neurobiological underpinnings of treatment resistance, and assessing the efficacy of specific interventions.

While the broader scope of DTD allows for a more diverse patient population, which is useful for understanding a wide range of responses, it also introduces additional variables and potential confounders. This diversity can sometimes dilute the specificity of conclusions about particular interventions. In contrast, the TRD framework offers a more standardized set of inclusion criteria, which produces clearer and more consistent results when evaluating specific treatments. This precision is especially beneficial when the research goal is to identify biomarkers of treatment resistance. For these reasons, I chose to focus on TRD in this work to allow for more rigorous examination of specific biomarkers associated with treatment resistance, minimizing confounding variables and enhancing the interpretability of the findings.

1.3.1. Treatment-Resistant Depression (TRD): (In)Definitions

Treatment-resistant depression is primarily characterized by patients with Major Depressive Disorder who do not adequately respond to standard treatments. Despite its clinical significance, a consensus remains elusive on its definitive characterization, reflecting the ongoing debate and complexity inherent in its conceptualization.

Different staging models and definitions exist, with some proposing specific criteria based on the number of failed antidepressant trials or the percentage of symptom improvement. These models aim to standardize the classification of patients and guide treatment.

The 2020 Royal Australian and New Zealand College of Psychiatrists clinical practice guidelines, discussed by Malhi et al. (2021), mark a pivotal shift in the approach to managing mood disorders. These guidelines advocate for a comprehensive strategy that extends beyond pharmacotherapy, integrating psychological and lifestyle interventions to tailor treatment to individual patient needs. The introduction of the MIDAS (Mood Disorder Algorithmic Stratification) framework signifies an innovative approach to enhancing treatment responsiveness, aiming to transition patients from a state of inadequate response to one of recovery and functional restoration.

Regulatory agencies like the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) offer specific guidelines, typically necessitating the failure of two distinct antidepressant treatments at appropriate doses and lengths (European Medicines Agency, 2013; Food and Drug Administration, 2018). In contrast, academic perspectives propose more nuanced definitions, considering the number of failed medication classes, the depth of response, and the inclusion of other treatment modalities like psychotherapy or neurostimulation (Sforzini et al., 2021), which adds complexity to the TRD definition and reflects the multifaceted nature of depression.

Besides efforts to reach a consensual definition for what TRD is, several staging models of TRD have been drawn. Thase and Rush's staging model categorizes TRD based on the number and type of failed antidepressant trials, offering a pragmatic framework yet facing challenges in specificity and operationalization (Thase & Rush, 1997). The Maudsley Staging Method incorporates symptom severity, functional impairment, and treatment history, providing a more comprehensive approach to assessing TRD (Fekadu et al., 2009). Similarly, the Massachusetts General Hospital (MGH) Staging Method focuses on treatment optimization and response, adding a detailed dimension to TRD definitions (Sackeim et al., 2019).

Another key discussion revolves around the operationalization of "adequate response" and whether TRD definitions should encompass responses to non-pharmacological treatments. Additionally, the integration of patient-centred considerations, including patient-reported outcomes, quality of life, and functional measures, is increasingly recognized as vital for a holistic approach to depression treatment (Sforzini et al., 2021).

In summary, TRD's evolving definition underscores a critical aspect of clinical management: the need to individualize treatment strategies. The traditional focus on pharmacological non-response has broadened to include resistance to a spectrum of interventions, acknowledging the multifaceted nature of depression. This expanded perspective is essential for clinicians to tailor their approach, considering the unique biochemical, psychological, and social factors influencing each patient's condition.

In this thesis, TRD was defined as no remission of symptoms with at least two adequate trials (in terms of dosage, duration – 6 weeks for each trial) of antidepressant of different pharmacologic classes, as well as the presence of moderate to severe symptoms.

1.3.2. The Quest for Biomarkers in TRD

The identification of reliable biomarkers holds the promise of transforming TRD management by offering insights into its biological underpinnings, enabling personalized treatment approaches, and providing objective measures to predict treatment outcomes.

The lack of consensus on TRD's definition not only affects clinical practice (Zanardi et al., 2024) but also poses significant challenges for research. Variability in definitions complicates the comparability of study results, impeding the advancement of knowledge and the development of innovative treatments. Biomarkers offer a tangible solution to this challenge, serving as objective, quantifiable indicators of biological processes that can be linked to the presence and severity of TRD. By identifying specific biomarkers, clinicians could predict which patients are likely to benefit from certain treatments and identify novel therapeutic targets for treatment-resistant depression, thus moving a step closer to personalized medicine.

Understanding the pathophysiological mechanisms underlying TRD is crucial for identifying potential biomarkers. Research suggests that TRD is associated with alterations in various biological systems, including the neuroendocrine, immune, and neurotransmitter systems (O'Connor et al., 2023). For instance, elevated inflammatory markers have been observed in patients with TRD, suggesting that inflammation could play a role in treatment resistance (Oliveira-Maia et al., 2024). Similarly, alterations in brain-derived neurotrophic factor (BDNF) levels (Kolasa & Faron-Górecka, 2023) and hypothalamic–pituitary–adrenal axis dysfunction have been linked to TRD (Li, 2023), providing potential targets for biomarker development.

The integration of biomarkers into TRD research can revolutionize study designs, enabling more precise patient stratification and outcome prediction. This precision is vital for elucidating the effectiveness of interventions and understanding the heterogeneous nature of TRD. In clinical practice, biomarkers can guide treatment selection, offering a more nuanced approach than the current trial-and-error method. For example, if a biomarker indicates a patient's likely non-response to a conventional antidepressant, alternative treatments like neuromodulation or novel pharmacotherapies could be prioritized. One tool which may uncover biomarkers is brain imaging. This technology can identify patterns of brain structure, activity and connectivity associated with disorders, symptoms, or treatments that with robust

testing could become objective biomarkers. This tool can also be used to uncover the underlying neurobiology of illness features or treatment response.

1.4. The Underlying Neurobiology of TRD

While the distinction between TRD and non-TRD patients is based on the failure to respond to treatment, there is a growing body of evidence suggesting that TRD can also be considered a distinct MDD subtype based on neurobiological features. Various imaging methodologies have been employed to explore the structural and functional abnormalities associated with TRD.

Studies employing gray matter volume assessments have revealed significant differences between remitting and non-remitting recurrent depression (Li et al., 2010). For instance, patients with non-remitting recurrent depression exhibit decreased gray matter volume in the left dorsolateral prefrontal cortex, which correlates with impaired facial and delayed verbal memory (Li et al., 2010). Additionally, chronic severe depression and TRD patients often show the smallest gray matter volumes in the frontotemporal regions, with illness duration negatively correlated with right medial frontal cortex volumes (Serra-Blasco et al., 2013).

Whole-brain cortical thickness analyses have demonstrated reduced cortical thickness in key regions such as the frontal cortex and anterior cingulate cortex among TRD patients. Specifically, reductions in the left frontal pole, left inferior frontal cortex, left anterior cingulate cortex, and left middle temporal cortex have been observed when compared to healthy controls (Chen et al., 2022). These structural abnormalities are linked to cognitive impairments, with cortical thickness in the left frontal pole and anterior cingulate cortex being positively associated with mean error rates in cognitive tasks (Chen et al., 2022).

Functional connectivity MRI studies have revealed altered connectivity in TRD patients compared to non-TRD MDD and healthy controls. For instance, decreased connectivity in the cognitive control network (CCN) and increased connectivity in the affective network (AN) have been observed, suggesting network-specific dysregulation in TRD (Miola et al., 2023). Additionally, TRD patients show distinct patterns of connectivity in the default mode network (DMN) and salience network (SN) compared to healthy controls (Miola et al., 2023), which are associated with symptoms such as rumination and impaired emotional regulation (Sun et al., 2023).

These findings support the notion of TRD as a distinct subtype of MDD, highlighting the importance of employing advanced imaging techniques to elucidate its underlying mechanisms. However, whether these alterations are distinct from those in patients who respond to treatments is currently unknown. This thesis aims to address this gap by comparing TRD with treatment-sensitive depression (TSD) to further clarify its neurobiological differences.

1.4.1. Neural Networks of Depression

Neural networks, also known as brain networks, are intricate systems of interconnected neurons that communicate with each other to perform various functions, including sensory processing, motor coordination, and higher cognitive processes (Bullmore & Sporns, 2009). These networks are fundamental to how the brain processes information and regulates behaviour. In the context of psychiatric illnesses such as depression, understanding these networks can provide crucial insights into the underlying neurobiology of these disorders. While there are several functional brain networks, as well as subcortical regions and the cerebellum contributing to the overall function of the brain, some of these networks are particularly relevant to depression and thus the focus of this thesis.

The **Default Mode Network (DMN)** is active when the brain is not focused on the outside world. It comprises several key regions, including the precuneus, posterior cingulate cortex, ventral anterior cingulate cortex, dorsal medial prefrontal cortex, bilateral inferior parietal lobules, bilateral middle temporal gyri, and left middle frontal gyrus (Laird et al., 2009). This network is primarily involved in self-referential thoughts, mind-wandering, and processing internal stimuli (Raichle et al., 2001). Dysfunction in the DMN has been implicated in depression, with alterations in connectivity patterns associated with symptoms such as rumination and negative self-referential processing (Sheline et al., 2009).

Depression has been associated with decreased activity in several regions of the DMN, including the dorsal cingulate and neocortical areas (such as the prefrontal, premotor, and parietal cortices), while exhibiting increased activity in ventral limbic and paralimbic regions. These findings support a cortical-subcortical imbalance theory (Drevets & Raichle, 1992; Mayberg et al., 1999). The DMN, particularly regions like the medial prefrontal cortex (mPFC), which encompasses the subgenual anterior cingulate cortex (sgACC), ventromedial

prefrontal cortex (vmPFC), and rostral anterior cingulate cortex (rACC), plays a critical role in implicit emotion regulation and self-referential processing (Etkin et al., 2011; Fales et al., 2008; Phillips et al., 2003).

The sgACC is especially noteworthy due to its involvement in processing negative emotional stimuli and its altered activity patterns in MDD, where increased sgACC activity is often observed alongside reduced activity in the dorsolateral prefrontal cortex (dlPFC) (Fales et al., 2008; Fu et al., 2008; Siegle et al., 2007). Understanding how these regions interact within the DMN, and their collective dysfunction can provide deeper insights into the characteristic symptoms of depression, such as rumination and negative self-referential thought patterns.

The **Salience Network (SN)** plays a key role in detecting and integrating relevant external and internal stimuli to guide behaviour (Seeley et al., 2007). The SN typically comprises several critical brain regions, including the fronto-insular cortex, the dorsal anterior cingulate cortex (dACC), the amygdala, and the temporal poles (Mulders et al., 2015). Dysfunction in the SN has been linked to aberrant processing of emotional stimuli and disruptions in cognitive control, which are common features of depression (Menon & Uddin, 2010). The SN shows decreased connectivity in depression, impacting engagement in rewarding behaviour (Tahmasian et al., 2013; Torrisi et al., 2018). Studies reveal abnormal functioning between and within this network with increased connectivity associated with higher depression scores (Sheline et al., 2010).

The **Cognitive Control Network (CCN)** is involved in executive functions such as attention, working memory, and cognitive control (Dosenbach et al., 2008). This network links the dlPFC, the dorsal dACC, and the dorsal parietal cortex (DPC) (Breukelaar et al., 2017). Altered connectivity within the CCN has been associated with cognitive deficits seen in depression, including difficulties in concentration and decision-making (Kaiser et al., 2015). The CCN, part of the corticostriatopallidal-limbic circuit, exhibits increased activity in frontal and parietal regions during attention-demanding tasks. Depressed patients show decreased task-related activity in the CCN but increased resting state functional connectivity, reflecting higher, more volatile activity at rest (Davidson et al., 2002; Fitzgerald et al., 2008; Siegle et al., 2007). Notably, the interplay between the CCN and the DMN is significant in depression, as the DMN is often more active during depressive episodes, engaging in self-referential thought processes that may detract from cognitive control and task-focused attention (Buckner et al., 2008; Raichle et al., 2001).

The **Affective Network (AN)**, alternatively referred to as emotion processing or limbic network, plays a crucial role in the regulation of emotions and mood, comprising key brain regions such as the amygdala, ventral striatum, anterior insula, and orbitofrontal cortex (Raichle et al., 2001). This network is particularly active during emotional and social cognitive tasks, reflecting its involvement in processing emotionally salient information. In individuals with MDD, increased activity and heightened resting state functional connectivity within the AN are commonly observed (Grimm et al., 2009; Sheline et al., 2010). This increased connectivity suggests an overactive emotional response system, which may lead to exaggerated emotional reactions and difficulties in emotional regulation. Furthermore, the AN's interactions with other brain networks, such as the DMN and the CCN, can significantly influence how emotions are processed and regulated. For instance, dysregulation in the AN may contribute to impaired cognitive control and persistent rumination, which are characteristic features of MDD. Overall, the AN's altered functioning is linked to the severity and persistence of depressive symptoms, underscoring its importance in understanding the neurobiological underpinnings of depression.

The **reward system**, encompassing regions like the striatum, ventral tegmental area (VTA), and prefrontal cortex, is crucial for processing rewards and motivating behaviour. In depression, dysfunctions in this system are particularly evident. Patients with depression often exhibit anhedonia, a reduced ability to experience pleasure, which is linked to abnormalities in the reward circuitry. For example, reduced functional connectivity between the ventral striatum and prefrontal cortex has been observed in depressed patients, which correlates with the severity of anhedonia and overall depressive symptoms (Pizzagalli et al., 2009). A critical component of the reward system that has gained attention in depression research is the habenula, a small structure involved in processing negative reward signals. The habenula inhibits dopamine release in response to negative outcomes, and its hyperactivity has been associated with depression. Studying the functional connectivity of the habenula can provide insights into how disruptions in reward processing contribute to depressive symptoms (Hikosaka, 2010).

1.4.1. Neural Networks in Treatment-Resistant Depression

Functional connectivity (FC) studies highlight differences between TRD and non-TRD patients in brain network connectivity, particularly in the DMN, SN, CCN, and the reward

circuits, which may underlie the neurobiological mechanisms differentiating TRD from non-TRD. Particularly, the DMN has been shown to play a central role in self-referential processing and emotional regulation. Alterations in the DMN, especially involving the rACC and the sgACC, are implicated in the persistent symptoms of TRD (Davey et al., 2012). Understanding these differences in DMN connectivity may elucidate the neurobiological mechanisms underlying TRD, thereby justifying a focused examination of the DMN and anterior cingulate cortex in this context.

High global connectivity strength during resting-state in DMN regions has been suggested as a significant neurobiological marker that could differentiate TRD from non-TRD patients (Çatal & Northoff, 2024). TRD has been associated with hypo and hyper connectivity within the DMN. Specifically, decreased FC within the DMN has been reported in TRD compared to treatment-sensitive depression (TSD) (de Kwaasteniet et al., 2015; Guo et al., 2013b; He et al., 2016; Ma et al., 2012). For example, He et al. (2016) found a significant network of reduced FC centred on the parahippocampal gyrus, which included reduced connections with the precuneus, posterior cingulate gyrus, and inferior parietal lobe, among others.

Decreased connectivity between the DMN and other brain regions has also been reported in TRD. This includes reduced connectivity between the DMN and sensory association areas, executive network regions, and affective limbic network regions (de Kwaasteniet et al., 2015; Guo et al., 2013a). For instance, decreased FC was found between the cognitive control network (dlPFC) and the posterior DMN (left and right angular gyrus) in TRD, indicating widespread reduced resting-state connectivity beyond specific neurocognitive networks.

In contrast to decreased FC, some studies have also reported increased FC in TRD compared to TSD within the DMN and between the DMN and other brain regions (Liu et al., 2011; Ma et al., 2012). For example, significantly increased resting-state connectivity was observed between the right insula (part of the salience network) and the posterior DMN, and between the left amygdala and the ACC in TRD (Liu et al., 2011).

The sgACC is a key region within the DMN and has been consistently associated with TRD. Dysfunction in the sgACC has been frequently observed in TRD, with abnormal activity patterns often normalized by effective antidepressant treatments, such as deep-brain stimulation (Mayberg et al., 2005). The sgACC is implicated in predicting treatment outcomes, as evidenced by a study that found increased activity and functional connectivity in the sgACC following electroconvulsive therapy (ECT), with greater baseline activity

correlating with better clinical responses in MDD patients (Liu et al., 2015). Additionally, functional connectivity between the sgACC and other brain regions such as the dlPFC has been associated with clinical improvement following rTMS treatment (Ge et al., 2023

The rACC is known for its involvement in conflict monitoring and emotion regulation, playing a pivotal role in adaptive treatment responses (Carter et al., 1998; Pizzagalli, 2011). It is important to distinguish the rACC from the sgACC, as they occupy different anatomical positions within the anterior cingulate cortex. Patients with depression often show elevated rACC activity at rest and reduced suppression during external tasks, alongside altered connectivity patterns, such as decreased connectivity between the DMN and the CCN (Jamieson et al., 2022). Understanding the distinct roles of the sgACC and rACC may provide deeper insights into the neurobiological mechanisms underlying treatment responses in depression. Moreover, the rACC's activity at rest has been associated with treatment response, with higher baseline activity predicting better response, and higher connectivity between the rACC and regions like the dlPFC, sgACC, and amygdala associated with poorer treatment outcomes (Jamieson et al., 2022). Additionally, stronger baseline rACC resting-state FC to left lateral parietal cortex has been linked to greater clinical improvement following rTMS (Ge et al., 2023). These findings suggest that the rACC plays a crucial role in depression and treatment response, with unique connectivity patterns associated with different aspects of the disorder and its treatment. The current thesis aims to extend this understanding by examining the functional connectivity of both the rACC and sgACC in TRD.

The habenula, a key node in the reward circuit, also shows distinct connectivity patterns in TRD. Hyperconnectivity of the left habenula with the left precuneus cortex and right precentral gyrus has been observed in TRD patients compared to remitted MDD individuals and healthy controls (Grehl et al., 2023). This hyperconnectivity may contribute to the persistent anhedonia and negative affect characteristic of TRD, indicating a unique relationship between the habenula and regions implicated in processing aversive stimuli and simulating positive future events. The habenula’s connectivity with the DMN, as explored in this thesis, is central to understanding the neural mechanisms underlying treatment resistance in TRD.

Additionally, brain areas involved in emotional (amygdala) and reward (striatal areas) processing and part of the DMN and SN respectively, display altered activity, connectivity,

metabolism, and structure in individuals with TRD compared to healthy volunteers and treatment responsive depressed patients in some studies (Kotoula et al., 2023). Increased FC of the inferior ventral striatum with multiple brain regions, including the middle frontal gyrus, cerebellum, parahippocampal gyrus, middle occipital gyrus, and lingual gyrus, has been observed in TRD compared to non-TRD (Sun et al., 2023). In the same study, the TRD group also showed a wider range of altered striatal function compared to healthy controls, with HAMD-17 scores positively correlated with FC between the right ventral rostral putamen and the left caudate (Sun et al., 2023).

The literature suggests that the reduced FC observed in TRD may indicate widespread connectivity impairments, potentially linked to specific cognitive processes in MDD. Depressive rumination, a key MDD symptom, involves repetitive, self-reflective focus on depressive symptoms, with maladaptive (brooding) and adaptive (reflective pondering) components (Morrow & Nolen-Hoeksema, 1990; Nolen-Hoeksema, 1991; Treynor et al., 2003). Notably, adaptive rumination has been associated with lower depressive symptom levels over time than maladaptive rumination (Treynor et al., 2003). In MDD, dominance of the DMN over the executive network is linked to higher maladaptive rumination and lower adaptive rumination (Hamilton et al., 2011). The salience network appears to regulate the switching between the DMN and executive network (Sridharan et al., 2008). Previous studies in MDD suggest increased FC between the salience network and the anterior/posterior DMN, along with decreased FC between the posterior DMN and the executive network (Manoliu et al., 2014; Mulders et al., 2015). These connectivity alterations, which appear in both MDD and TRD, suggest that the SN may play a critical role in mediating the interaction between the DMN and the executive network. Specifically, the impaired connectivity patterns observed in TRD indicate that the SN may be less effective at regulating the switch between the self-referential processes of the DMN and the goal-directed functions of the executive network. This dysfunction could contribute to the persistence of maladaptive rumination and cognitive deficits commonly seen in TRD, as the inability of the SN to facilitate this switching may hinder adaptive emotional regulation and cognitive control.

Additionally, decreased FC between the DMN and sensory areas in TRD may indicate impaired sensory integration during self-referential processing, particularly regarding positive life events. This decreased FC could be understood within the framework of the two-factor sensitization model in MDD, where a fixation on negative life events fosters the development

of abstract dysphoric schemas, further intensifying the focus on negative over positive events (Farb et al., 2015).

Overall, TRD is characterized by complex patterns of both decreased and increased connectivity within and between various brain networks. These findings underscore the multifaceted nature of functional connectivity alterations in TRD, emphasizing the need for further research to elucidate the underlying neurobiological mechanisms. Given the implications of the DMN in prior research this thesis will concentrate on the role of the DMN, specifically the sgACC, the rACC, and the habenula, as these regions have been consistently associated with TRD. Understanding the connectivity patterns and dysfunctions within these areas may provide valuable insights into the unique challenges faced by TRD patients and inform the development of targeted interventions tailored to their specific needs.

Table 1. *Comparison of findings from key studies on the neural networks of treatment-response in depression*

Study	Population	Method	Main Findings	Interpretation/Conclusion
Davey et al. (2012)	18 MDD (15–24 yrs), 20 controls	Resting-state fMRI, FC	MDD: ↑ connectivity sgACC–dmPFC, ↑ connectivity pgACC–dlPFC, ↓ connectivity pgACC–caudate	Altered cingulate connectivity supports sgACC role in affective processing and pgACC in cognitive regulation and motivation
Liu et al. (2015)	23 first-episode MDD, pre/post 8 ECT	Resting-state fMRI, ALFF, FC	Post-ECT: ↑ ALFF in sgACC & hippocampus; FC ↑ between sgACC and OFC, MTG, pgACC	ECT modulates sgACC activity/connectivity; antidepressant effects tied to sgACC–limbic network changes
Jamieson et al. (2022)	94 MDD, 91 controls	Resting-state fMRI, spectral DCM	MDD: ↑ inhibitory EC from rACC to dlPFC, AIC, dACC; Responders: distinct EC profiles (e.g., ↓ sgACC–amygdala inhibition)	rACC inhibitory hyperconnectivity in MDD; unique EC patterns may predict treatment response
Sun et al. (2023)	38 TRD, 42 nTRD, 39 HC	Resting-state fMRI, seed-based FC (striatum)	TRD: ↑ FC in inferior ventral striatum–OFC, cerebellum, PHG, occipital areas	Striatal network dysfunction specific to TRD, implicating altered reward/motivation circuitry
Nolen-Hoeksema (1991)	Community sample of adults	Longitudinal; depressive symptoms over time	Rumination predicts longer duration of depressive episodes	Foundational support for RST; rumination linked to episode duration
Hamilton et al. (2011)	Adults with MDD (n=17) and controls (n=17)	Resting-state fMRI; DMN-TPN balance metric	Greater DMN dominance = more brooding; MDD group had disrupted RFIC response to network transitions	Neural correlate of maladaptive rumination; highlights DMN overactivity and salience network dysfunction
Manoliu et al. (2014)	MDD patients (n=25) vs controls (n=25)	Resting-state fMRI; ICA	Reduced connectivity in right anterior insula (rAI); abnormal DMN-CEN switching; associated with symptom severity	Salience network dysfunction linked to rumination and depressive symptomatology

Table 2. *Summary of key findings from fMRI Studies in Treatment-Resistant Depression (TRD), based on the review from Katoula et al. (2023)*

Functional Domain / Network	**Finding**	**Consistently reported in**
Default Mode Network (DMN)	Abnormal baseline connectivity; reduced connectivity in TRD vs HVs; increased connectivity post-treatment (psilocybin, ketamine)	Lui et al., 2011; de Kwaasteniet et al., 2015; Carhart-Harris et al., 2017; Evans et al., 2018b
Salience Network (SN)	Higher baseline SN connectivity predicts treatment response (rTMS); altered SN activity in TRD compared to non-refractory depression	Ge et al., 2017
Central Executive Network (CEN)	Reduced CEN-DMN connectivity in TRD vs HVs and non-refractory depression	de Kwaasteniet et al., 2015
Anterior Cingulate Cortex (ACC)	Hypoactivation in emotional processing tasks; connectivity patterns (with DLPFC and DMN) predict treatment response	Kumari et al., 2003; Luan et al., 2019; Chen et al., 2019; Ge et al., 2020
Amygdala Reactivity	Blunted amygdala response during emotional tasks in TRD; psilocybin increases amygdala activity, linked to clinical improvement	Ferri et al., 2017; Roseman et al., 2018
Temporal & Limbic Regions	Greater synchrony at rest in TRD (vs HVs); elevated temporal/limbic activation in TRD during negative emotion processing	Wu et al., 2011; Kumari et al., 2003
Predictive Biomarkers	Baseline DMN and SN connectivity (Ge et al., 2017); subgenual/rostral ACC connectivity with lateral parietal and DLPFC (Ge et al., 2020) predict rTMS response	Ge et al., 2017, 2020

1.5. Aims and Goals

The overarching aim of this project is to advance our understanding of the neurobiological mechanisms underlying TRD that could be used to differentiate TRD from non-TRD patients. Building on the existing body of research, this project aims to address the following specific goals:

Default Mode Network (DMN): Investigate the functional connectivity within the DMN to identify specific alterations in TRD patients. Previous studies have shown abnormal DMN activity in depression; however, there is a notable gap in understanding how subregional heterogeneity within the DMN can differentiate TRD from TSD. This project specifically focusses on examining the connectivity of the rACC and sgACC with other DMN regions, as these areas have been linked to treatment resistance and rumination.

Reward System: Investigate the role of the habenula within the reward system and its connectivity with other brain networks. The lateral habenula has been shown to encode aversive stimuli and modulate dopaminergic activity, essential for effective reward processing and triggering feelings of pleasure. This is of particular interest as a core symptom of TRD is anhedonia–the inability to experience pleasure. Despite the growing body of research on the habenula's role in depression, there remains a significant gap in understanding how its connectivity with other brain networks, particularly the DMN, influences the expression of anhedonic symptoms in TRD. The novelty of this project lies in examining how the FC of the habenula differentiates TRD from TSD. By investigating these differences, we aim to elucidate the mechanisms by which abnormalities in reward processing contribute to anhedonic symptoms specifically in TRD patients.

Affective Network (AN): Examine the connectivity patterns within the AN to understand its role in response to emotional task demands and how these differ between TRD and non-TRD patients. The AN's involvement in processing emotional stimuli makes it a critical focus for understanding TRD-specific abnormalities. While previous research has established the AN's involvement in processing emotional stimuli, there remains a significant gap in understanding the specific connectivity patterns that differentiate TRD from non-TRD in the context of emotional processing tasks. Task-based fMRI was utilized to study the AN's connectivity during emotional processing tasks, providing insights into the neural basis of emotional dysregulation in TRD.

Clinical Factors: Investigate how these differences in brain network connectivity correlate with clinical symptoms. By understanding the relationship between neural connectivity patterns and clinical manifestations of TRD, the project aims to identify potential biomarkers for early diagnosis and treatment response prediction.

While the SN and the CCN are critical in the context of emotional regulation and cognitive control, they were not the primary focus of this thesis. The current study primarily interrogated the DMN, which has been shown to have significant implications in the pathophysiology of TRD. However, future work will evaluate the interactions between the SN, CCN, and DMN, particularly in resting-state and emotion processing contexts, to better understand their role in TRD and treatment outcomes.

By achieving these goals, this project aims to provide a comprehensive understanding of the neurobiological underpinnings of TRD, which could lead to the identification of novel therapeutic targets and more effective treatment.

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Chapter 2: The Project

2.1. Project and Thesis Overview

The increasing burden of treatment-resistant depression (TRD), coupled with advancements in neuroimaging technologies, provides a unique opportunity to investigate mechanistic factors that can characterize individuals with this challenging condition.

This project aims to study TRD using functional connectivity, an established MRI-based technique that analyses the brain's network connectivity patterns. Functional connectivity has the potential to uncover new brain signatures that might predict TRD before ADM treatments, facilitating better patient stratification and informing the development of novel therapeutic approaches.

This project will use functional connectivity to understand 1) neural characteristics of treatment resistant depression, and 2) mechanisms that characterise treatment resistant depressed patients from depression patients who respond to antidepressant medication treatments.

In Chapter 3, we examine the role of the habenula focusing on its connectivity with other brain regions, and how this could differentiate between treatment-resistant depression, treatment-sensitive depression (TSD) and healthy controls (HC). As outlined in the previous chapter, studying habenula connectivity is particularly relevant for understanding anhedonia and altered reward processing in TRD.

Chapter 4 investigates the anterior cingulate cortex (ACC), particularly its interactions within the default mode network (DMN) and with other networks. This chapter aims to clarify the role of the DMN connectivity patterns across TRD, TSD, and HC, to better understand the neural mechanisms that underlie treatment non-responsiveness.

Chapter 5 goes one step further by studying the functional connectivity of the DMN—specifically the rostral anterior cingulate cortex (rACC), a key hub of the DMN with a known role in emotion processing—during an emotion processing task. This approach is especially relevant for understanding dysfunctional negative and positive affect characteristic of TRD.

By successfully addressing these questions, this project aims to generate new knowledge on the mechanisms underlying treatment-resistant depression, enhancing our understanding of

how neural characteristics may differentiate TRD patients from those who respond to treatment. This chapter provides an overview of the study design, description of participants, and a rationale and description of the fMRI methods used to address the proposed research questions.

2.2. Participants

Data collection for this study was conducted from 2018-2021. Thirty-nine TRD and thirty-five TSD patients were recruited through general practitioner referrals and clinics. Thirty-eight HC participants were recruited through community advertisements. Data collection was conducted at Westmead Hospital, Department of Radiology and at the Brain Dynamics Centre, The Westmead Institute for Medical Research, in Sydney, Australia. TRD and TSD patients met DSM-5 criteria for primary diagnosis of MDD, according to the Structured Clinical Interview for the DSM-5 (SCID-5) (American Psychiatric Association, 2013).

TRD was defined as no remission of symptoms after at least two adequate trials (in terms of dosage, duration – 6 weeks for each trial) of antidepressant of different pharmacologic classes, as well as the current presence of moderate to severe depressive symptoms. Severity of the symptoms was characterized by a 21 item Hamilton Depression Rating Scale (HAMD-17) (Hamilton, 1960) score greater or equal to 16. TSD patients were defined as those who had responded to their current treatment with symptom remitted for at least two weeks as defined by a HAMD-21 score of less than or equal to 9 (equivalent to HAMD-17 score of or less than or equal to 7), prior to testing. HC were healthy individuals with no history of or current psychiatric illnesses, assessed using the SCID-5.

All participants were aged between 18 and 65 years old. Exclusion criteria included a) inability to provide consent, b) insufficient English proficiency, c) current primary diagnosis of eating disorder, psychosis, personality disorder or primary post-traumatic stress disorder, d) substance dependence for the past 3 months, e) pregnancy, f) history or current neurological disorder or prior brain injury, defined as a loss of consciousness of greater than 10 minutes or hospital admission for greater than 4 hours after head injury, g) diagnosis of bipolar disorder (assessed using SCID-5) or history of mania (assessed using the Young Mania Rating Scale, Young et al., 1978), h) Electroconvulsive Therapy (ECT) or Transcranial Magnetic Stimulation (TMS) in the last 6 months, i) contraindication to MRI.

For both patient groups, indices of illness severity (based on the Clinical Global Impression scale – Severity, CGI-S, scores) and chronicity (by collecting information about the age of onset) were assessed. These indices included age of onset, number of inpatient hospitalizations, length of remission period since last episode, number of previous depressive episodes, history of suicidal ideation and behaviour, and history of suicide attempt.

Information on past and current medication and other forms of treatment (e.g. ECT, or TMS) was also collected. Level of functioning was assessed by the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman et al., 1992).

2.3. Clinical Measurements

2.3.1. Hamilton Depression Rating Scale - 21 items (HAMD-21)

The Hamilton Depression Rating Scale (HAMD) is one of the most widely used clinician-administered assessments for evaluating the severity of depression. Developed by Max Hamilton in 1960, the HAMD-21 consists of 21 items that assess various symptoms of depression, such as mood, guilt, suicidal ideation, insomnia, and somatic complaints. It is administered through an interview where the clinician rates each symptom on a scale from 0 (not present) to 4 (severe). The total score helps quantify the severity of the patient's depression, with higher scores indicating more severe depression. For example, one of the items asks, "Feeling of guilt," where the clinician will score from 0 (absent) to 4 (guilt delusions). The HAMD-21 remains a "gold standard" in depression assessment, particularly in clinical trials of antidepressant treatments (Hamilton, 1960; Worboys, 2013). Although the 17-item version (HAMD-17) is widely used for its simplicity, the HAMD-21 offers a more complete measure of depression severity, making it particularly suitable for capturing the full spectrum of symptoms in clinical trials and research where a more detailed assessment is required. Its inclusion of more items may improve sensitivity in detecting treatment-related changes in symptomatology, especially for trials aiming to assess both core and peripheral symptoms of depression (Worboys, 2013).

2.3.1. Social and Occupational Functioning Assessment Scale (SOFAS)

The SOFAS is a clinician-administered scale used to assess the level of social and occupational functioning of an individual, specifically in relation to mental health conditions. It was introduced by Goldman et al. in 1992 as part of the DSM-IV criteria to provide a measure of functional impairment, independent of psychiatric symptom severity. The SOFAS evaluates the patient's ability to perform daily activities, maintain relationships, and function in work or school settings. It uses a scale from 1 (grossly impaired) to 100 (superior functioning) to rate global functioning. One example question would be: "How would you rate the patient's ability to maintain a job or educational responsibilities?". SOFAS ratings focus solely on functioning, excluding symptom severity or medical conditions, making it a unique tool to measure functional capacity in individuals with mental health disorders (Goldman et al., 1992; Samara et al., 2014).

2.3.1. Clinical Global Impression Scale - Severity (CGI-S)

The Clinical Global Impression - Severity (CGI-S) is a clinician-rated scale used to assess the overall severity of a patient's psychiatric condition. It is a brief, one-item scale that asks the clinician to rate the patient's level of illness based on their total clinical experience with the individual. The CGI-S uses a 7-point scale: 1 = "normal, not at all ill" to 7 = "among the most extremely ill patients.". The clinician considers various factors, including observed symptoms, behaviour, and functioning over the past week, to assign a severity score. For instance, a typical question posed by the CGI-S would be, "Considering your total clinical experience with this patient, how mentally ill is this patient at this time?". The simplicity and wide applicability of the CGI-S make it a useful tool for assessing severity across various psychiatric disorders, from depression to schizophrenia (Busner & Targum, 2007).

2.4. Neuroimaging Methods

2.4.1. fMRI Functional Connectivity to Study Brain (Dys)Function

<u>How Does fMRI Work?</u>

Functional Magnetic Resonance Imaging (fMRI) is an essential technique in neuroscience, offering insights into the brain's dynamic activities. Unlike structural MRI, which provides a static image of the brain's physical structure, fMRI captures neural activity by monitoring

changes in blood flow. The core principle behind this method is straightforward yet significant: when neurons are active, they require more oxygen, leading to an increase in blood flow to these areas to replenish oxygen levels. Neurons lack internal energy reserves, such as glucose or oxygen (Lv et al., 2018). When they become active, they rely on nearby capillaries to supply energy through the hemodynamic response, which increases regional cerebral blood flow and oxygen supply, often exceeding the neurons' immediate needs (Lv et al., 2018). This leads to a shift in the relative concentrations of oxyhemoglobin which have distinct magnetic properties: oxyhemoglobin is diamagnetic, while deoxyhemoglobin is paramagnetic. This difference affects the local magnetic field, making these changes detectable by MRI (Lv et al., 2018). This variation in blood flow is detected through the Blood Oxygen Level-Dependent (BOLD) contrast, an indirect indicator of neuronal activity (Ogawa et al., 1990).

The BOLD signal obtained from fMRI does not directly measure the electrical signals of neurons; rather, it reflects the subsequent changes in the levels of oxygenated and deoxygenated haemoglobin due to alterations in cerebral blood flow and volume. As such, fMRI provides a broad perspective of brain activity, illustrating patterns of neuronal engagement and the interactions among various brain regions during different tasks or while at rest (Logothetis, 2008).

The Emergence of Functional Connectivity Methods

Functional connectivity (FC) is the statistical relationship of BOLD signals between brain regions as measured by fMRI which captures the temporal synchrony of neuronal activity patterns across anatomically separated brain regions, offering insights into the brain’s communication pathways (Rosazza and Minati, 2012, de Schotten and Forkel, 2022, Huang et al., 2024). No single brain region is solely responsible for any brain function; instead, functions emerge from multiple regions working in sync. Functional connectivity can hence enhance traditional fMRI studies by offering a more comprehensive picture of brain function.

Brain dynamics, observed during both resting states and active emotional or cognitive tasks, show how different brain regions interact. This concept aligns with emergentism, which posits that "the whole is something besides the parts" and that understanding complex systems requires analysing both their components and the interactions between them (de

Schotten and Forkel, 2022). In neuroscience, there is a growing recognition that brain functions are emergent properties arising from interactions among various areas (Fox et al., 2005). It is now well-established that brain functions are supported by the coordinated activity of multiple brain regions, which interact in complex networks to facilitate various cognitive and emotional processes (Fox et al., 2005).

Viewing the brain as an interconnected network helps elucidate its functions but also dysfunctions. Before the advent of comprehensive neuroimaging technologies for studying the entire human brain, clinical neuroscience primarily focused on identifying dysfunctions in specific, localized brain regions rather than understanding the importance of connectivity between these regions. Historically, from Broca's studies of aphasia to investigations of patient's memory loss, many brain disorders were believed to originate from localized areas (Eickhoff et al., 2018). This focus was partly due to the limitations of earlier neuroscientific tools, which often led researchers to examine clinical problems related to the brain by studying specific brain regions with abnormal structures or functions. However, with the development of whole-brain imaging techniques and sophisticated quantitative analyses, research has increasingly highlighted the significance of widespread network dysfunctions across a broad spectrum of neurological and psychiatric conditions (Zhang et al., 2021).

The saying "regions that fire together, wire together" reflects the idea that measuring the coordination of activity between brain areas can indicate their degree of connectivity, even if these areas are not directly linked structurally (de Schotten and Forkel, 2022). When a brain region is deprived of its connections, it undergoes structural changes, such as pruning dendrites and synapses, leading to neuron degeneration or death (de Schotten and Forkel, 2022). Consequently, this results in both functional and structural breakdowns of the network, a phenomenon known as diaschisis (de Schotten and Forkel, 2022). This underscores the importance of connectivity in maintaining the functional and structural integrity of distant brain regions.

Furthermore, there is mounting evidence suggesting that coordinated dynamics within and between modular neural networks support the functional organization of the human brain (Fox et al., 2005). The term "connectome," introduced in 2005, marks a significant shift in neuroscience by highlighting the brain's functional networks, connectivity, and inter-regional interactions (Fornito and Bullmore, 2015). While the traditional focus of associating regional fMRI activity with specific tasks remains valuable, the increasing interest in connectomics

has spurred the use of functional connectivity methods in both task-based and resting-state fMRI studies. FC analyses have proved reliable in identifying these brain networks both at individual and group levels (Raimondo et al., 2021).

FC studies have unveiled how these intrinsic functional networks in the brain develop throughout the lifespan. Understanding normal FC development with age has provided crucial benchmarks for evaluating deviations during early development and degeneration in later life. This has led to the significant insight that many clinical conditions are associated with complex, distributed network-level changes in the brain, rather than merely focal abnormalities (Zhang et al., 2021). FC studies have contributed to a dimensional approach for investigating transdiagnostic clinical symptoms and have enhanced multimodal characterization and prediction of symptom progression across various conditions (Zhang et al., 2021). Clinical FC studies have significantly advanced our understanding of how brain function, widely distributed across numerous regions within a network, or across multiple networks, can be impacted by disease and disorder (Zhang et al., 2021). Beyond connectivity, approaches like Dynamic Causal Modelling (DCM) offer insight into how one brain region may influence another, addressing causality within these networks but this was beyond the scope of the current work.

In summary, fMRI-based functional connectivity methods have revolutionized our understanding of brain function and dysfunction. Brain connectivity involves more than merely transmitting signals between regions. Behaviour and cognition are products of interactions between cortical areas, necessitating integration between local and distant regions within densely interconnected networks. By examining how different brain regions interact as networks, rather than in isolation, this research aims to provide new insights into the complex organization of the depressed brain and how this organization may be altered in treatment-resistant depression.

2.4.1. fMRI Functional Connectivity Methodologies

Resting-State Functional Connectivity

Resting-state fMRI (rs-fMRI) is one of the primary methods used to study FC. It involves scanning the brain while the subject is not engaged in any specific task, allowing researchers to capture spontaneous intrinsic brain activity. This method is based on the observation that even in a resting state, the brain exhibits coherent, spontaneous fluctuations in the BOLD signal that reflect ongoing neural communication and the brain's intrinsic functional architecture (Fox & Raichle, 2007). Rs-fMRI provides a unique glimpse into the brain's intrinsic activity. Unlike task-based studies, where participants are engaged in specific cognitive tasks, rs-fMRI captures the brain in its natural "at-rest" state—though "rest" is somewhat of a misnomer. Even in this so-called resting state, the brain is far from inactive. It continuously engages in spontaneous activity that reflects its default mode of operation, revealing intrinsic or spontaneous functional connectivity (Biswal et al., 1995). Rs-fMRI is particularly useful for evaluation of neural circuits because it does not require task performance, making it accessible to various populations, including young children, the elderly, and patients with cognitive impairments (Greicius et al., 2003).

In this thesis, two analytical methods were employed using rs-fMRI to study FC: *Seed-Based Correlation Analysis* and *Independent Component Analysis (ICA).* The seed-based correlation analyses approach involves selecting an a priori defined region of interest (ROI) and calculating the temporal correlation of its BOLD signal with other brain regions. The resulting connectivity maps illustrate how activity in the seed region correlates with activity in the rest of the brain, revealing functional connections (Lowe et al., 1998). The choice of seed regions is often hypothesis-driven, based on prior knowledge or specific research questions. Hence, the reliance on a priori seed selection can introduce bias and may overlook novel connectivity patterns. In contrast, ICA is a data-driven technique that decomposes fMRI data into a set of spatially and temporal independent components, each representing a distinct neural network or artifact (Beckmann et al., 2005). ICA does not require predefined seed regions, allowing for the discovery of connectivity patterns across the entire brain. This method is particularly useful for identifying networks that are not well-captured by seed-based approaches and for separating neural signals from noise.

Chapter 3 utilizes seed-based connectivity analysis to explore habenula connectivity, which is critical for understanding reward processing differences between TRD and TSD. Chapter 4 combines seed-based and ICA approaches to evaluate both intra- and inter-network connectivity within the default mode network to further investigate its role in distinguishing TRD from treatment-sensitive depression. Through these complementary techniques, this

research provides a more comprehensive analysis of the connectivity patterns associated with TRD across different neural networks.

Task-Based Functional Connectivity

Task-based fMRI (tb-fMRI), in contrast to rs-fMRI, provides a more controlled exploration of brain connectivity by examining how regions interact during specific cognitive tasks. Task-based fMRI provides precise control over experimental conditions, enabling researchers to link brain activity and connectivity patterns directly to specific cognitive processes or behaviours (Petersen & Sporns, 2015). This specificity is crucial for understanding the neural basis of complex cognitive functions and for identifying task-related functional networks.

Psychophysiological Interaction (PPI) method is a technique that assesses how the relationship between the activity in a seed region and other brain regions changes in response to a specific task (McLaren et al., 2012). It involves modelling the interaction between the seed region's activity and the experimental condition, allowing researchers to evaluate task-related changes in functional connectivity. By examining these interactions, PPI helps to identify networks that are activated during specific cognitive tasks.

By combining insights from both rs-fMRI and tb-fMRI, we can appreciate the brain's dual nature: its stable, intrinsic architecture that supports a continuous baseline of activity, and its flexible, adaptive networks that can reorganize in response to specific demands. This dual approach is vital for a comprehensive understanding of brain function, emphasizing the importance of using multiple methodologies to capture the full complexity of neural networks.

The main analytical method used to study tb-fMRI FC in this thesis was: *Generalized Psychophysiological Interaction (gPPI).* gPPI is an extension of the PPI method that examines task-specific changes in functional connectivity (McLaren et al., 2012). Unlike traditional PPI, which models connectivity between a seed region and other brain regions based on a single task contrast, gPPI allows for multiple task conditions to be modelled simultaneously, providing a more nuanced view of how different cognitive states modulate connectivity. This method is particularly useful for exploring how specific task demands influence the interaction between brain regions.

In this research, Chapter 5 applies gPPI to examine task-based functional connectivity of the rACC during tasks designed to evoke emotional responses. This analysis is key to understanding how emotion processing, particularly in TRD, affects connectivity in the brain's DMN and related regions, and highlights the role of the rACC in emotion regulation and dysfunction in TRD.

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Chapter 3: Abnormal Habenula Functional Connectivity Characterizes Treatment-Resistant Depression

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Abnormal habenula functional connectivity characterizes treatment-resistant depression

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ABSTRACT

Background: A significant proportion of patients with major depressive disorder are resistant to antidepressant medication and psychological treatments. A core symptom of treatment-resistant depression (TRD) is anhedonia, or the inability to feel pleasure, which has been attributed to disrupted habenula function – a component of the reward network. This study aimed to map detailed neural circuitry architecture related to the habenula to identify neural mechanisms of TRD.

Methods: 35 TRD patients, 35 patients with treatment-sensitive depression (TSD), and 38 healthy controls (HC) underwent resting-state functional magnetic resonance imaging. Functional connectivity analyses were performed using the left and right habenula as seed regions of interest, and the three groups were compared using whole-brain voxel-wise comparisons.

Results: The TRD group demonstrated *hyperconnectivity* of the left habenula to the left precuneus cortex and the right precentral gyrus, compared to the TSD group, and to the right precuneus cortex, compared to the TSD and HC groups. In contrast, TSD demonstrated *hypoconnectivity* than HC for both connectivity measures. These connectivity values were significantly higher in patients with a history of suicidal ideation.

Conclusions: This study provides evidence that, unlike TSD, TRD is characterized by hyperconnectivity of the left habenula particularly with regions of the default mode network. An increased interplay between reward and default mode networks is linked to suicidality and could be a possible mechanism for anhedonia in hard to treat depression.

1. Introduction

First line treatments for major depressive disorder (MDD) consist of focussed psychological interventions and antidepressant medications (ADMs) (Malhi et al., 2021). Unfortunately, more than 60% of patients when first prescribed ADMs fail to achieve remission and a further 50% of these do not respond to second and subsequent rounds of ADMs (Taylor et al., 2019). Patients who fail to produce a significant clinical and functional improvement with at least 2 trials of antidepressants from different pharmacologic classes (adequate in terms of dosage, duration, and compliance) are considered to suffer from treatment-resistant depression (TRD) (Gaynes et al., 2020). This definition has

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also recently been reframed to include patients who fail to respond to neuromodulation and psychotherapies (Malhi et al., 2019). Months or years of trying various treatment regimens without finding relief results in increased emotional and financial burden to the individual, family and caregivers. TRD also causes the highest direct and indirect medical costs among MDD patients. Individuals with TRD are twice as likely to be hospitalized, when compared to patients with treatment-sensitive depression (TSD) and the cost of this hospitalization for TRD is more than six times the mean total cost for TSD (Greenberg et al., 2015). This stresses the importance of understanding the neural mechanisms underlying this disorder to not only achieve timely, effective treatment by identifying these individuals earlier but to also identify novel neural treatment targets.

TRD patients exhibit the same diversity of symptoms, course, history and co-occurring conditions as for TSD patients (Akil et al., 2018). These symptoms heavily interfere with the individual's life, and include impairments in cognition, sad mood, concentration difficulties, fatigue, and anhedonia (Kennedy & Ceniti, 2018). Anhedonia is broadly defined as lack of interest or the inability to experience pleasure (Delfino et al., 2021). It appears to be one of the main symptoms of TRD, with significant impact on course of treatment (Slupski et al., 2020). It has been considered as an independent somatic symptom in TRD and defined as a target for next generation treatments for these patients, including pharmacological and non-pharmacological interventions (Slupski et al., 2020). Identifying treatment-resistant forms of depression early in the course of the disease could potentially enable clinicians to manage more appropriate treatment in a timely manner.

Neuroimaging-based biomarkers offer potential to identify which depression profile will respond to particular treatments and predict treatment outcomes (Akil et al., 2018, Korgaonkar et al., 2020). In recent years, resting-state functional magnetic resonance imaging (rs-fMRI) has become widely applied in studying brain functional changes as it is more easily replicated and independent of task-related confounds. This approach measures temporal correlations of levels of blood-oxygen dependency signal in multiple brain regions to estimate their interactions and can be used to identify atypical intrinsic brain function in psychiatric disorders. Research suggests the functional architecture of the brain is made up from a collection of interacting but functionally dependent networks (Schaefer et al., 2017).

Previous fMRI studies indicate that abnormal circuitry underlying TRD may involve the affective, salience, auditory, visual networks, and the language processing cortex (He et al., 2016). Recent studies have shown, more specifically, abnormal functional connectivity (FC) in the habenular nucleus (Amiri et al., 2021), especially with the default mode network (DMN) (Luan et al., 2019) in TRD patients. This is very interesting given the putative functions of the habenula.

The habenula is a small midbrain structure in the pineal region divided into two nuclear complexes, the medial and the lateral habenula. Of those two compartments, the lateral habenula is thought to play a major role in the encoding of aversive stimuli and is strongly connected to both the reward system and motor regions (Hu et al., 2020, Metzger et al., 2019). There are a growing number of neuroimaging studies that suggest that abnormal lateral habenula function could be involved in the pathogenesis of psychiatric disorders, including MDD (Gosnell et al., 2019, Skandalakis et al., 2018, Zhu et al., 2019, Ambrosi et al., 2019, Wu et al., 2020). More specifically, evidence suggests that the lateral habenula is highly involved in the induction of depression-like symptoms, as the processing of negative-valence information (Yang et al., 2018) and anhedonia (Coccurello, 2019). Abnormalities in the lateral habenula are shown to be particularly associated with reward processing underlying anhedonia, or diminished sensitivity to rewarding stimuli, in depression (Coccurello, 2019). Circuitry wise, it may influence neurotransmission between dopaminergic neurons in the ventral tegmental area and the medial prefrontal cortex (mPFC) (Browne et al., 2018, Aizawa & Zhu, 2019).

Given that anhedonia is a core symptom of TRD, and that the habenula plays a crucial role in the development of anhedonia in patients with depression, it is important to understand if abnormalities in the habenula function are a distinguishing feature of TRD. Indeed, aberrant habenular functional connectivity has been found in patients with TRD. TRD patients were shown to have increased FC between the right habenula with medial superior frontal gyrus, anterior cingulate cortex and medial orbitofrontal gyrus, decreased FC of right habenula with corpus callosum (Luan et al., 2019) and with median raphe (Gosnell et al., 2019), increased FC in left habenula with the locus coerulus (Gosnell et al., 2019) and the inferior temporal gyrus, and decreased FC in left habenula with insula (Luan et al., 2019). There is also preliminary evidence linking abnormal habenular nucleus activity and the DMN (von Hohenberg et al., 2018), particularly in TRD patients (Luan et al., 2019). These findings indicate that dysfunction in habenular -related circuitry could be a key feature/marker for TRD. However, the literature is incomplete. Most previously reported findings cannot be generalized to all TRD patients, as most studies report results comparing TRD with healthy individuals rather than to TSD patients (Ge et al., 2019). Other studies limit their analyses to the responsiveness to a particular treatment being trialed e.g. psilocybin (Carhart-Harris et al., 2017), ketamine (Rivas-Grajales et al., 2021, Chen et al., 2019), transcranial magnetic stimulation (TMS) (Avissar et al., 2017), electroconvulsive therapy (ECT) (Waarde et al., 2015), making it difficult to generalize and to identify reliable diagnostic biomarkers for TRD.

The aim of this work is to investigate differences in habenular resting-state functional connectivity between TSD patients and TRD patients, across multiple treatment-types.

2. Materials and Methods

2.1. Participants

Thirty-nine TRD and thirty-five TSD patients were recruited through general practitioner referrals and clinics. Thirty-eight healthy individuals (HC) participants were recruited through community advertisements. Data collection was conducted at Westmead Hospital, Department of Radiology and at the Brain Dynamics Centre, The Westmead Institute for Medical Research, in Sydney, Australia.

TRD and TSD patients met DSM-5 criteria for primary diagnosis of MDD, according to the Structured Clinical Interview for the DSM-5 (SCID-5) (American Psychiatric Association, 2013). TRD was defined as no remission of symptoms with at least two adequate trials (in terms of dosage, duration – 6 weeks for each trial) of antidepressant of different pharmacologic classes, as well as the presence of moderate to severe symptoms. Severity of the symptoms was characterized by a 17 item Hamilton Depression Rating Scale (HAMD-17) (Hamilton, 1960) score greater or equal to 16. TSD patients were defined as symptom-remitted patients for at least two weeks, a HAMD-17 score of or less than or equal to 9. HC were healthy individuals with no psychiatric illnesses, assessed using the SCID-5. All participants were aged between 18 and 65 years old.

Exclusion criteria included a) inability to provide consent, b) insufficient English proficiency, c) current primary diagnosis of eating disorder, psychosis, personality disorder or primary post-traumatic stress disorder, d) substance dependence for the past 3 months, e) pregnancy, f) history or current neurological disorder or prior brain injury, g) ECT or TMS in the last 6 months, h) contraindication to MRI.

For both patient groups, indices of illness severity and chronicity were assessed. These indices included age of onset, number of inpatient hospitalizations, length of remission period since last episode, number of previous depressive episodes, history of suicidal ideation and behavior, and history of suicide attempt. Information on past and current medication and other forms of treatment (e.g. ECT, or TMS) was also collected. Level of functioning was assessed by the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman et al., 1992).

The research protocol was approved by the Western Sydney Local

Health District Human Research Ethics Committee and all participants provided written consent.

2.2. Imaging acquisition

MRI acquisitions were performed in a 3 Tesla MRI Scanner (Prisma, Siemens Medical Solutions, Germany), with a 64-channel array head coil.

Participants underwent a scanning protocol that included an 8-minutes resting-state protocol in which the participants were instructed to remain still, to relax and let their mind wander while looking at a fixation cross projected onto the screen. Functional T2*-weighted echo-planar images were acquired (repetition time (TR) = 1500 ms, echo-time (TE) = 33.0 ms, field of view = 255 mm; flip angle = 90°, phase encoding direction = A ≫ P, excitation = standard, 60 axial slices resulting in isotropic voxels of 2.5 mm^3 encompassing the whole brain). A three-dimensional T1-weighted structural images (TR/TE = 2400 ms/2.21 ms, field of view = 256 mm, flip angle = 8°, inversion time = 900 ms, phase encoding direction = A ≫ P, 192 sagittal slices resulting in isotropic voxels of 0.89 mm^3 encompassing the whole brain).

2.3. Imaging data analyses

Data processing and analyses were performed using Matlab R2018b (The Mathworks inc, Natick, Massachusetts), SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) and CONN functional connectivity toolbox v16b (http://www.nitrc.org/projects/conn/). Anatomical images were segmented into grey matter, white matter, and cerebrospinal fluid. Pre-processing of the functional images included realignment, unwrapping, motion correction, co-registration to native space structural data, smoothing with a 6-mm FWHM Gaussian kernel, and normalization to Montreal Neurological Institute (MNI) space. To eliminate the influence of residual noise components in the blood-oxygen-level-dependent (BOLD) signal, the data was also subject to a denoising process, using the default pipeline for denoising (including anatomical component-based noise correction procedure and default bandpass filtering [0.01, 0.1] Hz) andfunctional outlier detection tools in CONN (ART-based scrubbing). Scrubbing correction outputs were analysed to detect datasets with high-motion volumes of BOLD data, and subjects with a volume-to-volume index of head motion (head displacement from previous frame) higher than 1 mm were considered outliers. Four TRD participant datasets were excluded for excessive head motion during the scan.

Seed-based - a priori selection of regions of interest (ROIs) - FC analyses on the rs-fMRI data were performed, using the left and the right habenula as seeds. Due to the small size of the habenular nucleus, the left and right habenula seed ROIs were manually created for every subject (Luan et al., 2019). The T1-weighted structural images were used to visually identify the left and right habenula, using SPM12. In T1-W images, the habenula is clearly visible as two small triangular structures, hyperintense to the surrounding cerebrospinal fluid and grey matter, pointing into the third ventricle, on the epithalamus. Each functional ROI was a 3 × 3 × 3 mm cube placed around the central MNI coordinate in the habenula, identified individually for each participant.

For each seed, whole-brain voxelwise FC was quantified. In the first-level FC analyses, CONN calculated the functional connectivity values as bivariate Fisher's z-transformed correlation coefficients for the association between each seed BOLD timeseries and each voxel of the whole brain BOLD timeseries, using generalized linear model (GLM). The correlation coefficients were then used in subsequent second-level statistical analysis, and compared between the TRD, TSD, and HC groups through ANOVA tests of variance (for three groups). The statistical parametric maps were thresholded at the cluster-level false discovery rate (FDR)-corrected for multiple comparisons $p < 0.05$, using an initial voxel-wise $p < 0.001$.

Secondly, mean beta resting-state FC values were extracted from significant clusters found in the seed-to-voxel whole brain analyses to explore post-hoc paired contrasts.

2.4. Analyses of demographic and clinical factors

Statistical analyses were performed using SPSS software version 21 (IBM Corp, 2012).

All three groups were compared for age and gender (demographic variables), using a one-way ANOVA and chi square test, respectively. The TRD and TSD groups were compared for age of onset, age of first episode, depression severity (HAMD-17 score), functionality (SOFAS score), severity of worst depressive episode, number of previous depressive episodes, using student t-tests. The groups were also compared for history of hospitalizations, suicidal attempts and suicidal ideation, using chi square tests of independence.

We further explored the effect of group, age, age at first episode, length of time on ADMs, HAMD-17 score, and SOFAS score, and suicidal ideation in explaining the variance in the significant neural measures using a univariate GLM ANOVA, with group as the fixed factor and the demographic and clinical variables as co-variants in the model.

FC values were tested for correlations with clinical and demographic variables in the TRD group. The effect of history of suicidal ideation, history of suicidal attempt, and ECT treatment in explaining the variance of the neural measures were also explored, using independent samples t-tests to compare differences in the FC measures between those with and without history of suicidal ideation, suicidal attempt and ECT treatment. Histograms were generated to visualize the distribution of frequencies for the variables with significant differences between the groups.

All statistical tests were corrected for multiple comparisons, and all effects considered significant at the $p < 0.05$ significance level.

3. Results

3.1. Demographic and clinical characteristics

Demographic and clinical data for the final sample are summarized in Table 1. All three groups were comparable for age and gender. The TRD group was significantly worse in their depressive profile when compared with the TSD group, with a greater severity of depressive symptoms (severity of worst episode), poorer functioning (SOFAS) and more hospitalizations and suicide attempts. There were no significant differences between groups for motion during the scan, after the exclusion of the four TRD participants for excessive motion (Supplementary Section).

3.2. Functional connectivity analyses

The 3-way ANOVA identified significant connectivity differences between the three groups for the left habenula. There were significant connectivity group differences for the left habenula with the precentral gyrus and bilateral precuneus cortical regions (Table 2, Fig. 1). More details on supplementary FC results comparing only the TRD and TSD groups are presented on the Supplementary Section. Next, we extracted mean FC beta values for the voxels from the three significant clusters identified in the whole-brain analyses and compared them between the groups.

Post-hoc tests revealed hyperconnectivity of the TRD group relative to TSD and HC groups in the right precuneus cortex cluster, and relative to the TSD group in the left precuneus cortex and the right precentral gyrus clusters.

The TSD group on the other hand demonstrated significant hypo-connectivity than TRDs in the left precuneus and right precentral gyrus clusters, and relative to the HC group in all the three clusters.

Table 1
Demographic and clinical characteristics of participants from TRD, TSD and HC groups.

	TRD (35)	TSD (35)	HC (38)	F/t/X^2	sig
Demographics					
Age, Mean ± SD [Min-Max]	42.3 ± 14.1 [18.1–64.3]	37.2 ± 11.0 [20.0–57.4]	47.1 ± 14.3 [18.9–66.0]	n.s.	n.s.
Gender (M), N (%)	14 (40)	17 (48.6)	17 (44.7)	n.s.	n.s.
Clinical Profile					
Age of onset, Mean ± SD [Min-Max]	26.97 ± 13.13 [8–53]	21.66 ± 9.26 [8–50]	n.a.	n.s.	n.s.
Number of previous MDE, Mean ± SD [Min-Max]	8 ± 11 [1–40]	6 ± 6 [1–30]	n.a.	n.s.	n.s.
CGI-S - Severity of worst MDE, Mean ± SD [Min-Max]	6.77 ± 0.49 [5–7]	5.21 ± 1.36 [3–7]	n.a.	6.346	<0.001
Length of current episode, days, Mean ± SD [Min-Max]	117.1 ± 117.2 [14–365]	n.a.	n.a.	n.a.	n.a.
HAMD-21 score, Mean ± SD [Min-Max]	25.23 ± 6.46 [16–41]	4.18 ± 3.10 [0–9]	n.a.	15.819	<0.001
SOFAS score, Mean ± SD [Min-Max]	73.91 ± 16.18 [40–100]	89.58 ± 5.47 [78–95]	n.a.	−5.275	<0.001
History of Hospitalizations, N (%)	25 (71.4)	6 (17.1)	n.a.	20.902	<0.001
History of ECT, N (%)	10 (28.6)	0 (0)	n.a.	11.667	0.001
History of TMS, N (%)	3 (8.6)	0 (0)	n.a.	n.a.	n.a.
History of Suicidal Ideation, N (%)	28 (80)	26 (74.3)	n.a.	n.s.	n.s.
History of Suicidal Attempt, N (%)	19 (54.3)	5 (14.3)	n.a.	13.938	<0.001
Remission time (days), Mean ± SD [Min-Max]	n.a.	409 ± 1062 [14 – 6205]	n.a.	n.a.	n.a.

n.a. – not applicable, n.s. – not significant; SD – Standard Deviation; M – male; MDE – Major Depressive Episode; HAMD-21 – Hamilton Depression Rating Scale, 21 items; SOFAS – Social and Occupational Functioning Assessment Scale; CGI-S – Clinical Global Impression, Severity; ECT – Electroconvulsive Therapy; TMS – Transcranial Magnetic Stimulation; N – total number.

Table 2
Summary of the main findings from the seed-based functional connectivity analyses.

Source seeds	Brain regions	Side	Cluster size (voxels)	F value	Post-hoc	Peak MNI coordinates (mm)		
						x	**y**	**z**
Habenula		L						
	Precentral Gyrus	R	102	12.757	TRD, HC > TSD	62	2	42
	Precuneus Cortex	L	122	14.714	TRD, HC > TSD	−12	−76	48
	Precuneus Cortex	R	69	13.542	TRD > HC > TSD	18	−72	38

R – right, L – left; TRD – treatment-resistant depressive patients, TSD – treatment-sensitive depressive patients, HC – healthy controls.

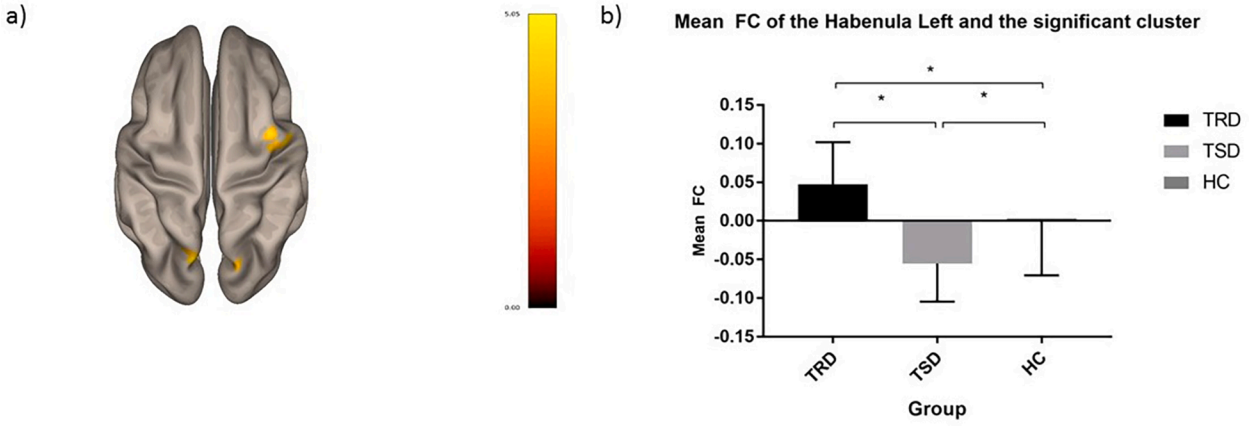

Fig. 1. Functional connectivity differences between the three groups. Illustration of the differences functional connectivity (FC) patterns of the left habenula between the three groups: (a) the left and the right precuneus cortex and right precentral gyrus FC with the left habenula showed differences between the treatment-resistant depression (TRD) group and the treatment-sensitive depression (TSD) group (TRD > TSD) (superior view); (b) means of FC of the left habenula ot the combined clusters described at (a) for TRD, TSD and healthy controls (HC). The color scale bar on (a) represents the strength of the t statistic. The "mean FC" (mean functional connectivity) on (b) represents beta values of functional connectivity between the two regions.

3.3. Correlations between FC and demographic and clinical measures

TRD patients with a history of suicidal ideation were shown to have higher FC in the left habenula – left and right precuneus cortex – right precentral gyrus clusters, when compared to patients without history of suicidal ideation ($t = 2.407$, $p = 0.038$) (see Fig. 2). As suicidal ideation was found to be significantly associated with the FC measures, a follow-up analyses as conducted to evaluate if it was a contributing factor for the groups differences found, by running a GLM ANOVA on the two clinical groups, controlling for suicidal ideation. The results ($F(2,68) = 40.02$, $p < 0.001$) indicate that there is an effect of group in the neural measure, controlling for suicidal ideation – so, differences between the clinical groups in the FC between the left habenula and the precuneus cortex remain beyond the effect of suicidal ideation.

There were no other significant effects (more details on Supplementary Section).

4. Discussion

This study identified a pattern of hyperconnectivity in TRD, especially between the left habenula and the bilateral precuneus cortex and the right precentral gyrus, which is not only abnormal (different from

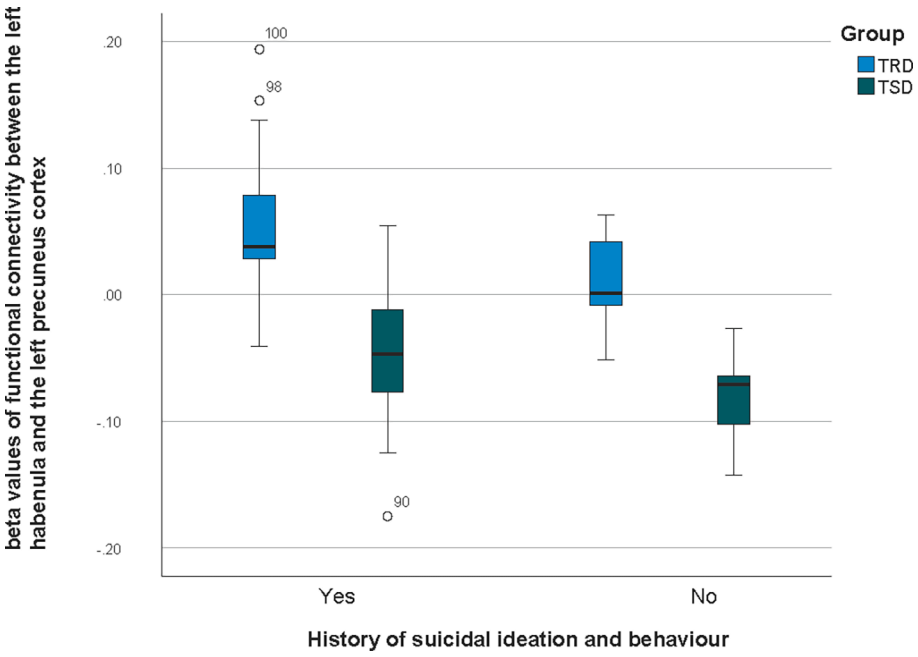

Fig. 2. Frequency distributions for functional connectivity between the left habenula and the precuneus cortex, in individuals with and without a history of suicidal ideation, in the TRD group and TSD group. Boxplots illustrating of the distrubition of frequencies for functional connectivity (FC) beta values between the left habenula and the left precuneus cortex, in patients with (Yes) and without (No) a history of suicidal ideation and behaviour. Frequency distributions are represented separately for the treatment-resistant depression (TRD) group and the treatment-sensitive depression (TSD) group.

HC) but also greater than TSD. These results suggest that abnormal resting state connectivity in the habenular circuitry might be a distinguishing feature of TRD, as compared to those patients who respond to treatment.

The precuneus is known to be part of the posterior default mode network, the functional neural system that controls internal rumination and switching between external and internal cognition (Cavanna & Trimble, 2006, Fransson & Marrelec, 2008). Our findings confirm that abnormal functional connectivity in the DMN distinguishes TRD from TSDs. Dysfunction of this network has been previously implicated in the maintenance of depressive states (Liu et al., 2021). Recent functional neuroimaging studies in healthy subjects indicate that the precuneus plays a central role in a broad range of highly integrated functions, episodic memory retrieval, and self-processing operations, namely first-person perspective taking and an experience of agency (or the ability to control external events through our own actions) (Cavanna & Trimble, 2006).

The reward circuit is critical for motivated behavior and the capacity to feel pleasure in response to an event so it is conceivable that abnormalities in this circuitry may underpin what is clinically known as anhedonia (Yang et al., 2021). Habenula function is thought to integrate dopaminergic and serotonergic inputs in to this, encoding reward value, probability and magnitude. As a critical node of the reward-related circuit, habenula hyperconnectivity might be associated with a higher influx of internal negative thoughts which could then feedback into the reward system leading to increased anhedonia and/or that sensory information is being overly processed as negative stimuli. It has also been reported that reduced engagement of the precuneus cortex is associated with difficulties in positive future scene simulation in individuals with anhedonia (Yang et al., 2021). Further, there is evidence of TRD patients characterized by impaired connectivity of the DLPFC and precuneus component of the attention and default mode networks (Williams et al., 2021). This indicates there may be abnormalities in the interaction between DMN and sensory information in the encoding of negative reward by the habenula, in patients with TRD. However it is important to note that our study used resting state fMRI and future work should explore these functional relationships using task based fMRI and cognitive behavioral data.

Abnormalities in connectivity between the reward network and the precuneus cortex are also thought to be related to other clinical symptoms of depression, such as suicidality (Zhang et al., 2016). Although we confirmed that, for this sample, suicidal ideation is not a contributing factor for group differences in FC, the higher levels of suicidal ideation in the TRD group compared to the TSD group may be associated with the hyperconnectivity of the habenular circuit involving regions of the DMN in the TRD group. The higher habenular FC found in people with a history of suicidal behaviour may also mediate a dysfunction in the mechanism that links the habenula with motor activity and contextual associative processing. This could be linked to its hyperconnectivity with the right precentral gyrus, as it is the location of the primary motor cortex. Furthermore, knowing that the habenula is closely linked to the function of reward processing, particularly with regards to encoding negative feedback on negative reward (Baker et al., 2016), our findings suggest that TRD patients exhibit alterations in the brain circuits mediating reward (interrelated with the default mode network) that may affect their proclivity for suicide. This is not necessarily due to decreased motivation, but rather an inability to engage in alternative strategies and actions, as the habenula acts a regulator for behaviour flexibility (Baker et al., 2016). Thus, chronic dysregulation of the habenula circuit seems to be associated with long-term changes in the dopaminergic, serotoninergic and norepinephrinergic activity that are in the background of dysfunctional coping strategies related to suicide-related behavior (Ambrosi et al., 2019).

Treatment studies also point to the importance of the habenula in TRD (von Hohenberg et al., 2018). The antidepressant effects of SSRIs may result from down-regulation of pre-treatment serotonin activity in terminal regions receiving serotonergic projection, such as the habenula. These findings further indicate an important role for the habenula in regulating serotonin levels that are relevant to the symptoms of depression and suggest the habenula as a potential target for antidepressant treatments (Zhao et al., 2015).

5. Limitations

Functional MRI studies on the habenula have several limitations. From an imaging perspective, the habenula volume is very small, ranging from approximately 29 to 36 mm3 in each hemisphere. This may be smaller than the voxel size of a standard fMRI, making its identification challenging. Ideally, lateral and medial habenula should be identified and segregated, as they are known to have differential

functions (Coccurello, 2019) – however, given the resolution limitation of the acquisition protocol used in this study, this was not feasible.We acknowledge that the habenula is a tiny structure and there is risk of signal bleeding from adjacent structures due to the limited resolution. Calculating the absolute volume of the left and right habenular nucleus for each participant would have also been a more accurate way of segmenting this region, and future work should use individual-specific habenula masks based on anatomic manual segmentation.

Secondly, it is likely that the habenular signal is contaminated by activity in adjacent structures, such as the medial dorsal thalamus or the epithalamic paraventricular nucleus. This work needs to replicated using more high-resolution fMRI scanning at possibly higher field strengths. A further limitation is that the sample size is relatively small and that the participants had been trialled on many different antidepressant medications. The response may also have been different in participants who were managed with non-pharmacological treatments. Although we collected information about current and past medication, it was based on patients' self-report, and it is likely that we may have not captured the medication history accurately. With an increased sample size, it may be possible to differentiate these subtypes, and we strongly suggest this to be done in future work. The generalizability of our findings also needs to be validated in independent cohorts.

6. Conclusions

These findings indicate that different responsiveness profiles in depression are associated with distinct pathophysiological mechanisms. Unlike TSD, TRD is characterized by hyperconnectivity of the habenula with regions of the default mode network and sensorimotor networks, which may be associated with the capacity to encode negative feedback, and the mechanisms of suicidal ideation. This may suggest that this connectivity feature could be a potential treatment target for hard to treat depression. Future research should also consider assessing this functional connectivity feature in depression patients prior to treatment resistance is determined and exploring if this feature characterizes non-remission to one type of treatment (i.e. ADMs) or if it is a general mechanism to resistance across multiple treatments. This may potentially enable clinicians to identify treatment-resistant forms of depression and initiate appropriate treatment options earlier.

CRediT authorship contribution statement

Ana Rita Barreiros: Data curation, Investigation, Resources, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Isabella Breukelaar:** Formal analysis, Writing – review & editing. **Prashanth Mayur:** Resources, Writing – review & editing. **Jagadeesh Andepalli:** Resources, Writing – review & editing. **Yoshiro Tomimatsu:** Conceptualization, Methodology, Writing – review & editing. **Kenta Funayama:** Conceptualization, Methodology, Writing – review & editing. **Sheryl Foster:** Resources, Writing – review & editing. **Philip Boyce:** Writing – review & editing. **Gin S. Malhi:** Writing – review & editing. **Anthony Harris:** Conceptualization, Project administration, Data curation, Resources, Writing – review & editing. **Mayuresh S. Korgaonkar:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anthony Harris received funding from Takeda Pharmaceutical Company for this project. There are no other financial disclosures related to the work. Mayuresh Korgaonkar received funding from Takeda Pharmaceutical Company for this project. There are no other financial disclosures related to the work. And all other authors has declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2022.102990.

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45

Chapter 4: Intra- and Inter-Network Connectivity of the Default Mode Network Differentiates Treatment-Resistant Depression from Treatment-Sensitive Depression

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Intra- and Inter-Network connectivity of the default mode network differentiates Treatment-Resistant depression from Treatment-Sensitive depression

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A R T I C L E I N F O

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A B S T R A C T

Understanding why some patients with depression remain resistant to antidepressant medication could be elucidated by investigating their associated neural features. Although research has consistently demonstrated abnormalities in the anterior cingulate cortex (ACC) – a region that is part of the default mode network (DMN) – in treatment-resistant depression (TRD), a considerable research gap exists in discerning how these neural networks distinguish TRD from treatment-sensitive depression (TSD). We aimed to evaluate the resting-state functional connectivity (rsFC) of the ACC with other regions of the DMN to better understand the role of this structure in the pathophysiology of TRD. 35 TRD patients, 35 TSD patients, and 38 healthy controls (HC) underwent a resting-state functional MRI protocol. Seed-based functional connectivity analyses were performed, comparing the three groups for the connectivity between two subregions of the ACC (the subgenual ACC (sgACC) and the rostral ACC (rACC)) and the DMN ($p < 0.05$ FWE corrected). Furthermore, inter-network connectivity of the DMN with other neural networks was explored by independent component (ICA) analyses ($p < 0.01$, FDR corrected). The results demonstrated hyperconnectivity between the rACC and the posterior cingulate cortex in TRD relative to TSD and HC ($F(2,105) = 5.335$, $p < 0.05$). ICA found DMN connectivity to regions of the visual network (TRD<TSD) and a parietal region of the DMN (TRD>TSD), differentiating the two clinical groups. These results provide confirmatory evidence of DMN hyperconnectivity and preliminary evidence for its interactions with other neural networks as key neural mechanisms underlying treatment non-responsiveness.

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1. Introduction

Major Depressive Disorder (MDD) is a prevalent psychiatric disorder resulting in significant negative outcomes. Although several therapeutic interventions are accessible for its management, attaining remission has proven challenging (Kverno and Mangano, 2021). Over 30 % of the patients with MDD fail to achieve complete remission after different levels of successive pharmacological treatment (Taylor et al., 2019), with decreasing likelihood of response at each subsequent antidepressant medication treatment trial. This is referred to as treatment-resistant depression (TRD). Alternative brain stimulation therapies are promising for patients who do not respond to standard treatment options (Wu et al., 2019), as they offer potential treatment avenues in the context of TRD, where the mechanisms are currently unknown and ideal therapeutic targets remain elusive.

Functional magnetic resonance imaging (fMRI) has been used to understand the neural mechanisms that underlie MDD, and research suggests that resting-state fMRI may elucidate neural mechanisms that can help predict the response to various forms of treatment (Ge et al., 2017). Functional connectivity (FC) is a widely used fMRI technique that enables the investigation of inter- and intra-brain network interactions and dependencies (Andreescu et al., 2013). FC measures the synchronised fluctuations of activity between brain regions across time. During rest, brain regions exhibit slow, correlated fluctuations which reflect the intrinsic architecture of the brain exposing how the primary functional networks of the brain are contributing to undirected thought patterns (Andreescu et al., 2013). Recent work has shown that it is possible to non-invasively modulate resting FC (Taylor et al., 2022) and to use it as a treatment intervention in psychiatric disorders such as TRD (Taylor et al., 2022) or post-traumatic stress disorder (PTSD) (Lieberman et al., 2023).

Depression is associated with altered FC within the default mode network (DMN) – a functional network consisting of several brain regions such as the posterior cingulate cortex, medial prefrontal cortex, medial temporal lobe, and inferior parietal lobe (Andreescu et al., 2013). In individuals with depression, there is often increased connectivity between anterior regions, such as the rostral anterior cingulate cortex (rACC) or the subgenual cingulate cortex (sgACC) and other DMN regions, such as the medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC) (Kaiser et al., 2015). This enhanced connectivity is thought to be related to excessive rumination and a heightened focus on negative self-referential thoughts, contributing to the persistent negative emotions characteristic of depression (Hamilton et al., 2015).

The DMN is not an isolated network; it interacts with various other networks, such as the cognitive control network (CCN) and somatomotor network, among others (Korgaonkar et al., 2014). The CCN is responsible for cognitive control processes like attention, working memory, and task execution, which are often impaired in depression (Korgaonkar et al., 2019). The interaction between the DMN and CCN is intriguing because they tend to exhibit an anti-correlation relationship, where one becomes more active as the other becomes less active (Whitton et al., 2018). This dynamic balance is crucial for shifting attention from internal thoughts (DMN) to external tasks (CCN). Disruptions in this balance could contribute to the cognitive inflexibility and difficulty disengaging from ruminative thoughts seen in depression (Whitton et al., 2018).

TRD is characterized by persistent rumination, a form of repetitive thought fixated on negative content, often from the past or present, leading to emotional distress (Machino et al., 2014). The DMN is implicated in these self-referential processes and is significantly altered in TRD patients (Hamilton et al., 2015; Machino et al., 2014; Li et al., 2013). Within the DMN, the ACC serves as a critical hub, showing increased activity during rest and self-referential tasks. Altered functional connectivity between the ACC and other DMN regions, such as the mPFC and PCC, has been observed in depression (Whitton et al., 2018). This altered relationship is theorized to play a role in regulating emotional processing and affective behavior, directly underpinning the persistent rumination seen in TRD (Fox & Raichle, 2007). Increased connectivity between the rACC and other DMN regions has been associated with excessive rumination and a heightened focus on negative self-referential thoughts, contributing to the maintenance of negative emotional states in depression (Greicius et al., 2007). There is evidence for a direct neural connection between the ACC and specific brain regions involved in regulating mood, depression, and the antidepressant response (Goldstein-Piekarski et al., 2018, Korgaonkar et al., 2014). Specifically, it has been suggested that the rACC has potential utility in identifying patients who may respond better to antidepressant treatments, irrespective of treatment modality (Mayberg et al., 1997; Pizzagalli, 2011; Fox et al., 2014). Increased pre-treatment rACC physiological activity represents a promising and nonspecific prognostic marker of treatment outcome in depression (Pizzagalli et al., 2018). We recently demonstrated that rACC activity was specifically related to level of treatment resistance using four large samples ranging from psychotherapy, to transcranial magnetic stimulation (TMS) and electroconvulsive therapy (ECT) (Prentice et al., 2023), possibly suggesting prior reports on rACC activation and treatment response could be mediated by treatment-resistance. There is also evidence for a direct association between the sgACC and treatment response in depression (Korgaonkar et al., 2014). For instance, deep brain stimulation targeting the sgACC is shown to be effective in reducing depressive symptoms, particularly anhedonia, in patients with TRD (Riva-Posse et al., 2014).

In summary, there is strong evidence in the literature suggesting abnormalities in connectivity related to specific subregions of the ACC – the rACC and the sgACC – and particularly that to other DMN regions in depression. Whether this neural circuitry affects response to treatment is still unclear, and there is a need to fully understand the significance of rACC and sgACC functional connectivity as a predictor of treatment response. The aim of this study is to investigate how functional connectivity of the rACC and sgACC with the DMN distinguishes treatment sensitive and treatment resistant depression, using resting-state fMRI. We hypothesized that TRD patients will have higher functional connectivity of the rACC and the sgACC with the DMN when compared to patients with depression who responded to treatment. First, we examined the functional connectivity of the rACC and sgACC with other regions of the DMN. Subsequently, we extended our analysis to investigate the inter-network connectivity between the DMN and the other brain regions, hypothesizing that TRD patients will show lower connectivity between the DMN and other networks of the brain, compared to treatment-sensitive depression (TSD) patients.

2. Materials & methods

2.1. Participants

Initially, 39 individuals with TRD and 35 individuals with TSD were recruited through a local network of specialist psychiatrists and clinics. Thirty-nine individuals with TRD were recruited, but only 35 were used in the final sample due to four participants being excluded for excessive head motion during the scan (see section 2.3 for details). TRD and TSD individuals met DSM-5 criteria for primary diagnosis of MDD, assessed through the Structured Clinical Interview for the DSM-5 (SCID-5) (APA, 2013). The inclusion criteria for TRD were: no remission of symptoms with at least two adequate trials (in terms of dosage, duration – 6 weeks for each trial) of antidepressant of different pharmacologic classes, as well as the presence of moderate to severe depressive symptoms (assessed by a rating greater or equal to 16 in the 21-item Hamilton Depression Rating Scale – HAMD-21) (Hamilton, 1960). Participants in the TSD group were identified as patients who had achieved complete remission of symptoms for at least two weeks (characterized by a HAMD-21 score of less than or equal to 9). The control group (HC) comprised of 38 healthy individuals recruited through community advertisements with no psychiatric illnesses, assessed using the SCID-5. All

participants were aged between 18 and 65 years old.

For both patient groups, indices of illness severity and chronicity were assessed. These indices included age of onset, number of inpatient hospitalizations, length of remission period since last episode, number of previous depressive episodes, history of suicidal ideation and behaviour, and history of suicide attempt. Information on past and current medication and other forms of treatment (e.g. ECT, or TMS) was also collected. Level of functioning was assessed by the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman et al., 1992).

HC and patient groups (TRD and TSD) were matched for sex, age, and education status. Exclusion criteria for all participants included a) inability to provide consent, b) insufficient English proficiency, c) current primary diagnosis of eating disorder, psychosis, personality disorder or primary PTSD, d) substance dependence for the past 3 months, e) pregnancy, f) history or current neurological disorder or prior brain injury, g) ECT or TMS in the last 6 months, h) contraindication to MRI.

Data collection was conducted at Westmead Hospital, Department of Radiology and at the Brain Dynamics Centre, The Westmead Institute for Medical Research, in Sydney, Australia. The research protocol was approved by the Western Sydney Local Health District Human Research Ethics Committee and all participants provided written consent.

2.2. MRI data collection

MRI scanning was carried out with a 3 Tesla MRI Scanner running VE11C software (Prisma, Siemens Medical Solutions, Germany), with a 64-channel head/neck array head coil.

Participants underwent an 8-minutes resting-state protocol, in which they were instructed to remain still, to relax and let their mind wander while looking at a fixation cross projected onto the screen. Functional T2*-weighted echo-planar images were acquired (repetition time (TR) = 1 500 ms, echo-time (TE) = 33.0 ms, field of view = 255 mm; flip angle = 90°, phase encoding direction = A≫P, excitation = standard, 60 axial slices resulting in isotropic voxels of 2.5 mm^3 encompassing the whole brain). A three-dimensional T1-weighted structural dataset was also acquired (TR/TE=2 400 ms/2.21 ms, field of view = 256 mm, flip angle = 8°, inversion time = 900 ms, phase encoding direction = A≫P, 192 sagittal slices resulting in isotropic voxels of 0.89 mm^3 encompassing the whole brain).

2.3. MRI data processing

MRI data were processed and analysed using Matlab R2018b (The Mathworks inc, Natick, Massachusetts), SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK), CONN functional connectivity toolbox v16b. Details of pre-processing steps are presented in our previous study (Barreiros et al., 2022). To reiterate, anatomical images were segmented into grey matter, white matter, and cerebrospinal fluid. Pre-processing of the functional images included realignment, unwrapping, motion correction, co-registration to native space structural data, smoothing with a 6-mm FWHM Gaussian kernel, and normalization to Montreal Neurological Institute (MNI) space. To eliminate the influence of residual noise components in the blood-oxygen-level-dependent (BOLD) signal, the data were also subject to a denoising process, using the default pipeline for denoising (including anatomical component-based noise correction procedure and default bandpass filtering [0.01, 0.1] Hz) and functional outlier detection tools in CONN (ART-based scrubbing). Scrubbing correction outputs were analysed to detect datasets with high-motion volumes of BOLD data (we excluded any participant who had more than 25 % of their volumes flagged as outliers), and subjects with a volume-to-volume index of head motion (head displacement from previous frame) higher than 1 mm were considered outliers. Four TRD participant datasets were excluded for excessive head motion during the scan.

2.4. Demographic and clinical characteristics

Demographic variables, age and gender, were compared for differences across all groups using a one-way ANOVA and chi square test, respectively. The TRD and TSD groups were compared for age of onset, age of first episode, depression severity (HAMD-21 score), functionality (SOFAS score), severity of worst depressive episode, number of previous depressive episodes, using student t-tests. The groups were also compared for history of hospitalizations, suicidal attempts and suicidal ideation, using chi square tests of independence.

2.5. Functional connectivity analyses

FC analyses on the resting-state fMRI data were performed, through a seed-based FC approach. In this approach, regions of interest (ROIs) are selected a priori. For this study, we selected the rACC and the sgACC as the seeds and investigated the FC between these seeds and other regions of the DMN (DMN intra-network connectivity).

The selection of rACC ROI anatomic landmark derived from the voxels reported by Pizzagalli and colleagues (2001), and also used in our prior studies on rACC (Prentice et al., 2023, Arns et al., 2015). Each functional rACC ROI was a 10 × 10 × 10 mm cube placed around the central MNI coordinate in this region (−10 45—5). FC values were calculated from the rACC voxel-wise to the rest of the brain for each participant in the dataset as bivariate Fisher's z-transformed correlation coefficients. This measures the association between the rACC seed BOLD timeseries and each voxel of the whole brain BOLD timeseries using generalized linear model (GLM).

The selection of sgACC ROI anatomic landmark derived from PET imaging studies localizing this region as Brodmann Area 25 (BA25) (Riva-Posse et al., 2014). The ROI for BA25 was generated from the AAL atlas (Rolls et al., 2020).

The rACC was selected based on its well-documented involvement in emotion regulation and its potential as a biomarker for treatment response (Mayberg et al., 1997; Pizzagalli, 2011). In contrast, the sgACC was chosen due to its significant role in the pathophysiology of depression and its connections with other brain regions implicated in mood regulation. This approach allowed us to capture the comprehensive network dynamics involved in TRD and TSD.

The ROIs for the DMN mask used for this analysis were selected based on published literature (Laird et al., 2009). Using a combination of activation likelihood estimation, Laird and collaborators (2009) assessed statistically significant convergence of neuroimaging results and identified core regions in the DMN. Through this *meta*-analytic work, they identified 9 anatomical regions as part of the DMN (coordinates listed in Talairach space): the precuneus cortex (−4, −58, 44), the posterior cingulate (−4, −52, 22), the ventral anterior cingulate (2, 43, −8), the right inferior parietal lobe (52, −28, 24), the medial prefrontal cortex (−2, 50, 18), the right middle temporal gyrus (46, −66, 16), the left middle frontal gyrus (−26, −36, 28), the left inferior parietal lobule (−56, −36, 28), and the left middle temporal gyrus (−42, −66, 18). We created an image mask that consisted of 9 sphere ROIs of 3-mm radius each, centered in the coordinates described for each DMN anatomical region described above, transformed into the MNI space. Pearson correlation coefficients between the average time series of the rACC and the time series of all voxels of the DMN mask were calculated.

Given our hypothesis regarding the DMN, we then explored the FC between the rACC and the sgACC and the DMN compared between the two patient groups (TRD and TSD) for our primary analysis of interest. The analysis of rACC and sgACC to DMN connectivity between the groups was performed in a step-wise manner focusing on comparing the two clinical groups using two-sample t-tests (TRD vs TSD). To further unpack connectivity differences between the two clinical groups, as a secondary analysis, each of the clinical groups was contrasted with the HC group (TRD vs HC, HC vs TSD) for significant connectivity measures from the primary analysis.

The statistical parametric maps threshold was set $p < 0.05$ at the cluster-level family-wise error (FWE)-corrected for multiple comparisons, and an initial whole-brain voxel-wise $p < 0.001$. The minimum cluster size necessary to be considered relevant was 20.

2.6. Independent component analyses

Subsequently, independent component analyses (ICA) were performed, in order to investigate differences in connectivity between the DMN and other brain networks, between the groups. ICA were performed on the functional data of individual subjects and extract subject-specific component maps, using the group-ICA in the CONN toolbox. This data-driven approach enabled the identification of functionally independent networks within each participant and facilitated the examination of the variability in these networks across subjects.

Prior to conducting ICA, we estimated the number of components in the dataset using the Group ICA of fMRI Toolbox (GIFT) v3.0b. For each subject, we estimated the components using minimum description length criteria and then calculated the average number of components across the dataset using the mean of the individual subject results. The group-ICA analysis was conducted using default settings for CONN 18b, with GICA3 back-projection and G1 FastICA with dimensionality reduction set to 64. We determined that there were 23 independent components (ICs) based on the component estimation.

Then, we matched the 23 ICs to a spatial template of neural networks provided by the CONN functional network atlas. The correlation coefficient values indicated the predominant network regions for each component. To evaluate connectivity differences related to each intrinsic connectivity network (ICN), we performed group comparisons. The statistical threshold was set to voxel-wise $p < 0.001$ at an uncorrected level to define the voxel size. Then, we applied a cluster-wise correction at a threshold of false discovery rate $p < 0.05$ to determine significant clusters.

2.7. Clinical factors

In order to analyse the associations between the FC measures and treatment resistance in the TRD group, we computed a variable to reflect chronicity of the disorder, by calculating the number of years from age of onset to current age, which is referred to as "number of years since onset". The relationships between the mean FC values for the significant ROIs and different clinical factors (depression severity, functionality, severity of worst depressive episode, number of previous depressive episodes, and number of years since onset) in the two patient groups separately were analysed using Pearson correlation coefficients.

All statistical tests were corrected for multiple comparisons, using a Bonferroni adjustment. All effects were considered significant at the $p < 0.05$ significance level. Statistical analyses were performed using SPSS software version 21 (IBM Corp, 2012).

3. Results

3.1. Demographic and clinical characteristics

Demographic and clinical data for the final sample are summarized in Table 1. All three groups were comparable for age and gender. As expected, groups were significantly different in some clinical variables, reflecting a more severe clinical profile for TRD patients; although the groups were not different for the number of past depressive episodes, the TRD group had higher rates of history of hospitalizations, history of ECT, and history of suicidal attempts.

There were no significant differences between groups for motion during the scan, after the exclusion of the four TRD participants for excessive motion.

3.2. DMN intra-network connectivity

3.2.1. rACC

Results showed significant differences between the TRD and the TSD groups for the connectivity of rACC with the PCC ($t = 3.83$, p = 0.004, $k = 40$, −2—54 20, family-wise error corrected; large effect size $d = 0.861$) (See Table 2). Subsequent post-hoc cluster analyses revealed significantly lower connectivity of the rACC and PCC in the TSD when compared to the TRD. When compared to the HC group, the TSD group showed significantly lower connectivity between the rACC and the PCC ($t(71) = -2.649$, p = 0.010, with a moderate effect size $d = -0.621$). There were no significant differences between the TRD and the HC, for the connectivity between the rACC and the other regions of the DMN (Table 2).

Table 1
Summary of demographic and clinical characteristics of the sample.

	TRD (35)	TSD (35)	HC (38)	F/t/ X^2	sig
Demographics					
Age, Mean ± SD [Min-Max]	42.3 ± 14.1 [18.1–64.3]	37.2 ± 11.0 [20.0–57.4]	47.1 ± 14.3 [18.9–66.0]	n.s.	n.s.
Gender (M), N (%)	14 (40 %)	17 (48.6 %)	17 (44.7 %)	n.s.	n.s.
Clinical Profile					
Age of onset, Mean ± SD [Min-Max]	26.97 ± 13.13 [8–53]	21.66 ± 9.26 [8–50]	n.a	n.s.	n.s.
Number of previous MDE, Mean ± SD [Min-Max]	8 ± 11 [1–40]	6 ± 6 [1–30]	n.a	n.s.	n.s.
Severity of worst MDE, Mean ± SD [Min-Max]	6.77 ± 0.49 [5–7]	5.21 ± 1.36 [3–7]	n.a	6.346	0.000
Length of current episode, days, Mean ± SD [Min-Max]	117.1 ± 117.2 [14–365]	n.a.	n.a	n.a.	n.a.
HAM-D-21 score, Mean ± SD [Min-Max]	25.23 ± 6.46 [16–41]	4.18 ± 3.10 [0–9]	n.a	15.819	0.000
SOFAS score, Mean ± SD [Min-Max]	73.91 ± 16.18 [40–100]	89.58 ± 5.47 [78–95]	n.a	−5.275	0.000
Antidepressant medication treatment past, N (%)	31 (88.6 %)	30 (85.7 %)	n.a.	76.152	< 0.001
Antidepressant medication treatment current, N (%)	19 (54.3 %)	22 (62.9 %)	n.a.	37.393	< 0.001
Length of time on antidepressant medication, years, Mean ± SD [Min-Max]	4.44 ± 3.70 [0.17 – 12.0]	4.18 ± 4.16 [0.12 – 17.0]	n.a.	n.s.	n.s.
History of Hospitalizations, N (%)	25 (71.4 %)	6 (17.1 %)	n.a	20.902	0.000
History of ECT, N (%)	10 (28.6 %)	0 (0)	n.a	11.667	0.001
History of TMS, N (%)	3 (8.6 %)	0 (0)	n.a	n.a.	n.a.
History of Suicidal Ideation, N (%)	28 (80 %)	26 (74.3 %)	n.a	n.s.	n.s.
History of Suicidal Attempt, N (%)	19 (54.3 %)	5 (14.3 %)	n.a	13.938	0.000

n.a. – not applicable, n.s. – not significant; SD – Standard Deviation; M – male; MDE – Major Depressive Episode; HAMD-21 – Hamilton Depression Rating Scale, 21 items; SOFAS – Social and Occupational Functioning Assessment Scale; CGI-S – Clinical Global Impression, Severity; ECT – Electroconvulsive Therapy; TMS – Transcranial Magnetic Stimulation; N – total number.

Table 2
Summary of the main findings from the seed-DMN functional connectivity analyses.

Source seed	DMN region	Contrast	Cluster size (voxels)*	Cluster P value**	Peak voxel P value (unc.)***	Cluster analyses F/t	p	η2/d	Post-hoc
sgACC	PCC	ANOVA	81	ns	0.009	3.607	0.031	0.064	TRD, HC>TSD
		TRD vs TSD	81	ns	0.002				
		TRD vs HC	ns						
		HC vs TSD	81	ns	< 0.001				
rACC	PCC	ANOVA	116	ns	0.001	6.497	0.002	0.110	TRD, HC>TSD
		TRD vs TSD	40	0.004	< 0.001	3.601	< 0.001	0.861	
		TRD vs HC	ns						
		HC vs TSD	ns						

*considered only k > 10.
**p value threshold < 0.05, Family-wise error (FWE) corrected.
***uncorrected p values – did not survive family-wise error correction for multiple comparisons.
TRD – treatment-resistant depressive patients, TSD – treatment-sensitive depressive patients, HC – healthy controlsns – not significant.
DMN Mask includes 8 regions: precuneus, posterior cingulate cortex (PCC), ventral anterior cingulate, right (R) inferior parietal lobe, medial prefrontal cortex, R middle temporal gyrus, left (L) middle frontal gyrus, L inferior parietal lobule, L middle temporal gyrus.

3.2.2. sgACC

Results showed significant connectivity differences between the TRD and the TSD groups for the sgACC with the PCC ($p < 0.002$, $k = 75$, −2—48 20, uncorrected). Subsequent post-hoc cluster analyses revealed differences between the two patient groups (TRD and TSD) on the connectivity between sgACC and DMN ($t(68) = 2.457$, $p = 0.017$, with a moderate effect size $d = 0.587$), with the TSD group showing significantly lower connectivity when compared to the TRD. When compared to the HC group, the TSD group showed significantly lower connectivity between the sgACC and the PCC ($t(71) = -2.703$, $p = 0.009$, with a moderate effect size $d = -0.633$). There were no significant differences between the TRD and the HC, for the connectivity between the sgACC and the DMN.

3.3. DMN inter-network connectivity – Independent component analyses

In this study, ICA was used to identify functionally independent networks within each participant based on their fMRI data. An independent component (IC) represents a spatial pattern of brain activity that is statistically independent from other components in the analysis. Each IC is characterized by a spatial map showing regions of the brain that exhibit synchronous activity and a corresponding time course reflecting the fluctuations in activity over time. In our analysis, we identified 23 ICs, two of which were highly associated with the DMN. These ICs, labelled IC#6 and IC#15, were found to have correlations (r) of 0.33 and 0.42, respectively, with the DMN. The correlation coefficient indicates the strength of association between each IC and the DMN, with higher values indicating a stronger association. Whole brain voxel analyses were conducted to identify significant connectivity patterns

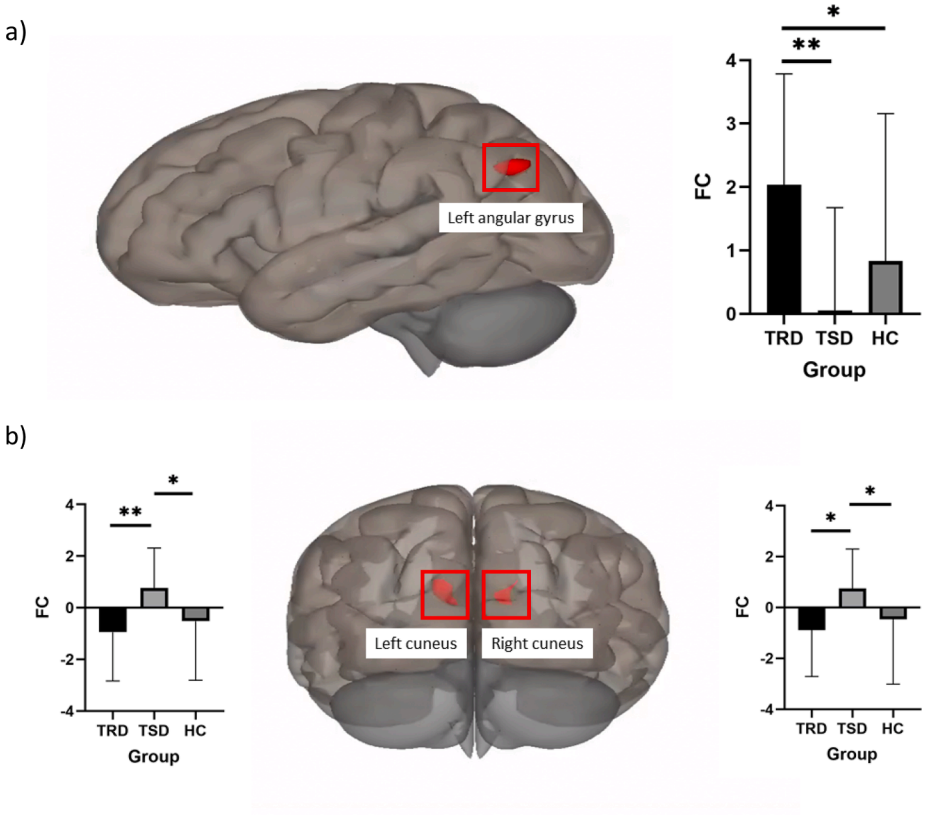

Fig. 1. Inter-network connectivity differences between groups.

related to each DMN component. A cluster size threshold greater than 20 was applied with voxel-level $p < 0.001$, cluster-level $p < 0.05$ FDR corrected, leading to the identification of clusters for the best-matching DMN component.

No significant group differences were observed for ICA#6, indicating that this component did not show robust connectivity differences that met the predefined threshold criteria. For ICA#15, the best match component (see Supplementary Figure 1), we identified 3 clusters showing significant connectivity patterns differentiating the two clinical groups (Fig. 1). These clusters included regions of the visual network (left (k = 144) and right (k = 90) cuneus), showing higher FC in the TSD group compared to the TRD group, and a parietal region of the DMN network (left angular gyrus ($k = 117$), indicating higher FC in the TRD group compared to the TSD group.

Post hoc comparisons indicated that the TRD group showed significantly higher connectivity between the best-matching DMN component and the left angular gyrus compared to both the TSD and HC groups with no differences between TSD and HC; and the TSD group showed higher connectivity compared with both the TRD and the HC groups between the DMN component and the left and right cuneus cortex (see summary in Table 3) with no differences between TRD and HC. No significant results were found for whole brain voxel wise comparisons for TRD with HC and TSD with HC.

This illustration offers a comprehensive visualization of the different clusters that contribute to the resting-state functional connectivity differences between the three groups (Treatment-Resistant Depression (TRD) and Treatment-Sensitive Depression (TSD) on the averaged connectivity of ICA#15 (the best-match default mode network component) with the rest of the brain. Image a) represents the cluster showing TRD>TSD FC (left lateral view), and image b) represents the clusters showing TSD>TRD FC (posterior view).

3.4. Clinical factors

There were no significant effects of clinical variables and the rACC result or the DMN ICAs.

Chronicity of the disorder was significantly anti-correlated with the sgACC-PCC FC result, in the TRD group ($r = -0.370$, $p < 0.05$). When looking at the TSD group only, sgACC-PCC FC result is positively correlated with length of time on ADM ($r = 0.433$, $p < 0.05$). There were no other significant effects of other clinical variables on the sgACC result.

4. Discussion

This study aimed to investigate how the functional connectivity of the default mode network differentiates TRD from TSD, using resting-state fMRI. We focused on the functional connectivity of the rACC and the sgACC, two regions that have previously been identified as key regions in TRD, with other regions of the DMN. We explored whether the functional connectivity of these regions differentiates between treatment-response and treatment-resistance. We also used a data driven approach to investigate the inter-network connectivity between the DMN and other brain regions. The results evidenced a pattern of hyperconnectivity for the rACC and the sgACC with the PCC, hyperconnectivity of the angular gyrus with the rest of the DMN, and hypoconnectivity of the DMN with the visual network in the TRD group relative to TSD.

Consistent with previous research (Hamilton et al., 2015, Machino et al., 2014, Li et al., 2013), we observed altered functional connectivity between the rACC and the PCC, differentiating between TRD and TSD patients. This connectivity difference was found between the TSD and both TRD and HC groups, but not between TRD and HC, suggesting this as a marker of treatment response specifically, rather than depression severity. For the sgACC, results showed a similar trend, at an uncorrected level. This finding is consistent with pharmacological treatment findings from previous studies, showing that individuals with greater baseline rACC-PCC connectivity are more likely to show a positive response to antidepressant treatment (Dichter et al., 2015, Korgaonkar et al., 2014). However, the lack of significance at a corrected level may be attributed to several factors, including the exploratory nature of this analysis, the relatively small sample size, and the complexity of fMRI data. Correcting for multiple comparisons in fMRI studies is essential to minimize the risk of false positives, but it can also increase the likelihood of false negatives, particularly in studies with limited statistical power or subtle effects. Therefore, while the uncorrected trend in sgACC connectivity is intriguing and aligns with previous research, further studies with larger sample sizes are needed to confirm these findings and elucidate their clinical implications.

Despite the well-established theory of hyper-DMN connectivity and excessive rumination in TRD, our study did not find significant differences in connectivity between the sgACC and rACC with other DMN regions in the TRD group compared to the HC group. Several potential explanations for this discrepancy can be considered.

First, it is possible that the hyperconnectivity within the DMN in TRD is not uniformly present across all patients, but rather in a subset of individuals. This variability could result in non-significant findings when analyzing the entire TRD group. Additionally, the connectivity alterations in TRD may involve more complex, dynamic patterns that are not fully captured by static resting-state fMRI measures. For instance, fluctuations in connectivity over time or context-dependent connectivity changes may play a role in the pathology of TRD, which would require more advanced analytic techniques to detect.

Moreover, the role of other networks and regions outside the traditional DMN in TRD should be considered. For example, the interaction between the DMN and networks such as the salience network and the cognitive control network might be more critical in driving the symptoms of TRD. Disruptions in these inter-network dynamics could contribute to the persistence of depressive symptoms and rumination, even if intra-DMN connectivity differences are not pronounced.

Finally, it is important to note that the mechanisms underlying TRD are likely multifactorial and involve a complex interplay of various neural circuits. The absence of significant findings in the sgACC and rACC connectivity does not negate the theory of hyper-DMN

Table 3
Summary of the main findings from Independent Component Analyses (ICA), for differences between the three groups on functional connectivity between the best matching DMN component (ICA#15) and other brain regions.

Contrast	MNI coordinates (x y z)	Voxels	Region	Neural network	F value	P value	Post-hoc
TSD>TRD	−10—92 26	144	Left Cuneus Cortex	Visual	7.430	< 0.001	TSD>TRD, HC
	10—84 20	90	Right Cuneus Cortex	Visual	6.112	0.003	TSD>TRD, HC
TRD>TSD	−44—72 38	117	Left Angular Gyrus	Default Mode Network	9.302	< 0.001	TRD>TSD, HC
TRD>HC	–	–	–	–	ns	ns	–
HC>TRD	–	–	–	–	ns	ns	–
TSD>HC	–	–	–	–	ns	ns	–
HC>TSD	–	–	–	–	ns	ns	–

Effects significant at cluster-size False Discovery Rate $p < 0.05$; cluster size $k > 20$.
TRD – treatment-resistant depression group, TSD – treatment-sensitive depression group, HC – healthy controls groupns – not significant.

connectivity but suggests that our understanding of the neural correlates of TRD needs to be nuanced and expanded to include broader network interactions and individual variability.

The anomalous neural activity implicated in TRD may not change in response to pharmacological treatments and has made non-pharmacological neuromodulation interventions increasingly attractive (Hitti et al., 2020). In line with the main findings of this study, fMRI studies have demonstrated activity of sgACC as a predictor of response to electroconvulsive therapy (ECT), a brain stimulation technique effective in TRD.

Clinical research investigating the underpinnings of resistance to the different types of treatment could provide additional insights into the concept of treatment-resistance. For instance, research on different DBS targets has the potential to enhance our understanding of treatment response mechanisms. In recent years, various brain stimulation locations have been studied for their potential efficacy, including the subcallosal cingulate (SCG) white matter (Mayberg et al., 2005), the ventral capsule/ventral striatum, the nucleus accumbens, the lateral habenula, the inferior thalamic peduncle, and the medial forebrain bundle (Riva-Posse et al., 2014, Zhou et al., 2018, Drobisz et al., 2019, Hitti et al., 2020). The ACC is the portion of the cingulum that lies ventral to the corpus callosum. The rACC/sgACC and PCC sit on opposing ends of the cingulum white matter bundle, which has been previously linked to treatment response (Korgaonkar et al., 2014, Bracht et al., 2015). The cingulum bundle is a significant white matter tract that connects frontal, parietal, and medial temporal regions, as well as linking subcortical nuclei to the cingulate gyrus. The relevance of the cingulum bundle to the limbic system was emphasized by Papez (1937) in his influential model of emotion, which highlighted its association with the cingulate gyrus. Disruptions in this pathway have been associated with altered functional connectivity patterns, such as hyperconnectivity of the rACC and sgACC with the PCC observed in TRD (Bubb et al., 2018). Nonetheless, efforts to integrate functional and anatomical knowledge of this pathway remain scarce, and this study represents a significant step in comprehending this highly complex pathway.

Chronicity, or the duration of depressive symptoms, is a key factor in understanding the progression and treatment response of depression. In this study, higher chronicity was associated with lower sgACC-PCC connectivity in the TRD group. However, the finding of lower sgACC-PCC connectivity in the TSD group compared to both the TRD and HC groups suggests that this specific connectivity pattern may be a marker of treatment sensitivity rather than chronicity alone. Important to note that there were no differences between TRD and HC which supports this theory. It's possible that while chronicity plays a role in connectivity alterations, other factors, such as the specific neural circuitry involved in treatment response and resistance, may override the effect of chronicity alone. Additionally, the impact of chronicity on sgACC-PCC connectivity may be nonlinear or influenced by other variables not measured in this study, contributing to the observed discrepancy. Future research incorporating longitudinal assessments and more comprehensive clinical and neuroimaging measures may help elucidate the complex relationship between chronicity, sgACC-PCC connectivity, and treatment response in depression.

The results from the ICA analyses emphasize the critical role of DMN connectivity with other brain networks in understanding the neural underpinnings of TRD. Our findings indicate that the patterns of connectivity between the DMN and other networks, such as the visual network are crucial in distinguishing individuals with TRD from those with TSD. In our study, we identified significant connectivity patterns in three clusters that differentiated the clinical groups: left and right cuneus, and the left angular gyrus. Notably, the TSD group exhibited higher functional connectivity between the DMN and the cuneus regions compared to the TRD group, while the TRD group showed higher FC between the DMN and the left angular gyrus compared to the TSD group.

The cuneus cortex, part of the visual network, is involved in mid-level visual processing and is also influenced by extraretinal effects such as attention, working memory, and reward expectation (Zhang et al., 2013; Fischer et al., 2019; Liu et al., 2022; Cechetto & Topolovec, 2002). The cuneus has been shown to be associated with reward attainment, with a positive correlation between cuneus activation and clinical symptoms of depression and anxiety (Liu et al., 2022). This finding aligns with the notion that reward processing is a core feature of major depressive disorder (Fischer et al., 2019). Specifically, in adolescent depression, dysfunctional reward processing is a significant concern, with resilient adolescents exhibiting different activation patterns in the cuneus during reward processing compared to those who have remitted from depression (Fischer et al., 2019).

Additionally, a *meta*-analysis of fMRI studies on reward-related processing in MDD has shown that the cuneus, along with other brain regions, preferentially responds to positive stimuli (Zhang et al., 2013). This increased activation in cortical regions, including the cuneus, during reward processing in MDD highlights its potential role in the neural circuitry of reward expectation and response to visual stimuli (Zhang et al., 2013). The higher FC observed in the TSD group between the DMN and the cuneus regions suggests that individuals with TSD may have enhanced reward processing capabilities. This enhanced connectivity might contribute to their responsiveness to treatment by supporting reward-related processes that are often impaired in depression. Additionally, the fact that there were no significant differences between the TRD and HC for this cluster suggests an effect of response to treatment in TSD, which should be evaluated in treatment studies with pre- and post-treatment imaging data.

Conversely, the TRD group's higher FC between the DMN component and the left angular gyrus was observed. The angular gyrus is traditionally considered a part of the DMN, which is involved in various higher-order cognitive functions such as memory retrieval, semantic processing, and social cognition (Kang et al., 2023). The identified higher connectivity between the DMN component and the left angular gyrus in TRD patients highlights the potential dysregulation within the DMN itself, reflecting an altered functional integration of this region within the network. This can be seen as an indication of the angular gyrus's role in the broader context of TRD pathology. The angular gyrus, especially in the left hemisphere, is crucial for recollection and the retrieval of detailed episodic memories (Bellana et al., 2023). Neuroimaging studies have shown that activity in the left angular gyrus is strongly associated with how well a memory representation matches the original encoded stimulus (Bellana et al., 2023). In TRD, the hyperconnectivity between the DMN and the left angular gyrus could reflect an over-reliance on or dysfunction in memory retrieval processes, potentially leading to persistent negative ruminations and cognitive rigidity seen in TRD (Bellana et al., 2023). Additionally, the angular gyrus's involvement in visual-spatial attention, memory retrieval, and semantic processing suggests that its altered connectivity in TRD might contribute to the deficits in these cognitive functions, further exacerbating depressive symptoms (Kang et al., 2023).

Moreover, the angular gyrus has also been implicated in the response to antidepressant treatments (particularly electroconvulsive therapy – ECT) in MDD patients. Studies indicate that ECT can modulate the functional connectivity between the habenula and the left angular gyrus, with changes in this circuit correlating with clinical improvements (Gao et al., 2021). This underscores the angular gyrus's role in the broader neural circuitry underlying depression and its treatment.

The hyperconnectivity observed between the DMN and the left angular gyrus in TRD patients indicates a potential compensatory or maladaptive mechanism within the DMN. This could signify an attempt by the brain to manage or integrate negative emotional and cognitive states through the angular gyrus, which is intricately involved in memory and semantic processing. However, this increased connectivity may also represent a failure of the DMN to appropriately regulate its internal processes, leading to persistent depressive symptoms. The absence of significant differences between TSD and HC groups further supports the notion that the left angular gyrus connectivity alterations

are specific to TRD, providing a neural correlate for the persistent and treatment-resistant nature of the disorder.

However, we acknowledge certain limitations in this study. It is important to highlight that the results from the analyses using the sgACC as a seed were not significant after correcting for cluster level family wise error for multiple comparisons and should be interpreted with caution. The cross-sectional design limits our ability to establish causality between altered functional connectivity and treatment resistance. Future longitudinal studies, particularly those incorporating pre- and post-treatment scans, are warranted to explore the dynamic changes in connectivity patterns and their associations with treatment response.

The sample size, although carefully matched, is still relatively small, necessitating caution when generalizing the results. Also, the heterogeneity of the sample constitutes a major limitation in the study design. This study only focused on the symptoms featured on the HAMD-21 and did not explore in-depth symptoms of rumination, a feature that is particularly relevant given its functional role in the DMN. There is a potential confounding effect of depression severity on our results. Ideally, comparing the TRD group with an MDD group currently experiencing depression but not yet categorized as treatment-resistant would provide clearer insights. However, predicting which MDD patients will develop treatment resistance remains challenging. Additionally, the observed effects might be influenced by the multiple treatments received by the TRD group. Future longitudinal studies that track MDD patients from the onset of treatment through multiple interventions are necessary to better understand the features underlying treatment resistance. A lack of longitudinal study design also limits us from drawing definitive conclusions regarding trait versus state markers. Whether the identified connectivity patterns could be predictive markers for treatment resistance and guide early intervention remains to be tested. Future research could also benefit from evaluating dynamic functional connectivity of the DMN. Thirdly, there is a potential confounding effect of depression severity on our results. Ideally, comparing the TRD group with an MDD group currently experiencing depression but not yet categorized as treatment-resistant would provide clearer insights. However, predicting which MDD patients will develop treatment resistance remains challenging. Additionally, the observed effects might be influenced by the multiple treatments received by the TRD group. Future longitudinal studies that track MDD patients from the onset of treatment through multiple interventions are necessary to better understand the features underlying treatment resistance.

Finally, our study design primarily captures the neural correlates associated with treatment resistance at a single time point, preventing us from drawing definitive conclusions regarding trait versus state markers. Further longitudinal investigations are warranted to explore the temporal stability of these connectivity patterns and their potential utility as predictive markers for early intervention in depression treatment. This limitation underscores the need for future research endeavors to delve deeper into the dynamic nature of DMN connectivity and its interaction with other neural networks, shedding light on the potential for trait-specific markers and, consequently, more personalized and timely treatment strategies for individuals with depression.

5. Conclusions

In conclusion, this study contributes to the growing body of research investigating the neurobiological underpinnings of treatment-resistant depression. We demonstrate distinct patterns of functional connectivity in the cingulate cortex that differentiate TRD from TSD. Specifically, hyperconnectivity within the DMN, particularly involving the anterior to posterior cingulate cortex connections, may be a key characteristic of treatment response. These findings highlight the relevance of targeting the DMN in the development of novel treatment strategies for TRD. Furthermore, this study revealed a pattern of hypoconnectivity between the DMN and other key brain networks, particularly the visual network. By elucidating the neural circuits that underlie treatment resistance, our findings may pave the way for the development of targeted interventions, such as DBS, that can modulate these aberrant connectivity patterns and potentially improve treatment outcomes for TRD patients (Clark et al., 2020, Riva-Posse et al., 2014).

CRediT authorship contribution statement

Ana Rita Barreiros: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Isabella A. Breukelaar:** Writing – review & editing, Methodology, Formal analysis. **Amourie Prentice:** Writing – review & editing, Conceptualization. **Prashanth Mayur:** Writing – review & editing, Resources. **Yoshiro Tomimatsu:** Methodology, Conceptualization. **Kenta Funayama:** Methodology, Conceptualization. **Sheryl Foster:** Writing – review & editing, Resources. **Gin S. Malhi:** Writing – review & editing. **Martijn Arns:** Writing – review & editing, Conceptualization. **Anthony Harris:** Writing – review & editing, Resources, Project administration, Data curation, Conceptualization. **Mayuresh S. Korgaonkar:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2024.103656.

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Chapter 5: Role of The Rostral Anterior Cingulate Cortex in Emotion Processing in Treatment Resistant Depression

5. Abstract

5.1. Introduction

Treatment-resistant depression (TRD) represents a significant clinical challenge, characterized by a persistent lack of response to standard antidepressant treatments. This condition profoundly affects patients' quality of life and stresses the need for a deeper exploration into the complex brain dynamics that contribute to its persistence.

The investigation of emotion processing in TRD is particularly crucial given its central role in depressive symptomatology (Fan et al., 2023). Emotion processing deficits, characterized by impairments in recognizing, interpreting, and responding to emotional stimuli, are a hallmark of major depressive disorder (MDD) (Fitzgerald et al., 2008, Fu et al., 2004, Vanderlind et al., 2020, Anderson et al., 2011), including its treatment-resistant forms (Fan et al., 2023). Studies have shown that patients with TRD often exhibit altered neural responses to emotional stimuli, reflecting dysregulation in key brain areas and networks involved in emotion processing (Roseman et al., 2018, Murrough et al., 2015, Loureiro et al., 2021).

The understanding of the pathophysiology of depression has shifted to a model based on dysregulation of neural networks, rather than a single neuroanatomical location (Kaiser et al., 2015). Central to these investigations are neural networks like the default-mode network (DMN), which is known for its role in self-referential thought processes and mind-wandering. The DMN exhibits peak activity during periods of restful introspection and is diminished when engaging in tasks that require external goal-oriented focus (Buckner et al., 2008). This reduction in activity, or 'deactivation,' is believed to indicate a shift from internal processing to increased attention on external tasks (Buckner et al., 2008, Harrison et al., 2011).

The rostral anterior cingulate cortex (rACC), considered a hub of the DMN (Fair et al., 2009, Ge et al., 2020), is intricately linked with regions and networks vital for emotional, cognitive, somatic, and social functions. It acts as a central processor for diverse information streams, enabling fluid transitions between introspective and task-focused cognitive states (Harrison et

al., 2022, Lichenstein et al., 2016). Even during specific task engagement, the rACC plays a key role in modulating connectivity with networks that are inherently linked, potentially influencing the adaptive interplay between the DMN and other critical neural networks (Jamieson et al., 2024, Leonards et al., 2024).

Abnormal functioning of the rACC is closely associated with detrimental features of depression such as persistent negative thinking, biased negative perception, attentional disruptions, and impaired regulation of emotions (Gotlib et al., 2010, Pizzagalli, 2011, Price and Drevets, 2010). These dysfunctions are particularly relevant in the context of TRD, where the rACC exhibits reduced activity at rest in patients who do not respond to treatments, compared to those who do, across various therapeutic modalities including pharmacotherapy (Pizzagalli et al., 2001), sleep intervention (Kito et al., 2008), and Transcranial Magnetic Stimulation (TMS) (Gotlib et al., 2008). The atypical activity of the rACC at rest is implicated in the development, persistence, and relapse of depressive episodes (Gotlib et al., 2008, Lichenstein et al., 2016) with heightened rACC activity as a central neural mechanism underlying rumination and persistent thought patterns (Zhou et al., 2020, Greicius et al., 2007, Sheline et al., 2010).

Studies indicate that elevated rACC activity might serve as a dynamic marker for antidepressant treatment responsiveness, influenced by the degree of treatment resistance (Hunter et al., 2013). This relationship is nuanced, showing variability with the levels of treatment resistance. Our previous work extends these findings, demonstrating that patients who were unresponsive to more intensive treatments like repetitive TMS and electroconvulsive therapy (ECT) display higher rACC activity at rest relative to those treated with first-line therapies such as psychotherapy and antidepressants (Prentice et al., 2023), which suggests rACC activity could be mediated by level of treatment resistance. However, a significant gap remains in our understanding of how these neural networks function during active emotional processing tasks.

While resting-state fMRI has revealed much about the role of rACC in TRD, task-based fMRI can provide additional insights into the interplay of the rACC and the key brain regions that are activated and modulated in response to emotional stimuli. This approach is particularly relevant for understanding the neural basis of emotional dysregulation in TRD. Therefore, the goal of our investigation was to extend our understanding of the neural underpinnings of TRD, particularly the role of the rACC beyond resting-state conditions and

provide a more comprehensive view of the involvement of this region in the disorder's complex neurobiology. Specifically, this study evaluated the functional connectivity of the rACC in treatment-resistant depression, during supraliminal and subliminal level of processing of both negative and positive emotions. Our study included both healthy individuals and treatment responsive patients with depression for comparison.

Emotion processing also critically involves the Affective Network (AN), which includes regions such as the amygdala, anterior insula, hippocampus, and subgenual anterior cingulate cortex (sgACC) (Adolphs, 2022; Korgaonkar et al., 2019). The AN is engaged in the dynamic evaluation of emotionally salient stimuli, integrating sensory, cognitive, and memory-related aspects to generate appropriate emotional responses (Adolphs, 2022). Therefore, in addition to whole-brain seed-to-voxel analyses, we conducted exploratory ROI-to-ROI analyses targeting key AN regions, including the amygdala, hippocampus, insula, and sgACC. These analyses aimed to determine whether disruptions within the AN might explain emotion processing deficits in TRD, complementing findings from the primary analyses

Neuroimaging studies have revealed that the perception of emotion in faces is a complex process, engaging a network of brain regions working together dynamically across time and space, rather than being confined to isolated neural activities in singular areas (Vuilleumier et al., 2007). Furthermore, these studies highlight the involvement of both supraliminal and subliminal pathways in emotional processing, where supraliminal processing involves the conscious perception of emotions, and subliminal processing occurs without conscious awareness, both of which play crucial roles in how we perceive and respond to emotional facial expressions (Williams et al., 2004).

The processing of positive and negative emotional faces in the brain engages overlapping yet distinct neural circuits, reflecting the nuanced ways our brains interpret and respond to different emotional stimuli (Haxby et al., 2000). Understanding these differences is crucial for insights into various psychological conditions. For example, positive emotional faces such as happy facial expressions engage the brain's reward system (Haber et al., 2010). This system is less activated by negative emotional faces, which instead may activate brain areas associated with threat detection and stress response (Haber et al., 2010). The rACC, a crucial region for emotional regulation and cognitive processing, appears to exhibit atypical activity suppression during tasks, which Leonards and collaborators (Leonards et al., 2024) associated with disturbances in adaptive neural communication and the dynamic balance between

internal and external cognitive modes. These disruptions may form the basis of the maladaptive cognitions and biased emotional processing characteristic of depression (Leonards et al., 2024). This region is involved in emotion processing and regulation for both positive and negative emotions. However, its engagement can vary depending on the complexity of the emotion processing task and the specific emotional context (Bush et al., 2000).

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5.2. Materials and Methods

5.2.1. Participants

Initially, 39 individuals with TRD and 35 individuals with TSD were recruited through a local network of specialist psychiatrists and clinics. TRD and TSD individuals met DSM-5 criteria for primary diagnosis of MDD, assessed through the Structured Clinical Interview for the DSM-5 (SCID-5) (Shabani et al., 2020). The inclusion criteria for TRD were no remission of symptoms with at least two adequate trials (in terms of dosage, duration – 6 weeks for each trial) of antidepressant of different pharmacologic classes, as well as the presence of moderate to severe depressive symptoms (assessed by a rating greater or equal to 16 in the 21-item Hamilton Depression Rating Scale – HAMD-21 (Hamilton, 1960). Participants in the TSD group were identified as patients who had achieved complete remission of symptoms for at least two weeks (characterized by a HAMD-21 score of less than or equal to 9). The control group (HC) comprised of 38 healthy individuals recruited through community advertisements with no psychiatric illnesses, assessed using the SCID-5. All participants were aged between 18 and 65 years old.

For both patient groups, indices of illness severity and chronicity were assessed. These indices included age of onset, number of inpatient hospitalizations, length of remission period since last episode, number of previous depressive episodes, history of suicidal ideation and behaviour, and history of suicide attempt. Information on past and current medication and other forms of treatment (e.g. ECT, or TMS) was also collected. Level of functioning was assessed by the Social and Occupational Functioning Assessment Scale (SOFAS) (Morosini et al., 2000).

HC and patient groups (TRD and TSD) were matched for age, sex and education status. Exclusion criteria for all participants included a) inability to provide consent, b) insufficient English proficiency, c) current primary diagnosis of eating disorder, psychosis, personality disorder or primary post-traumatic stress disorder, d) substance dependence for the past 3 months, e) pregnancy, f) history or current neurological disorder or prior brain injury, g) ECT or TMS in the last 6 months, h) contraindication to MRI.

Data collection was conducted at Westmead Hospital, Department of Radiology and at the Brain Dynamics Centre, The Westmead Institute for Medical Research, in Sydney, Australia.

The research protocol was approved by the Western Sydney Local Health District Human Research Ethics Committee and all participants provided written consent.

5.2.2. MRI Data Collection

Functional imaging was performed on a Siemens Prisma 3T MRI system running VE11C software and utilising a 2D GRE EPI sequence with a 64-channel head/neck array coil. Whole brain data for both faces tasks was acquired in the axial oblique plane with 47 interleaved slices of 3mm thickness/0mm gap parallel to the AC-PC line. Other parameters were as follows: TR/TE=2500ms/27ms, Flip angle= 90°, FOV=240mm, matrix=86 x 86, voxel size = 3mm x 2.8mm x 2.8mm, parallel imaging r=2, number of volumes = 120. Total acquisition time was 5 minutes 13 seconds.

5.2.3. Task Description

The emotion processing tasks utilized in this study were based on established paradigms previously described in (Korgaonkar et al., 2019) and (Williams et al., 2015). These tasks involve the presentation of facial expressions categorized into various emotional states: threat-related emotions (fear and anger), disgust, loss-related emotions (sadness), reward-related emotions (happiness), and neutral expressions. The stimuli are derived from a standardized series of facial expressions and are modified for central positioning at eye level.

There were two tasks: supraliminal emotional face processing, and subliminal emotional face processing, each analysed separately.

In the subliminal task, each emotional face is briefly presented for 16.7 ms, followed by a perceptual mask of a neutral face for 150 ms and an interstimulus interval of 1233.3 ms. This presentation is crafted to be below the level of conscious awareness. Behavioral psychophysical testing, as detailed in Williams et al. (2015), confirms that this duration is sufficient to meet the criteria for subliminal threshold detection.

The supraliminal task differs in presentation duration, with each emotional face displayed for 500 ms, based on evidence that this duration ensures conscious processing of the emotion stimulus (Williams et al., 2015). The interstimulus interval in this condition is 750 ms. For

both subliminal and supraliminal task presentations, faces are grouped in blocks by emotion, with each block containing eight faces and repeated five times, totalling 240 stimuli.

Throughout both paradigms, participants are instructed to focus on each face, with no specific behavioural responses required during scanning. This approach is consistent with findings that passive processing of emotional stimuli can elicit significant brain activation (Costafreda et al., 2008).

5.2.4. MRI Data Processing

MRI data was processed and analysed using Matlab R2018b (The Mathworks inc, Natick, Massachusetts), SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK), CONN functional connectivity toolbox v16b. Details of pre-processing steps are presented in our previous study (Barreiros et al., 2022). Four TRD participant datasets were excluded for excessive head motion during the scan. Five participants who did not complete the Faces tasks were identified and subsequently excluded from the analysis. This resulted in 31 TRD, 35 TSD and 37 healthy controls for final analyses.

5.2.5. Demographic and Clinical Characteristics

All three groups were compared for age and gender (demographic variables), using a one-way ANOVA and chi square test, respectively. The TRD and TSD groups were compared for age of onset, age of first episode, depression severity (HAMD-21 score), functionality (SOFAS score), severity of worst depressive episode, number of previous depressive episodes, using student t-tests. The groups were also compared for history of hospitalizations, suicidal attempts and suicidal ideation, using chi square tests of independence.

5.2.6. Functional Connectivity (FC) Analyses

Generalized PsychoPhysiological Interaction (gPPI) analyses, as employed in this study, are designed to explore task-specific changes in the BOLD signal across different brain regions

over time. The gPPI approach is particularly suited for studies involving multiple task conditions, such as the varied emotional stimuli in our tasks.

In this analysis, the interaction term is created by convolving each task condition's onset times, representing different emotions, with the hemodynamic response function. This term is then multiplied by the seed region's estimated neural activity, derived from the deconvolved BOLD signal. The gPPI model is distinct from standard PPI in that it includes the interaction factors from all conditions simultaneously in the estimation model in order to better account for between-condition overlaps. In this analysis, PPI effects (interaction terms in PPI model) are relative to the baseline state (the baseline state is defined by the zero values of the interaction term), so they provide a relative measure of connectivity characterizing differential task-specific effects.

One critical contrast employed was "Happy" versus "Neutral," where "Neutral" represents emotionally neutral faces. This contrast was chosen to elucidate the neural mechanisms underlying positive emotion processing. By comparing brain activity associated with viewing happy faces against neutral faces, we aimed to identify the specific brain regions and their interconnections that are selectively engaged in the perception and processing of positive emotional stimuli.

Additionally, we analysed the aggregated negative valenced conditions (“Sadness”, “Anger”, “Fear”, and “Disgust”) versus "Neutral" to investigate the networks involved in negative emotion processing. By averaging together these negative conditions and contrasting them with neutral emotion processing, we aimed to capture a broad perspective of the brain's response to negative stimuli.

In this study, the primary gPPI analysis employed a seed-to-voxel whole brain approach to comprehensively examine the role of the rACC in emotional processing in this population.

Additionally, exploratory ROI-to-ROI analyses focused on the functional connectivity within the AN were conducted (See Appendix E).

The selection of rACC ROI anatomic landmark derived from the voxels reported by Pizzagalli and colleagues (2018) and used in our prior studies on rACC (Prentice et al., 2023). Each functional rACC ROI was a 10 × 10 × 10 mm cube placed around the central MNI coordinate in this region ((-10 45 -5). FC values were calculated from the rACC voxel-

wise to the rest of the brain for each participant in the dataset as bivariate Fisher's z-transformed correlation coefficients. This measures the association between the rACC seed BOLD timeseries and each voxel of the whole brain BOLD timeseries using generalized linear model (GLM).

The statistical parametric maps threshold was set $p < 0.05$ at the cluster-level family-wise error (FWE)-corrected for multiple comparisons, using an initial whole-brain voxel-wise $p < 0.001$. We used an arbitrary threshold of a minimum cluster size of 20 as necessary to be considered relevant.

5.2.7. Clinical Factors

To analyse the associations between the FC measures and treatment resistance in the TRD group, we computed a variable to reflect chronicity of the disorder, by calculating the number of years from age of onset to current age, which is referred to as "number of years since onset". The relationships between the mean FC values for the significant ROIs and different clinical factors (depression severity, functionality, severity of worst depressive episode, number of previous depressive episodes, and number of years since onset) in the two patient groups separately were analysed using Pearson correlation coefficients.

All effects considered significant at the $p < 0.05$ significance level corrected for multiple comparisons, using a Bonferroni adjustment. Statistical analyses were performed using SPSS software version 21 (IBM Corp, 2012).

5.3. Results

5.3.1. Demographic and Clinical Characteristics

Demographic and clinical data for the final sample are summarized in Table 1.

Table 3. *Summary of demographic and clinical characteristics of the sample.*

	TRD (32)	**TSD (34)**	**HC (37)**	**F/t/X^2**	**sig**
Demographics					
Age, Mean ± SD [Min-Max]	42.4 ± 13.8 [18.1-64.3]	37.4 ± 11.1 [20.0-57.4]	42.29 ± 14.1 [18.9-65.9]	n.s.	n.s.
Gender (M), N (%)	12 (38)	17 (50)	17 (44.7)	n.s.	n.s.
Clinical Profile					
Age of onset, Mean ± SD [Min-Max]	29.97 ± 13.52 [8-53]	21.68 ± 9.40 [8-50]	n.a	n.s.	n.s.
Number of previous MDE, Mean ± SD [Min-Max]	8 ± 11 [1-40]	6.11 ± 6.16 [1-30]	n.a	n.s.	n.s.
Severity of worst MDE, Mean ± SD [Min-Max]	6.56 ± 1.13 [5-7]	5.52 ± 1.28 [3-7]	n.a	3.318	0.002
HAM-D-17 score, Mean ± SD [Min-Max]	23.66 ± 6.62 [15-39]	3.9 ± 2.92 [0-9]	n.a	15.611	0.000
SOFAS score, Mean ± SD [Min-Max]	74.45 ± 15.98 [40-100]	89.59 ± 5.56 [78-95]	n.a	-5.052	0.000
History of Hospitalizations, N (%)	22 (68)	6 (17.6)	n.a	17.625	0.000
History of ECT, N (%)	8 (25)	0 (0)	n.a	9.672	0.002
History of TMS, N (%)	2 (6.3)	0 (0)	n.a	n.a.	n.a.
History of Suicidal Ideation, N (%)	25 (78)	26 (76.5)	n.a	n.s.	n.s.
History of Suicidal Attempt, N (%)	17 (53)	5 (14.7)	n.a	12.439	0.000

n.a. – not applicable, n.s. – not significant; SD – Standard Deviation; M – male; MDE – Major Depressive Episode; HAMD-21 – Hamilton Depression Rating Scale, 21 items; SOFAS – Social and Occupational Functioning Assessment Scale; CGI-S – Clinical Global Impression, Severity; ECT – Electroconvulsive Therapy; TMS – Transcranial Magnetic Stimulation; N – total number.

All three groups were comparable for age and sex. As expected, groups were significantly different in some clinical variables, reflecting a more severe clinical profile for TRD patients. Although the depression groups weren't different for the number of past depressive episodes, the TRD group had higher rates of history of hospitalizations, history of ECT, and history of suicidal attempts.

There were no significant differences between groups for motion during the scan, after the exclusion of the four TRD participants for excessive motion.

5.3.1. Whole Brain Seed-Based Connectivity Analyses – rACC

Supraliminal Processing:

Functional connectivity differences among the three groups were significant in the context of processing happy versus neutral faces. Specifically, in the whole-brain analysis, a significant difference was observed for connectivity of the rACC with the hippocampus (p=0.036, FDR corrected, see Figure 1). Post-hoc cluster analyses performed based on extracted beta connectivity values from the significant rACC-hippocampus connectivity found significant differences between the TRD relative to both TSD and HC groups. TRD patients had reduced connectivity compared to TSD (t = -5.24, p = 0.000) and HC (t = -3.47, p = 0.001) (see Table 2). There were no significant differences in connectivity between TSD and HC (p = 0.061) (see Table 2).

There were no significant differences in the FC whole-brain analyses of the rACC for the supraliminal processing of negative emotions versus neutral faces.

Subliminal Processing:

Significant differences between the three groups in the functional connectivity of the rACC for the subliminal task were found for the processing of happy versus neutral faces. Significant differences were identified for connectivity of the rACC and three clusters in the

brain: the cerebellum (p=0.001, FDR corrected), the middle temporal gyrus (p=0.001, FDR corrected), and the temporal fusiform gyrus (p=0.04, FDR corrected) (see Table 2, Figure 2).

Post-hoc tests revealed significant differences between all three groups for the connectivity between rACC and the cerebellum and between the rACC and the middle temporal gyrus. TRDs had hyperconnectivity compared to TSD and HC groups for both the rACC-cerebellum connectivity (TRD>TSD: t=4.816, p<0.001; TRD>HC: t=2.415, p=0.018) and the rACC-middle temporal gyrus connectivity (TRD>TSD: t=5.154, p<0.001; TRD>HC: t=3.260, p=0.002). TSD on the other hand had lower connectivity compared to HC for the cerebellum cluster (t=-2.610, p=0.011) and the middle temporal gyrus cluster (t=-2.338, 0.022).

For the third cluster i.e. connectivity between the rACC and the temporal fusiform gyrus, both TRD and TSD groups had lower connectivity than HC but were not different from each other (see Table 2).

For negative versus neutral emotional faces, there was a significant group effect for connectivity between the rACC and the dorsal ACC (See Figure 3). Posthoc analyses revealed that group differences were significant only during the processing of disgust with both the TRD and TSD groups with greater connectivity compared to healthy controls (p<0.001, FDR corrected) but not different from each other (See Table 2).

Table 4. *Whole-brain seed-based functional connectivity of the rostral anterior cingulate cortex during supraliminal and subliminal emotion processing tasks.*

Task	Contrast	Brain region (aal)	P (FDR corrected)	Cluster size (k)	Peak voxel coordinates (x y z)	Post-hoc
Supraliminal task	Positive > Neutral	Hippocampus	0.036	98	40 -24 -12	HC, TSD > TRD
	Negative > Neutral	-	-	-	-	-
Subliminal task	Positive > Neutral	Cerebellum	0.001	106	-50 -46 -50	TRD > HC > TSD
		Middle temporal gyrus	0.001	99	-36 12 -42	TRD > HC > TSD
		Temporal fusiform cortex	0.04	54	40 -40 -24	HC > TRD,TSD
	Negative > Neutral	Dorsal anterior cingulate cortex	<0.001	80	06 24 24	n.s. (averaged) Disgust: TRD, TSD > HC

Figure 1. Differences between the groups for the rACC functional connectivity during supraliminal processing of emotional stimuli

This illustration offers a visualization of the cluster that contribute to differences between the three groups (Treatment-Resistant Depression (TRD) and Treatment-Sensitive Depression (TSD) and Healthy Controls (HC) on the functional connectivity (FC) of the rostral anterior cingulate cortex (rACC) during the supraliminal processing of positive emotional stimuli, in contrast with the processing of emotionally neutral stimuli.

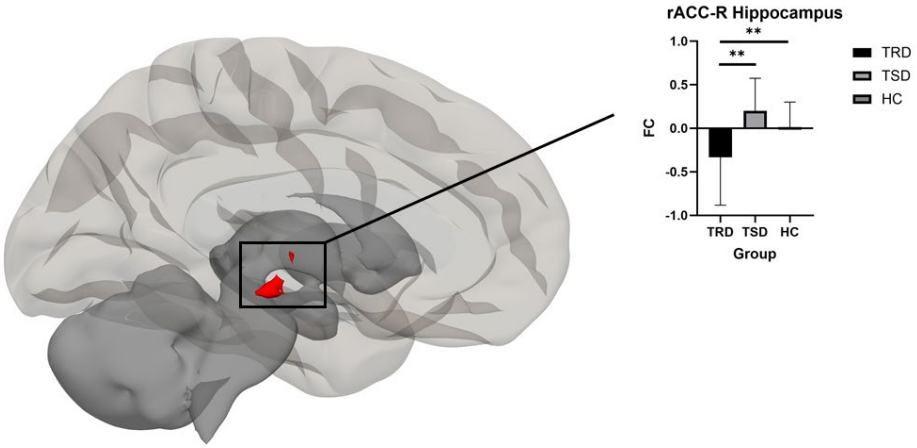


Figure 2. Differences between the groups for the rACC functional connectivity during subliminal processing of positive emotional stimuli

This illustration offers a visualization of the cluster that contribute to differences between the three groups (Treatment-Resistant Depression (TRD) and Treatment-Sensitive Depression (TSD) and Healthy Controls (HC) on the functional connectivity (FC) of the rostral anterior cingulate cortex (rACC) during the subliminal processing of positive versus neutral emotional stimuli.

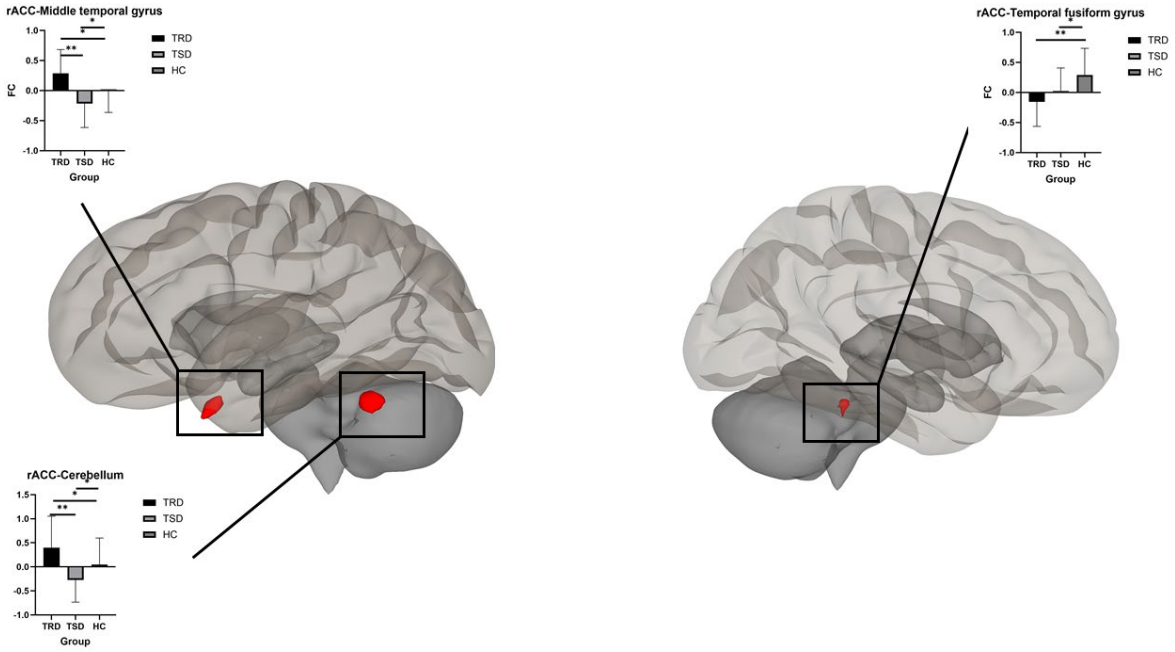
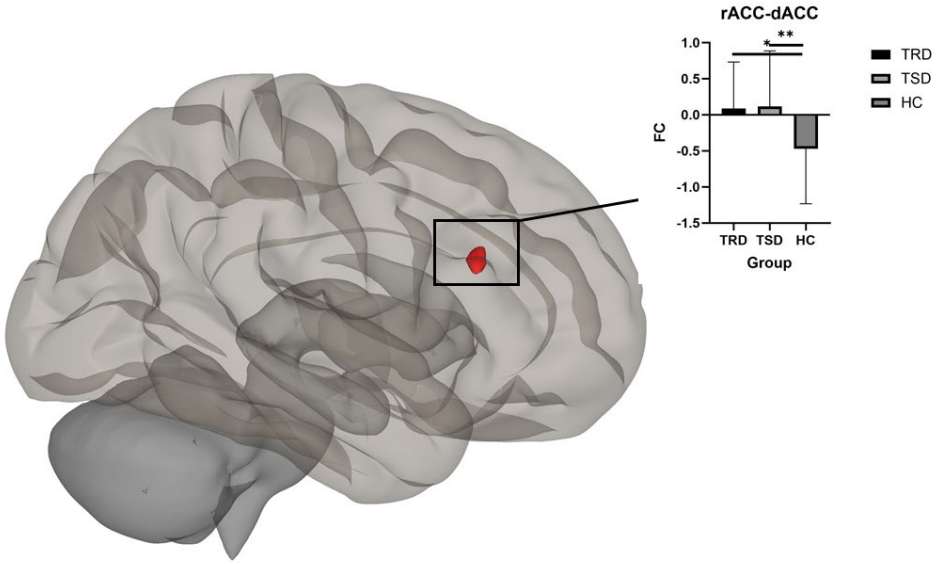


Figure 3. Differences between the groups for the rACC functional connectivity during subliminal processing of negative emotional stimuli

This illustration offers a visualization of the cluster that contribute to differences between the three groups (Treatment-Resistant Depression (TRD) and Treatment-Sensitive Depression (TSD) and Healthy Controls (HC) on the functional connectivity (FC) of the rostral anterior cingulate cortex (rACC) during the subliminal processing of negative versus neutral emotional stimuli.



Due to known biases of neutral facial cues to be interpreted as negative due to psychopathology, we also conducted all analyses contrasting each emotion and implicit baseline i.e. rest presented in Supplementary Material (Appendix E). We also evaluated group differences in connectivity beyond the rACC for other key neural regions involved in emotion processing (amygdala, hippocampus, insula, pregenual and subgenual ACC) in supplementary.

Additionally, results from the supplementary ROI-to-ROI analyses exploring Affective Network connectivity are available in Supplementary Materials (See Appendix E).

5.3.1. Clinical Factors

There were no significant correlations between the clinical variables and the functional connectivity measures.

5.4. Discussion

This study aimed to elucidate the role of the rostral anterior cingulate cortex in the neural underpinnings of emotion processing in TRD. We wanted to evaluate connectivity related to this region during emotion processing, extending from existing knowledge on altered functional connectivity of the rACC – a key region of the default mode network - in TRD during rest (Ge et al., 2020).

Given the central role of the AN in emotional regulation, we also conducted supplementary ROI-to-ROI analyses targeting key AN regions, including the amygdala, hippocampus, insula, and subgenual anterior cingulate cortex (sgACC). These analyses explored whether affective processing deficits in TRD could be linked to disrupted connectivity within this network. Despite the AN's theoretical relevance, no significant findings emerged for the supraliminal processing of negative versus neutral faces or positive versus neutral faces. However, during the subliminal processing of positive emotions, we observed reduced connectivity between the right hippocampus and sgACC in the TRD group compared to healthy controls. This specific finding highlights the possibility of subtle AN dysregulations in TRD that may not manifest consistently across tasks.

In our study, we investigated the functional connectivity underpinnings of the processing of positive and negative emotional expressions, contrasting with the processing of emotionally neutral faces. Additionally, we used both a supraliminal emotion perception task and a subliminal emotion perception task to allow us to measure both explicit and implicit level of emotion processing (Murrough et al., 2015).

For negative versus neutral emotional faces, our analyses revealed a significant group effect only for the supraliminal task and this was for connectivity between the rACC and the dorsal ACC. Posthoc analyses revealed that group differences were significant only during the processing of disgust facial expressions. Both the TRD and TSD groups exhibited greater connectivity between the rACC and the dorsal anterior cingulate cortex compared to healthy controls but not different from each other which suggests a shared neural mechanism underlying the processing of negative emotions, particularly disgust, in both TRD and TSD. The enhanced connectivity between the rACC and dACC might indicate an increased demand for cognitive control and emotional regulation in these groups, reflecting the heightened negative emotional reactivity that is a hallmark of treatment-resistant and treatment-sensitive depression (Hamilton et al., 2012). The lack of significant difference between TRD and TSD groups suggests that this connectivity alteration might be a trait feature in depressive disorders and irrespective of response to treatment.

In contrast for positive vs neutral emotional faces, we saw group differences for both the supraliminal and subliminal processing tasks. For the supraliminal processing task, our analysis revealed a pattern of hypoconnectivity between the rACC and the hippocampus when individuals with TRD processed happy emotions compared to neutral faces, that distinguished them from both the TSD and healthy control groups. This observation aligns with existing literature highlighting hypoconnectivity in reaction to emotional faces in patients with major depression who were non-responsive to antidepressant medication treatments (Kaiser et al., 2015). The observed hypoconnectivity in TRD when processing happy relative to neutral faces could reflect a diminished capacity to modulate neural responses to positive stimuli, a characteristic that might contribute to the anhedonia often seen in TRD, as an expression of impaired positive affect regulation (Pizzagalli et al., 2014). Existing functional neuroimaging literature corroborates these valence-specific alternations in emotion processing in TRD. Patients with TRD compared with healthy volunteers showed reduced responses to positive emotion within the caudate and insula (Murrough et al., 2015).

Anhedonia, or the diminished ability to experience pleasure, is a core feature of major depressive disorder, and it is particularly pronounced in TRD (Pizzagalli, 2014). The rACC has been implicated in the regulation of emotions and in modulating responses to reward-related stimuli. The link between such neural connectivity patterns and anhedonia is supported by findings that suggest alterations in reward processing circuits are associated with anhedonia in depression (Pizzagalli, 2014). Specifically, reduced activation in the ventral striatum, an area closely connected to the rACC and involved in reward processing, has been correlated with the severity of anhedonia in MDD (Pizzagalli, 2014). However, we did not observe connectivity differences between the rACC and the ventral striatum for our tasks which might suggest this neural mechanism is better evident in tasks that directly tap reward processing functions.

The connectivity of the rACC with the hippocampus, an area which is crucial for memory formation and retrieval, suggests a pathway through which emotional experiences are integrated and stored. This connectivity is believed to play a role in how emotional memories influence current emotional states and decision-making (Admon and Pizzagalli, 2015). The hippocampus is involved in the cognitive processing of emotional memory, so the decreased functional connectivity between the rACC and hippocampus may be related to abnormal regulation of emotional memory in TRD (Hao et al., 2020). Interestingly, our findings did not indicate significant differences in the FC of the rACC with the hippocampus between TSD and HC, suggesting that this altered connectivity might be specific to TRD. Additionally, literature on the connectivity between the DMN and hippocampus indicates hyperconnectivity in MDD patients (Kaiser et al., 2015). In our study, we not only found that this hyperconnectivity is absent in patients who are currently depressed (the TRD group), but it is also not present in the TSD group (patients with depression who recovered), highlighting a unique connectivity pattern in TRD.

Previous literature also has shown increased hippocampal response to positive stimuli associated with treatment response (Fu et al., 2007), suggesting that high levels of activity in the hippocampal region at baseline may indicate a resilience in neural responsivity in those patients who subsequently showed a clinical improvement (Fu et al., 2007). Therefore, hypoconnectivity between the rACC and the hippocampus, a region involved in memory and emotion processing (Mayberg et al., 1997, Phillips et al., 2003), may signal a disrupted neural circuit that contributes to the diminished sensitivity to positive stimuli seen in TRD. This

disruption could undermine the ability of individuals with TRD to engage with and retain positive emotional experiences, further perpetuating the cycle of depression.

One of the most notable findings on the subliminal task was the hyperconnectivity between the rACC and the cerebellum also during happy face processing, where connectivity was greatest in TRD compared to HC and TSD. This result is particularly interesting as it introduces a novel aspect of cerebellar involvement in TRD. Traditionally associated with motor function and coordination, the cerebellum has increasingly been recognized for its role in cognitive and emotional processing (Shmahmann, 2019). The heightened connectivity observed in TRD could suggest an adaptive or maladaptive mechanism where the cerebellum compensates or exacerbates emotional regulation difficulties, particularly in the non-conscious processing of positive emotions. This finding prompts further investigation into how the DMN might interact abnormally with the cerebellum in TRD, potentially disrupting normal emotional regulation.

Additionally, we also observed hyperconnectivity of the rACC with the middle temporal gyrus in the subliminal processing of positive emotions, that distinguished TRD from both HC and TSD. While previous literature commonly associates major depression with decreased activity in the middle temporal gyrus (Stuhrmann, Suslow and Dannlowski, 2011), the observed rACC hyperconnectivity to this region in TRD suggests a distinct neural pattern that differentiates it from TSD. The middle temporal gyrus is implicated in the semantic processing of emotions and social cognition (Oslon et al., 2007). The increased connectivity could reflect an over-engagement or faulty modulation of this region when processing happiness subliminally, pointing to a possible neural basis for the altered perception and integration of positive social cues in TRD.

The third significant finding during the subliminal processing of positive emotions involved the right temporal fusiform gyrus, where HC exhibited greater connectivity than both TRD and TSD. This aligns with existing research indicating reduced fusiform gyrus activity in depression during the processing of facial emotions (Stuhrmann, Suslow and Dannlowski, 2011). The fusiform gyrus plays a crucial role in face recognition and the interpretation of facial expressions. Its reduced involvement in both TRD and TSD groups suggests a shared impairment in processing emotional faces at a non-conscious level, potentially contributing to the social cognition deficits observed in depressive disorders in general rather than specifically to TRD.

The distinct patterns of connectivity observed during supra versus subliminal processing tasks imply a disconnection in how emotions are processed at different levels of awareness in TRD. Non-conscious processing tasks revealed significant connectivity between the rACC and areas typically associated with automatic emotional responses, such as the cerebellum and middle temporal gyrus. This suggests that individuals with TRD may have heightened automatic emotional reactivity, particularly in response to positive stimuli, which is not accessible to conscious awareness and could contribute to the persistent negative emotional bias seen in this disorder. In contrast, supraliminal emotion processing did not show these patterns, indicating that the rACC plays a role in both levels of processing in TRD but engages different regions. Specifically, during subliminal tasks, the rACC connects with automatic processing regions, while during conscious tasks, this region has altered hyperconnectivity with the hippocampus, which is involved in deliberate emotional processing. The lack of observed differences in automatic processing regions during the supraliminal task could be because the task is not sensitive enough to capture these mechanisms, rather than these processes being normal in TRD. The differences seen in the subliminal task highlight the complexity of emotional processing in TRD. Collectively these findings underscore a complex network dysfunction in TRD, where the rACC's impaired modulation of neural responses to positive stimuli and its altered connectivity with key regions of the emotion processing network, namely the hippocampus and the middle temporal gyrus.

We acknowledge several limitations in this study. First, the cross-sectional design constrains our ability to infer causality between the observed alterations in functional connectivity and treatment resistance. To better understand the dynamic changes in connectivity and their relationship with treatment outcomes, future research should adopt longitudinal designs. This would also help untangle trait vs state markers of treatment resistance. Additionally, despite careful matching, our sample size is relatively modest, urging caution in the extrapolation of our findings. Studies with larger and more diverse cohorts are essential for corroborating our results. This study's focus on emotion processing through facial expression tasks also presents a limitation. Face processing is a multifaceted function, engaging a network of brain regions often termed the "core face network," including the fusiform face area, occipital face area (Gauthier et al., 2000), and superior temporal sulcus (Haxby et al., 2000). Not all relevant regions within this network were examined in our analysis as our focus was primarily on emotion processing. Future research should distinguish between functional connectivity in

face processing and broader emotion processing by employing diverse emotional processing paradigms. The same applies for emotion processing versus emotion regulation. The sample's heterogeneity is another notable limitation, along with the study's focus on HAMD-17 symptoms, excluding a thorough investigation of anhedonia. This aspect is critical for a clinically meaningful interpretation of our findings especially considering that differences were for processing of happy emotions.

In conclusion, our findings, along with the broader literature, suggest a critical role for the rACC in the disrupted emotional processing observed in TRD. Abnormalities in the connectivity of the rACC with the hippocampus and the middle temporal gyrus might reflect an impaired ability to regulate negative emotions and to properly integrate emotional memories, which are essential for adaptive responses to environmental stimuli and for the efficacy of antidepressant treatments. The findings suggest that TRD involves distinct neural pathways for processing emotional stimuli under conscious and non-conscious conditions. Specifically, non-conscious processing seems to involve heightened connectivity between the rACC and brain regions like the cerebellum and the middle temporal gyrus in TRD compared to TSD and controls. This may indicate that while these regions more responsive or exhibit altered connectivity in TRD, these changes occur outside of conscious awareness. Such disruptions in automatic emotional processing could contribute to persistent symptoms, such as pervasive sadness or anhedonia, despite treatment. In other words, although the rACC may be involved in automatic emotional responses, the current findings do not allow us to conclude that it fails to integrate these responses into conscious emotional regulation. While its altered connectivity - particularly reduced coupling with the hippocampus - may reflect a dysfunction in pathways relevant to emotion regulation, the evidence is not sufficient to attribute this specifically to a failure of integrative processing. Rather, these findings may indicate that the rACC's role in coordinating between affective and cognitive networks is disrupted in TRD, potentially contributing to impaired emotion regulation, but further longitudinal and whole-brain network studies are needed to test this mechanistic hypothesis more directly. Addressing these neural circuitry disruptions holds promise for advancing our understanding of the role of the rACC in positive emotion processing in TRD, paving the way for interventions that more precisely target the neural bases of the disorder's core symptoms.

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Chapter 6 - Overall Discussion

In this final chapter, I will first provide an overview of the unique contributions of each thesis chapter. I will then address how they collectively answer the primary research question, their wider implications in the field of psychiatry, their limitations, and recommendations for future work. Overall, this work provides insights into the neurobiology of treatment-resistant depression (TRD), distinguishing it from treatment-sensitive depression (TSD) and highlighting the role of functional connectivity in elucidating its underlying mechanisms.

6.1. Overview of Findings

In Chapter 3, we investigated the neural circuitry associated with the habenula in TRD during rest. Given the role of the habenula in reward processing, the goal of this study was to elucidate the possible intrinsic neural mechanism underlying anhedonia in TRD that is present during stimuli-free internal mentation. Our findings revealed that TRD patients exhibited hyperconnectivity of the left habenula with the precuneus cortex and the precentral gyrus compared to treatment-sensitive depression patients and healthy controls. This increased connectivity of the habenula to key regions of the default mode network (DMN), a network responsible for processing negative thoughts, may represent the altered neural feedback loop underlying anhedonia in TRD. Additionally, we found this altered neural circuit to be associated with suicidality, which is more prevalent in patients with TRD. This provides new understanding of the relationship between neural circuitry and clinical outcomes in this population.

In Chapter 4, we examined the role of anterior cingulate cortex (ACC) connectivity in differentiating TRD from TSD and healthy controls during rest. The ACC is consistently linked to emotion regulation and self-referential thought processes, both of which are disrupted in TRD. By clarifying the ACC's connectivity patterns across TRD, TSD, and healthy controls (HC), we enhanced the understanding of the neural mechanisms that contribute to treatment non-responsiveness. Specifically, our results found that TRD patients displayed hyperconnectivity between the rostral ACC (rACC) and the posterior cingulate cortex (PCC) i.e. between the anterior and posterior components of the DMN, alongside altered inter-network connectivity with visual and parietal regions, relative to TSD and HC. These findings reveal novel insights into the role of the DMN in TRD, emphasizing that

altered connectivity within this network may be a defining neural feature of treatment resistance. This study underscores the critical role of network-level alterations, particularly in the DMN, with a focus on the connectivity between the rACC and PCC, in the pathophysiology of TRD. This altered connectivity within the DMN may reflect disrupted neural processes critical for emotion regulation and self-referential thinking, which could partially explain the persistent symptoms observed in TRD.

In Chapter 5, we explored the role of the DMN, in particular the connectivity of the rACC node of the DMN, during both subliminal and supraliminal processing of emotional stimuli, aiming to better understand the neural mechanisms underlying emotion processing deficits in TRD. Our findings indicated that TRD patients exhibited increased rACC connectivity to the cerebellum and other subcortical regions during subliminal processing of positive emotions, but decreased connectivity with the hippocampus during supraliminal processing of positive emotional information. While our main focus was on DMN connectivity, supplementary analyses targeted the Affective Network (AN), given its theoretical relevance in emotional regulation. Although ROI-to-ROI analyses of the AN did not yield significant findings in most contrasts, we found decreased connectivity between the right hippocampus and sgACC in TRD during subliminal processing of positive emotions. These findings offer novel insights into the connectivity alterations that could be associated with mechanisms disrupting positive (not negative) emotion processing in TRD, potentially contributing to the persistence of anhedonia in this population.

In the present study, effect sizes were calculated and reported for all significant findings to aid interpretation of the magnitude of group differences (see Table 5). Cohen's d was used for pairwise comparisons (e.g., TRD vs. TSD), and partial eta squared (η^2) was used for GLM-based analyses, such as one-way ANOVAs across the three groups. Across analyses, the Cohen's d values ranged from 0.21 to 0.24, and partial eta squared values ranged from 0.024 to 0.247. These values reflect small to moderate effects, consistent with the modest sample size and known variability in neuroimaging data. While these small effect sizes still suggest potentially meaningful group differences in neural activity and functional connectivity associated with treatment response in depression, they should be interpreted with caution. Small samples can lead to inflated or unstable estimates of effect size. Replication in larger, well-powered studies is necessary to validate and further clarify the robustness and generalisability of these preliminary findings.

6.2. Research Question - Answer

The primary research question addressed in this thesis was: **"What are the neural connectivity mechanisms that characterize treatment-resistant depression and differentiate it from treatment-sensitive depression?"**

This project investigates the neurobiological underpinnings of treatment-resistant depression through functional connectivity analysis, shedding light on the distinct neural patterns that differentiate treatment-resistant depression from treatment-sensitive depression. The results underscore the critical role of inter-network connectivity in understanding the complexities of these mood disorders.

Collectively, the findings across the three chapters of this thesis present a comprehensive picture of the role of the DMN and its interactions with other neural circuits in the pathophysiology of TRD. Chapter 3 highlights significant connectivity between the precuneus - a key DMN region - and the habenula, a critical component of the reward system, suggesting that disruption in self-referential processing and reward evaluation may contribute to the anhedonia observed in TRD. This hyperconnectivity is not only distinct from healthy controls but also more pronounced than in TSD patients, suggesting that disruptions within habenular circuitry could serve as a pivotal feature differentiating TRD from those who respond to treatment. Chapter 4 revealed hyperconnectivity between the rACC and PCC within the DMN, indicating altered communication within the DMN. Additionally, Chapter 5 built on this knowledge by revealing hypoconnectivity between the rACC and the hippocampus in TRD patients, which may hinder the storage and retrieval of positive emotional memories. This finding underscores the DMN's crucial involvement in emotion processing. Collectively, these findings suggest that dysregulation within the DMN - manifested through both inter-network and intra-network connectivity abnormalities - plays a pivotal role in the mechanisms underpinning TRD. The altered interactions within this network across different contexts, including general rumination and emotional processing, emphasize the importance of a network-oriented approach in understanding the neurobiological underpinnings of TRD. This thesis builds on foundational insights from previous research work which identified the rACC as a predictor of treatment response in depression due to its pivotal role in the DMN and its connections to task-positive networks (Pizzagalli, 2011). This thesis goes further by detailing specific rACC connectivity patterns

unique to TRD, offering a nuanced understanding of how these connections may hinder treatment efficacy or, alternatively, may emerge as a byproduct of cumulative treatment failures. By examining rACC hyperconnectivity with regions like the PCC and altered interactions with other networks, the findings add a layer of specificity that could explain why certain individuals remain unresponsive to conventional treatments.

The precuneus, an integral part of the posterior DMN, is implicated in internal cognitive processes, such as self-referential thinking and episodic memory retrieval (Sestieri et al., 2011). Our findings align with previous research that suggests that DMN dysfunction is associated with the maintenance of depressive states, thereby reinforcing the notion that altered connectivity within this network may underlie key symptoms of TRD, such as anhedonia and rumination (Hamilton et al., 2015).

Despite the established theories linking hyper-DMN connectivity to excessive rumination in TRD (Hamilton et al., 2015), we found that the TRD group did not exhibit significant differences in connectivity of the sgACC or rACC compared to HC. One likely reason could be that DMN hyperconnectivity is not uniformly captured across all individuals with TRD, potentially due to variability within this population and the relatively small sample size of our study. A second possibility is that this difference would be better captured using more nuanced methodologies, for example those that evaluate dynamic connectivity patterns over time. Another possibility is the differing pattern of treatments may have modified the responsiveness of this network in a less predictable way.

The findings from this thesis also emphasize the necessity of considering inter-network connectivity as a critical aspect of understanding the neural basis of treatment-resistant depression. The traditional focus on single brain regions or specific connectivity pathways does not capture the complexity of neural networks involved in mood regulation. Instead, examining inter-network dynamics - how different networks communicate and coordinate with one another - provides a more comprehensive perspective on the neurobiological mechanisms underlying TRD. Inter-network interactions, particularly between the DMN and other networks such as salience, reward and cognitive control networks may be critical in driving the persistence of depressive symptoms (Menon, 2011).

Our findings from Chapter 4 reveal significant alterations in the inter-network connectivity of the default mode network in patients with TRD, relative to TSD and HC. Increased connectivity between the DMN and other brain regions, including those involved in visual

and parietal processing. Specifically, our independent component analysis revealed that TRD patients exhibited reduced connectivity between the DMN and regions of the visual network, while showing increased connectivity between the DMN and a parietal region within the DMN. These connectivity differences between TRD and TSD groups suggest that treatment resistance may be linked to specific patterns of inter-regional connectivity within and between major functional networks. This maladaptive reorganization may contribute to the persistence of depressive symptoms by disrupting the coordinated functioning of these critical networks.

The results from Chapter 5 confirm the significance of altered rACC connectivity in emotional processing in TRD. Specifically, during the subliminal processing of positive emotions, TRD patients demonstrated increased rACC connectivity to the cerebellum and middle temporal gyrus compared to TSD and HC. In contrast, during supraliminal processing of positive emotions, significant hypoconnectivity between the rACC and hippocampus was observed in the TRD group relative to TSD and HC. These patterns of altered connectivity in response to positive emotions could contribute to the persistent anhedonia commonly seen in TRD patients, as abnormal connectivity between the rACC, involved in emotion regulation (Szekely et al., 2017) and the hippocampus, important for the recall and contextualisation of emotion, could represent a failure to gauge the positive emotions (Pronier et al., 2023). This may underlie the reduced capacity for experiencing positive emotions and pleasurable feelings in TRD.

Furthermore, our Supplementary findings from Chapter 5 (see Appendix E) suggest that while the AN plays a critical theoretical role in emotion regulation, its functional disruptions in TRD may be best understood through its interactions with other networks rather than as an isolated network. This aligns with prior research emphasizing the dynamic interplay between the AN, DMN, and CCN in emotion processing and cognitive control (Menon, 2011). In particular, AN regions such as the hippocampus and sgACC may engage dynamically depending on task demands and emotional context.

Importantly, our findings also point to the role of chronicity in connectivity alterations, suggesting that prolonged depressive symptoms, as indicated by clinical measures of chronicity, may influence the connectivity of the DMN in TRD patients. We found correlations with altered connectivity between the sgACC andPCC within the DMN. This correlation underscores the potential impact of the duration and severity of depressive

episodes on the neural mechanisms underlying treatment resistance. However, the absence of significant differences between the TRD and HC groups could also indicate that the neural abnormalities observed in TRD are related to the mechanisms of treatment response rather than the chronicity of the disorder. This suggests that while chronicity may influence connectivity patterns, it is the specific neural circuits involved in treatment response that are primarily responsible for the differences seen between the TSD and HC groups. For instance, increased sgACC-PCC connectivity could reflect an adaptive response to long-term emotional dysregulation rather than an inherent marker of TRD.

Across Chapters 3 to 5, the findings converge on a pattern of network-specific dysconnectivity in TRD, with emerging distinctions from both treatment-sensitive depression TSD and healthy controls HC. Resting-state analyses (Chapters 3 and 4) revealed TRD-associated hyperconnectivity between the habenula and regions such as the precuneus and precentral gyrus, alongside relative hypoconnectivity within the DMN, particularly pronounced in TSD. Inter-network analyses further demonstrated widespread hypoconnectivity in TRD across several canonical networks, including the DMN, cognitive control, and salience networks. During task-based emotional processing (Chapter 5), individuals with TRD exhibited a blunted hippocampal connectivity in response to positive stimuli during supraliminal tasks, and a divergent pattern during subliminal tasks - characterized by cerebellar and temporal hyperconnectivity and reduced fusiform connectivity - suggesting atypical implicit processing of emotional valence. Collectively, these findings support a model in which TRD involves both regional hyper- and hypo-connectivity, varying by network and task context. This pattern underscores the heterogeneity of underlying neurobiological mechanisms in TRD and highlights the potential for functional connectivity profiles to aid in mechanistic subtyping.

Our findings of correlations with demographic and clinical factors align with previous research indicating that the neurobiological signatures of depression can evolve over time and may vary significantly depending on individual patient characteristics (Fried & Nesse, 2015, Hamilton, Chen & Gotlib, 2013). For instance, results from Chapter 3 have shown that TRD patients with a history of suicidal ideation exhibit heightened FC in the left habenula compared to those without such a history (Barreiros et al., 2022). The habenula's role in modulating behaviour and its interactions with regions like the precuneus and the precentral gyrus underscore the complexity of its involvement in depression and suicidality. Increased connectivity between the habenula and these areas could indicate a dysfunction in the

mechanisms that link emotional processing to motor responses, potentially contributing to impaired behavioural flexibility and adaptive strategies. This dysregulation may not only heighten the perception of negative experiences but also hinder the ability to envision positive future scenarios, exacerbating feelings of hopelessness and despair. Consequently, chronic alterations in the habenula's functioning and its connectivity with the default mode network and other cognitive circuits may perpetuate a cycle of negative affectivity and increase the risk of suicidal behaviour.

Furthermore, residual heterogeneity and potential confounding factors remain challenges in neuroimaging studies of TRD. While we controlled for core clinical variables such as depression severity and episode duration, other relevant influences - such as psychosocial stressors, comorbid psychiatric diagnoses (e.g., anxiety, personality disorders), and detailed lifetime depression history - were not systematically measured in this study. Prior research indicates that these factors can significantly affect functional connectivity, particularly in emotion regulation networks (Kaiser et al., 2015; Drysdale et al., 2017). The omission of these variables may contribute to unexplained variability in connectivity patterns observed in our sample. Future studies incorporating comprehensive clinical and psychosocial assessments are needed to clarify their role and reduce residual confounding in neurobiological models of TRD.

The heterogeneity observed in functional connectivity alterations in TRD may arise from several interacting factors. First, relatively stable impairments in connectivity, potentially driven by genetic predispositions or early neurodevelopmental influences, may create a vulnerability to depression. Second, repeated depressive episodes and associated psychosocial stressors may lead to learned maladaptive connectivity patterns, reinforcing dysfunctional emotional processing circuits. Third, compensatory changes may occur whereby increased connectivity between certain brain regions develops in response to impaired activity in others, representing neural adaptations aimed at maintaining function. Although these mechanisms are critical to understanding the neurobiology of TRD, the present study’s cross-sectional design limits our ability to parse these contributions. Longitudinal and multimodal investigations will be required to delineate how stable, learned, and compensatory changes interact over time and influence treatment resistance.

An important conceptual question arising from this work is whether TRD represents a distinct subtype of depression, characterized by unique neurobiological mechanisms, or whether it

reflects a more severe position on a continuous spectrum of depressive symptom severity. The current findings, highlighting specific connectivity disruptions in TRD that differ from TSD and HC, suggest some degree of categorical distinction in neural circuit dysfunction. For example, the observed hyperconnectivity between the precuneus and habenula, and altered rACC connectivity patterns, may reflect neurobiological features that are qualitatively different rather than merely quantitatively more severe. However, the overlapping features between TRD and TSD - such as variability in DMN connectivity and some shared connectivity alterations - also support a continuum model, where TRD could represent the extreme end of a severity gradient influenced by cumulative clinical and neurobiological burden. This is consistent with evidence that the neurobiological correlates of depression evolve with illness progression and treatment exposure (Fried & Nesse, 2015; Hamilton et al., 2013). Given the cross-sectional nature of this thesis, definitive conclusions about categorical versus dimensional classification cannot be drawn. Longitudinal studies are needed to clarify whether the connectivity patterns identified here precede treatment resistance as trait markers (supporting a subtype model) or emerge as a consequence of illness chronicity and treatment failure (supporting a severity continuum). In either case, adopting a network-oriented approach may provide a framework to better define TRD both clinically and biologically, facilitating tailored interventions.

Finally, large-scale neuroimaging studies, such as those conducted by ENIGMA (e.g., Schmaal et al., 2017) and UK Biobank (e.g., Cox et al., 2019), have consistently demonstrated that structural and functional brain alterations associated with depression tend to be subtle and exhibit small effect sizes. This raises important concerns regarding the sensitivity and specificity of neuroimaging measures as clinically useful biomarkers. Sensitivity refers to the ability of a biomarker to correctly identify individuals with the condition of interest, while specificity pertains to correctly excluding those without it. Both are critical to ensuring that biomarkers are reliable and meaningful in clinical settings. Many observed neuroimaging abnormalities in depression lack sufficient sensitivity and specificity, and are often shared across multiple psychiatric disorders, further complicating their utility. To establish neuroimaging findings as valid biomarkers, rigorous external validation in independent samples is essential. Without replication and confirmation, initial findings risk being sample-specific and not generalizable to broader populations. Future research must prioritize such validation, alongside detailed evaluation of biomarker performance metrics, to determine their true clinical applicability. In this context, the results presented in this thesis

should be interpreted with caution, recognizing the current limitations in biomarker sensitivity, specificity, and validation.

In summary, this research elucidates the distinct functional connectivity patterns that differentiate treatment-resistant depression from treatment-sensitive depression, highlighting the complexity of its neurobiological underpinnings. The observed hyperconnectivity within specific neural circuits, particularly involving the habenula, precuneus, and the default mode network, offers critical insights into the mechanisms that may perpetuate depressive symptoms and hinder antidepressant treatment response. These findings not only advance our understanding of TRD but also underscore the necessity of adopting a network-oriented approach to investigating mood disorders.

Table 5. *Summary of main findings across chapters, with confidence assessment based on effect size, statistical correction and consistency with literature.*

Chapter	Task	Contrast	Region(s)	Effect Size (Cohen's *d* / η^2)	Statistical Correction	Cluster Size	Summary of findings	Consistent with Literature?	Confidence Level
3	Resting-state FC	Habenula seed-based FC	Precentral Gyrus (R), Precuneus (L/R)	*d* = .195–.219 (small)	Cluster-level corrected	69–122 voxels	Hyperconnectivity in TRD	Yes (habenula-prefrontal and DMN changes in TRD supported)	Moderate–High
3	Resting-state FC	ANOVA (TRD×TSD×HC)	Inferior Parietal, ITG, Insula, etc.	η^2 = .211–.244 (moderate)	FDR corrected, $p < .001$	109–184 voxels	Hypoconnectivity in TSD	Yes (networks like DMN, Salience, Cognitive Control involved in depression)	High
4	Resting-state FC	DMN intra-network - rACC seed-based FC	Posterior Cingulate Cortex (PCC)	*d* = .578 (moderate)	FWE corrected	1 cluster	Hypoconnectivity in TRD	Yes (DMN abnormalities well documented in TRD)	High
4	Resting-state FC	ICA inter-network FC	DMN–FPCN, DMN–Salience, etc.	*d* = .243–.247 (small)	FDR corrected	4 networks	Hypoconnectivity in TRD	Yes (network-level dysconnectivity is core to TRD models)	High
5	Supraliminal emotional processing	Positive > Neutral	Hippocampus	η^2 = .247 (moderate)	FDR corrected	98 voxels	Hypoconnectivity in TRD	Yes (hippocampal involvement in emotion regulation and depression)	Moderate–High
5	Subliminal emotional processing	Positive > Neutral	Cerebellum, MTG, Fusiform	η^2 = .195–.208 (moderate)	FDR corrected	54–106 voxels	Hyperconnectivity in TRD	Emerging support (especially cerebellar and MTG contributions)	Moderate
5	Subliminal emotional processing	Negative > Neutral	dACC	η^2 = .024 (small)	FDR corrected	80 voxels	No sig differences between TRD and TSD	Inconsistent; post-hoc NS	Low–Moderate

6.3. Implications for the Field of Psychiatry

The broader impact of TRD extends beyond individual patients, affecting healthcare systems, therapeutic approaches, and overall quality of life. As noted by Havlik et al. (2024), addressing TRD requires a comprehensive, multidimensional strategy that takes into account its varied effects. Patients with TRD frequently endure prolonged illness, characterized by ongoing symptoms, functional challenges, and reduced quality of life, reinforcing the need for treatment strategies that target the root causes of treatment resistance rather than just managing symptoms (Li et al., 2023).

Building on the progress made from neuroimaging studies, several potential applications for neural connectivity in clinical settings are emerging. Firstly, moving beyond DSM-based diagnostic manuals, functional connectivity offers promise as a neural system-level biomarker that could aid in diagnosing and tracking the progression of psychiatric conditions (Zhang et al., 2021). The identification of specific functional connectivity patterns as potential biomarkers for TRD may lead to more personalized treatment approaches, which could enable clinicians to tailor interventions based on an individual's neurobiological profile (Tozzi et al., 2024).

Furthermore, these insights contribute to a more comprehensive understanding of depression as a heterogeneous disorder, where one-size-fits-all treatment strategies may be insufficient. The findings presented in this work collectively demonstrate that TRD is characterized by distinct alterations in functional connectivity, highlighting key mechanisms underlying this condition. While these results offer valuable insights into neural correlates associated with treatment resistance, it is essential to consider that the observed alterations may be influenced by prior treatments or the chronicity of depression. The mechanisms identified in this thesis can guide future longitudinal studies, which should focus on tracking patients to assess how these connectivity changes develop over time and determine their potential as predictors of treatment resistance. This approach will not only deepen our understanding of TRD but also lay the groundwork for targeted interventions that leverage these established mechanisms. Baseline FC assessments could have potential to predict individual patients' future clinical trajectories, such as their likelihood of developing certain conditions, symptom changes, or treatment responses. Predictive markers derived from FC could be based on fMRI scans conducted before treatment begins or on how FC adapts shortly after treatment initiation.

Secondly, FC itself can be targeted for treatment using neuromodulation techniques like transcranial magnetic stimulation (TMS) (Brady et al., 2019) or real-time neurofeedback (Barreiros et al., 2024, Koush et al., 2013). Progress has already been made in using baseline FC measurements to optimize TMS targeting, with evidence suggesting that FC-guided approaches can improve treatment outcomes in major depressive disorder (Cash et al., 2021, 2019; Fox et al., 2013). Although neurofeedback traditionally focuses on regional activity, recent advancements have expanded its application to regulate interactions between brain regions or networks, guided by FC patterns, showing superior outcomes (Ramot et al., 2017; Watanabe et al., 2018). A systematic review conducted as part of this thesis (see Appendix A, Barreiros et al., 2024) highlights that fMRI-based neurofeedback interventions can induce significant changes not only in the neural activation of the targeted region but also in large-scale connectivity patterns. fMRI-neurofeedback targeting single regions reported significant changes in both within-network and between-network connectivity, underlining the potential of neurofeedback to rectify neural changes underlying depression beyond the target region. It is essential to consider the network context when examining disorder-related markers of pathophysiology. The clinical manifestation of a disease is influenced not only by areas of primary pathology but also by how this pathology impacts distant, interconnected systems (Segal et al., 2023, Zhang et al., 2021).

Lastly, this work underscores the importance of multidisciplinary approaches in psychiatric research, combining neuroscience in clinical practice to advance our understanding of complex mental health disorders.

6.4. Limitations

There are several limitations of this thesis that should be considered. Firstly, the sample sizes in the neuroimaging studies were relatively small, which may limit the generalizability of the findings (Chen et al., 2023). Previous imaging studies of TRD have also faced limitations in sample size, with a maximum sample size of 41 (Miola et al., 2023). Depression is a highly heterogeneous condition, with varying clinical profiles and neural patterns that may influence the results. Recent studies, such as Drysdale et al. (2017) and Tozzi et al. (2024), have identified distinct subtypes of depression based on functional connectivity profiles, indicating that different connectivity patterns may underlie various depressive phenotypes. Future research with larger, multicentre samples is necessary to validate the current findings and

explore how connectivity-based subtypes might intersect with treatment resistance, potentially offering more personalized insights into TRD.

Secondly, the cross-sectional nature of the studies restricts causal inferences regarding the relationship between functional connectivity and treatment resistance. The role of neuroimaging is expected to expand in the future to enhance diagnostic specificity and improve predictions regarding treatment response. However, at the present time, neuroimaging has limited utility for benefiting patients on an individual level, particularly in predicting the transition from an "at-risk" mental state to a full syndrome or in resolving diagnostic uncertainties. For instance, abnormalities in the DMN, such as hyperconnectivity or disrupted connectivity, have been reported across various psychiatric conditions, including depression, anxiety disorders, and schizophrenia. This overlap complicates the ability to ascribe DMN alterations to a specific diagnosis, highlighting the broader challenge of achieving cross-diagnostic validity in biological models of psychiatric disorders. There is a notable lack of studies that facilitate cross-diagnostic comparisons, which further complicates the situation. Consequently, there are currently no neuroimaging-based biomarkers available to assist in the differential diagnosis of major depressive disorder, presenting a substantial hurdle that must be addressed for neuroimaging to achieve clinical applicability.

Several significant conceptual and methodological challenges must be addressed before functional connectivity can be reliably implemented as a clinical tool. A key challenge in understanding TRD lies in its heterogeneity, which complicates the ability to capture variability effectively with FC. For instance, two patients with the same diagnosis can present markedly different symptom profiles in terms of type and severity, suggesting the existence of subtypes within depression. Efforts to subtype depression using FC, such as those by Tozzi et al. (2024), show promise; however, they also highlight substantial obstacles, including stability, reliability, and variability in FC measurements across studies. MRI measures are particularly sensitive to inconsistencies introduced by different scanners, field strengths, acquisition protocols, and hardware (e.g., coils), making replication challenging across clinical sites. Integrating FC measures while addressing these reliability issues may eventually improve our understanding of depression’s heterogeneity and pave the way for more precise, network-based approaches to diagnosis and treatment. Lastly, methodological barriers, such as head motion artifacts, challenges in acquiring longer scans, and difficulties in achieving larger sample sizes, must also be overcome to enhance the reliability of FC as a clinical measure.

Patients with TRD differ significantly in terms of clinical characteristics, comorbidities, and histories of treatment exposure (Li et al., 2023). Our study design did not fully address this heterogeneity, and it is likely that neurobiological findings may depend heavily on the specific characteristics of the sample. For instance, variations in age, duration of illness, severity of depressive episodes, treatment history (types of medications, previous response to neurostimulation interventions) and comorbid psychiatric conditions (such as anxiety or personality disorders) are all factors that can influence connectivity patterns and complicate the interpretation of results. On the other hand, though, having a less stringent criteria approach is more representative of the real-world challenges associated with antidepressant resistance (Li et al., 2023) and allowed us to capture a more diverse and real-world representative sample of TRD. Furthermore, given the growing recognition of symptom-level heterogeneity in depression, future studies with larger and more clinically diverse samples are warranted to investigate how specific symptom dimensions (such as anhedonia) may modulate functional brain networks and contribute to neurobiological variability within TRD.

Moreover, the choice of TRD definition used in our study impacts the generalizability of findings (Li et al., 2023). By focusing solely on medication-resistant patients, we potentially excluded those with treatment resistance to other modalities, such as ECT or TMS. This narrow focus limits our understanding of whether the neural circuits associated with resistance to pharmacotherapy are the same as those in patients resistant to neuromodulation. For example, the administration of neurostimulation treatments can modify brain circuitry, potentially creating a pattern of resistance that complicates future therapeutic interventions. This is critical because patients who are resistant to different treatments may exhibit distinct neurobiological signatures, which could alter both clinical management and outcomes. To address these differences effectively, various TRD staging models have been proposed (Li et al., 2023). For example, recent findings from our team (see Appendix B, Prentice et al., 2023) indicate that patients more resistant to neurostimulation treatments like TMS and ECT exhibit higher rACC-theta activity compared to less resistant patients undergoing psychotherapy or antidepressant treatment. Post hoc analyses in this work also showed similar patterns in delta, beta, and gamma bands, suggesting that neurobiological markers related to resistance could guide treatment selection more effectively in clinical practice. Therefore, expanding the scope of future research to include neurostimulation and psychotherapy resistance would provide a more comprehensive understanding of TRD and refine biomarker-based treatment strategies (Prentice et al., 2023). This expanded approach could also help address the role of

chronicity in connectivity alterations, as seen in our results. Chronic depressive symptoms may contribute to lasting changes in brain circuitry, particularly in regions like the rACC, which are implicated in emotional regulation and treatment response. By examining a more diverse sample, including patients who have undergone different treatment modalities, future research could offer a more comprehensive model of how functional connectivity relates to variability of symptoms across TRD.

Finally, all neuroimaging analyses controlled for current depression severity by adjusting for HAMD scores to account for potential confounding effects of symptom burden. However, this study was cross-sectional with a single assessment per participant and no follow-up data were collected, limiting our ability to evaluate remission or changes over time. Consequently, it remains unclear which neurobiological alterations observed are state-dependent versus trait markers of TRD. Future longitudinal research is needed to clarify which findings remain stable after symptom remission and which are transient markers of the depressed state.

These obstacles have led to the overall and by far main limitation of the thesis which is the relatively limited replicability of these findings, which, in turn, questions the generalizability of neurobiological models and limits their diagnostic use. Discrepancies can result from heterogeneity of depressive disorders, inclusion/exclusion of comorbidity, sample size, methodological differences, clinical differences in the patients included (e.g. number of episodes, length of illness, degree of treatment resistance, presence and length of pharmacological treatment), and analysis techniques. While these challenges are widespread across the neuroimaging field, ongoing advancements in imaging technologies, large-scale collaborative studies, and integrative approaches hold promise for addressing these limitations and paving the way for more robust and clinically relevant discoveries.

6.5. Future Directions

Given the ongoing advances in the neuroimaging field, the future goal remains to be able to predict treatment outcomes in patients with TRD by identifying neural markers that correlate with treatment response. Understanding the mechanisms of both existing and novel interventions, including neurostimulation therapies, ketamine, and psychedelics, has become a focal point of research in psychiatry. These novel interventions, in particular, have shown promise in offering relief for individuals with TRD, but the precise neural processes

underlying their efficacy remain poorly understood. Neuroimaging techniques such as functional MRI and electrophysiology could provide insights into how these interventions influence neural circuits involved in mood regulation. Understanding the neural mechanisms of these treatments is crucial not only to improve their effectiveness but also to personalize approaches to care. Further exploration of neuroimaging as a tool to predict treatment outcomes could pave the way for more targeted, evidence-based strategies to address TRD and other mood disorders.

There is an urgent need for innovative therapeutic approaches that can be personalized to individual patients, especially in light of the significant heterogeneity observed in functional connectivity patterns among those with treatment-resistant depression. This variability highlights the importance of developing personalized treatment strategies. Advances in neuroimaging techniques, such as functional MRI, alongside the integration of big data and machine learning, could play a key role in exploring this heterogeneity and identifying distinct subtypes of TRD. By analysing individual connectivity profiles, we could improve our understanding of how different brain networks contribute to treatment resistance and tailor interventions more precisely. These advancements could not only enhance treatment outcomes but also pave the way for the development of targeted therapies that are more effective in addressing the unique needs of each patient.

Future research should focus on several other key areas to further explore the neurobiology of TRD. Longitudinal studies that track changes in functional connectivity over the course of treatment could provide insights into the dynamic nature of these neural patterns and their relationship to treatment response. One of the strengths of this study is its direct comparison of TRD, TSD, and HC, which allows for a clearer differentiation between the neural mechanisms underlying treatment resistance and those associated with treatment response. By contrasting these three groups, this work offers valuable insights into the specific neural pathways and connectivity patterns that characterize treatment resistance, which is crucial for understanding why some patients do not respond to conventional treatments. However, future research should expand on this by incorporating pre- and post-treatment comparisons using FC in fMRI studies. This would allow for a more comprehensive understanding of how neural network dynamics evolve in response to treatment and whether specific connectivity patterns serve as mediators of treatment efficacy. Such longitudinal designs would be critical in distinguishing between predictors of treatment response and the effects of the treatment itself on the brain’s neural circuits.

Future research on the DMN continues to explore how this network interacts with others to support cognition and emotion and how disruptions in its connectivity can lead to psychopathology. Understanding the DMN's role in both health and disease has the potential to inform new therapeutic interventions targeting network-level dysfunction. By examining how different brain regions interact as networks, rather than in isolation, these methods have provided new insights into the complex organization of the brain and how this organization may be altered in various neurological and psychiatric conditions. Moving forward, further refinement of these methods could involve dynamic functional connectivity approaches to assess fluctuations in network connectivity over time, or Directed Causal Modelling (DCM), which helps identify causal interactions between brain regions. Additionally, integrating multimodal imaging data - such as combining fMRI with electrophysiological measures like electroencephalography or magnetoencephalography - can provide complementary insights into both the spatial and temporal dynamics of brain networks, offering a more comprehensive view of network function in TRD. These approaches could, for example, enable the identification of network signatures that are not observable through single-modality imaging alone. Furthermore, employing advanced computational techniques, such as machine learning, could facilitate the analysis of large, complex datasets by identifying subtle patterns of network connectivity linked to treatment response or symptom progression. For instance, machine learning models could be trained to detect connectivity patterns that distinguish between TRD and non-resistant forms of depression.

Future research should also focus on confirming and clarifying the involvement of the DMN in TRD and how the specific DMN alterations relate to these different aspects of internal mentation and depressive symptoms such as rumination. It is crucial to directly probe these and other psychological processes that are widely implicated in MDD, such as reward and affective processes, in TRD. Unfortunately, to date there are no task-based fMRI studies in TRD to address this and future work using tasks designed to probe these functions could be helpful.

As the field moves forward, multi-site studies utilizing "big data" approaches are essential to ascertain how clinically meaningful FC findings can be at the individual level. Dataset sizes are rapidly increasing due to larger samples, enhanced spatial and temporal resolution, and the integration of multimodal data alongside fMRI. However, challenges inherent to big data analytics, such as developing efficient data-sharing protocols and optimizing the analysis of high-dimensional FC features, must be addressed. Moreover, future studies may use

prospective longitudinal neuroimaging designs to delineate which effects are present in treatment-naive patients and which effects are the result of disease progression.

Finally, given the central role of network dysregulation in the pathophysiology of TRD, investigating novel interventions that target specific brain networks like the DMN and fronto-limbic circuits may lead to significant advancements in treatment. Studies such as Ge et al. (2020) demonstrate that functional connectivity in the rACC may serve as a predictor for therapeutic response in rTMS. These findings support the idea that the modulation of the DMN, including intra-network connections like the rACC-PCC, holds promise as a therapeutic target (Mayberg et al., 2005).

fMRI neurofeedback represents another innovative approach to treating TRD by enabling patients to gain real-time insights into their brain activity, potentially leading to modulations in neural networks associated with depression (See Appendix A, Barreiros et al., 2024). However, this approach remains in its early stages and requires further investigation to determine its effectiveness, long-term sustainability, and direct relationship with clinical improvement. The findings of this thesis offer valuable insights for refining neurofeedback interventions, particularly in identifying connectivity patterns that could serve as effective targets for feedback. For example, by focusing neurofeedback protocols on specific patterns of hyperconnectivity observed in TRD - such as the habenula's connection with the precuneus cortex and the rACC's altered connectivity with DMN regions—neurofeedback could be tailored to help patients modulate these networks directly. By incorporating the connectivity patterns identified in this thesis, future neurofeedback protocols could target relevant networks with greater specificity, potentially enhancing efficacy for TRD. For instance, focusing neurofeedback on the rACC connectivity alterations observed in TRD may potentially assist patients in better regulating self-referential and emotion-processing functions, both critical areas impacted in treatment-resistant cases.

Lastly, examining how rACC activity fluctuates across different treatment modalities, allowing for a more nuanced understanding of its implications for treatment efficacy (see Appendix B - Prentice et al., 2023). Such longitudinal studies could track the evolution of rACC activity and its relationship with treatment response, contributing to personalized treatment strategies.

Through these combined efforts, the next steps could significantly expand our understanding of TRD and pave the way for innovative treatment approaches that address the complex nature of this debilitating condition.

6.6. Conclusion

The aim of this thesis was to elucidate the role of functional connectivity in understanding treatment-resistant depression. Through a comprehensive examination of the neurobiological underpinnings of TRD, this work highlights the importance of recognizing the unique neural signatures associated with treatment resistance. The findings of this thesis not only advance the overall understanding of neural mechanisms in TRD, but these results could guide future work to pave the way for more effective and personalized treatment strategies that address the needs of individuals suffering from this challenging condition.

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Appendices

Appendix A

fMRI neurofeedback for the modulation of the neural networks associated with depression

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fMRI neurofeedback for the modulation of the neural networks associated with depression

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ABSTRACT

Objectives: Functional magnetic resonance imaging (fMRI) neurofeedback has emerged as a potential treatment modality for depression, but little is known about its mechanism of action. This study aims to investigate the efficacy of fMRI neurofeedback in modulating neural networks in depression.
Methods: Following PRISMA guidelines, a systematic review was conducted focusing on fMRI neurofeedback interventions in depression. A comprehensive search across multiple databases yielded 16 eligible studies for review.
Results: The review demonstrated that fMRI neurofeedback can modulate BOLD activity even in strategy-free protocols and within a single session, with a significant learning effect evident over sessions. Neurofeedback targeting specific regions led to changes in connectivity across broad neural networks, including the default-mode and executive control networks, with effects being region-specific. However, methodological diversity and the absence of standardized protocols in the reviewed studies highlighted the need for more uniform research approaches.
Conclusions: fMRI neurofeedback shows promise as a modulatory technique for depression, with the potential to induce significant changes in neural activity and connectivity of networks implicated in depression.
Significance: The review underscores the necessity for standardized, reproducible neurofeedback protocols with control groups to enhance research comparability and generalizability.

1. Introduction

Conventional treatments for major depression, such as pharmacotherapy and psychotherapy, although effective for many individuals, do not provide adequate relief for everyone (Kessler et al., 2003). There is a need for innovative and targeted interventions that can modulate neural mechanisms that are implicated in depression in a personalised way.

Functional magnetic resonance imaging (fMRI) neurofeedback (NF) is a promising technique that allows individuals to self-regulate their brain activity by receiving real-time feedback based on fMRI signals (Ruiz et al., 2014), promoting adaptive changes in neural functioning (Koush et al., 2017). The convergence of advancements in neuroimaging technology, increased understanding of neural mechanisms underlying psychiatric disorders, and the growing interest in personalized and patient-centred care has fuelled interest in fMRI neurofeedback in psychiatry, including major depression (Johnston et al., 2020).

Abbreviations: fMRI, Functional Magnetic Resonance Imaging; BOLD, Blood Oxygen Level-Dependent; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; MDD, Major Depressive Disorder; EEG, Electroencephalography; fNIRS, Functional Near-Infrared Spectroscopy; FC, Functional Connectivity; ROI, Region of Interest; vlPFC, Ventrolateral Prefrontal Cortex; DMN, Default Mode Network; ECN, Executive Control Network; NF, Neurofeedback; CG, Control Group; EG, Experimental Group; HC, Healthy Control; pgACC, Pre-Genual Anterior Cingulate Cortex.

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Since its inception in 2003, fMRI neurofeedback has garnered significant interest due to advancements in real-time analysis capabilities. In fMRI neurofeedback, the region of interest (ROI) or connectivity between regions is determined based on expected behavioral changes, identified using a functional localizer task or anatomical information from previous research. Participants are then guided to improve control over their region-of-interest activation or network connectivity by receiving real-time performance feedback.

In a general fMRI neurofeedback protocol, initially, functional MRI data is collected from the participant, typically focusing on specific ROIs, multiple ROIs, or the entire brain. This data acquisition phase may involve a task-based functional localizer to identify target regions or networks relevant to the desired behavioral change. Subsequently, the acquired data is processed in real-time using standard neuroimaging software packages. Feedback is computed based on changes in ROI signal intensity, correlations between multiple ROIs, or via a classification algorithm trained on offline fMRI data obtained prior to the neurofeedback session to establish baseline activity or calibrate the feedback system. The computed feedback signal is then transformed into a format suitable for real-time presentation, often as a continuous visual representation displayed to the participant within the MRI scanner environment. Participants can observe their neural response dynamics and actively engage in self-regulation to modulate the feedback signal, thereby initiating a closed-loop feedback cycle aimed at achieving the predetermined brain activity pattern (Fede et al., 2020). Additionally, neurofeedback protocols often include "transfer runs," during which participants practice self-regulation without real-time feedback to assess whether learned control over brain activity can be maintained independently.

In addition to univariate analyses of region-of-interest activation or connectivity regulation, decoded neurofeedback employs a multivariate approach involving spatial activity patterns. In decoded neurofeedback, feedback represents the likelihood of achieving a predetermined target brain activity pattern rather than the achieved activation or connectivity change (Tursic et al., 2020). Participants learn to elicit the predetermined state by receiving feedback on how similar their brain activity pattern is to the target. Neurofeedback is applied both in healthy individuals for cognitive performance enhancement and in clinical populations as an intervention for various conditions, including depression (Tursic et al., 2020).

By directly targeting and modulating dysfunctional neural networks, fMRI neurofeedback interventions have shown promise in reducing depressive symptoms and altering neurobiological features associated with depression (Mathiak and Keller, 2021, Linden, 2014, Young et al., 2017, Johnston et al., 2020). A recent *meta*-analysis by D'Agati et al. (D'Agati et al., 2021) examined the efficacy of neurofeedback interventions, including fMRI neurofeedback, for major depressive disorder. However, their *meta*-analysis focused on the overall effectiveness of various neurofeedback modalities for depression, including not only fMRI but also other techniques such as electroencephalography (EEG) and real-time functional near-infrared spectroscopy (fNIRS). As a result, the specific impact of fMRI neurofeedback on neural measures in depression was not thoroughly explored. Secondly, while the *meta*-analysis considered the general efficacy of neurofeedback interventions for the improvement of depressive symptoms, it did not provide in-depth insights into the specific neural mechanisms underlying the observed antidepressant effects. Understanding the neurobiological changes associated with fMRI neurofeedback in depression is crucial for elucidating the underlying mechanisms and optimizing treatment approaches.

This systematic literature review exclusively includes fMRI-based studies on depression. By focusing on fMRI neurofeedback, the review aims to improve our understanding of the underlying neural mechanisms and their impact on functional brain activity.

2. Materials and methods

2.1. Study design

The current systematic review was designed, conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Protocols (PRISMA) 2015 guidelines (Page et al., 2020, Moher et al., 2009). The protocol was registered on PROSPERO (Barreiros et al., 2022).

2.2. Search strategy

A comprehensive search was conducted in five scientific databases PubMed, ScienceDirect, Web of Science, Scopus, and PsycINFO. The search strategy incorporated the following key words: "fMRI" OR "functional magnetic resonance imaging" OR "functional MRI", AND "depression" OR "major depression" OR "major depressive disorder", AND "neurofeedback". The inclusion criteria required studies to be written in English, be peer-reviewed, use fMRI neurofeedback interventions, include at least one cohort of patients with unipolar major depressive disorder, and report fMRI results. Studies on electroencephalography-based neurofeedback interventions and other bio-feedback interventions were excluded as were review studies to ensure a focus on original experimental research. Duplicates, animal studies and publications which did not provide access to full-text were also excluded. The search reported here was first undertaken in September 2022 and updated for any new work in December 2023, without imposing any publication date limit.

The screening and selection process were conducted using Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. https://www.covidence.org.).

2.3. Data extraction and analyses

Initially, titles and abstracts were screened to determine their relevance to the research question and predefined inclusion/exclusion criteria. Subsequently, a full-text review was conducted for potentially eligible studies. Two independent reviewers (ARB & IBB) assessed the articles to ensure consistency and minimize bias.

Once the final set of included studies was determined, relevant data were extracted using a customized data extraction tool. This form captured key information, including study characteristics, participant demographics, intervention details, outcome measures, and results.

Data analysis was performed systematically, and a qualitative synthesis of findings was conducted, involving summarizing and interpreting the results of individual studies. We reviewed studies regarding the BOLD signal response to the NF training and the differences in the methodology used in these studies, by extracting and summarizing (a) Study characteristics—study cohort, experimental design, selection process –, (b) Participants characteristics – sample size, age, gender, exclusion criteria, medication, current episode, comorbidities –, (c) neurofeedback protocol – instructions, regulation strategy, stimuli, feedback display, feedback source, feedback ROI, sham feedback ROI, number of neurofeedback runs, number of neurofeedback sessions, practice run, and transfer run.

The main outcomes of the review are the change in the functional MRI measures (e.g. functional connectivity, BOLD activation) compared between groups, conditions or time points.

3. Results

3.1. Search and screening

The initial search resulted in the identification of 201 studies. After removing duplicates, the number was reduced to 136. These studies underwent a screening process based on predefined inclusion and

exclusion criteria, resulting in the exclusion of 109 articles (see Fig. 1).

From the remaining 27 studies, an eligibility assessment was performed. Four studies did not meet the criteria for fMRI neurofeedback studies, six studies did not report results on functional MRI, and one study did not include a cohort of patients with depression. As a result, a total of 16 studies were deemed eligible for inclusion in the systematic review.

3.2. Demographic and clinical characteristics of the sample

Table 1 provides insights into the demographic and clinical characteristics of the examined populations. These studies encompassed a range of depressive conditions, such as Major Depressive Disorder (MDD) (N = 10 studies; 310 participants), Recurrent MDD (N = 3 studies; 67 participants), and Mild Depression (N = 3 studies; 23 participants). The total number of participants across the studies varied, with sample sizes ranging from 6 to 43 individuals. On average 76.8 % of the participants were female in the studies – noting that only 8 of the 16 studies presented information on participants' gender ratio. Among the studies that reported medication information, 6 out of the 16 studies indicated that participants were currently medicated, while 9 studies included participants not currently medicated.

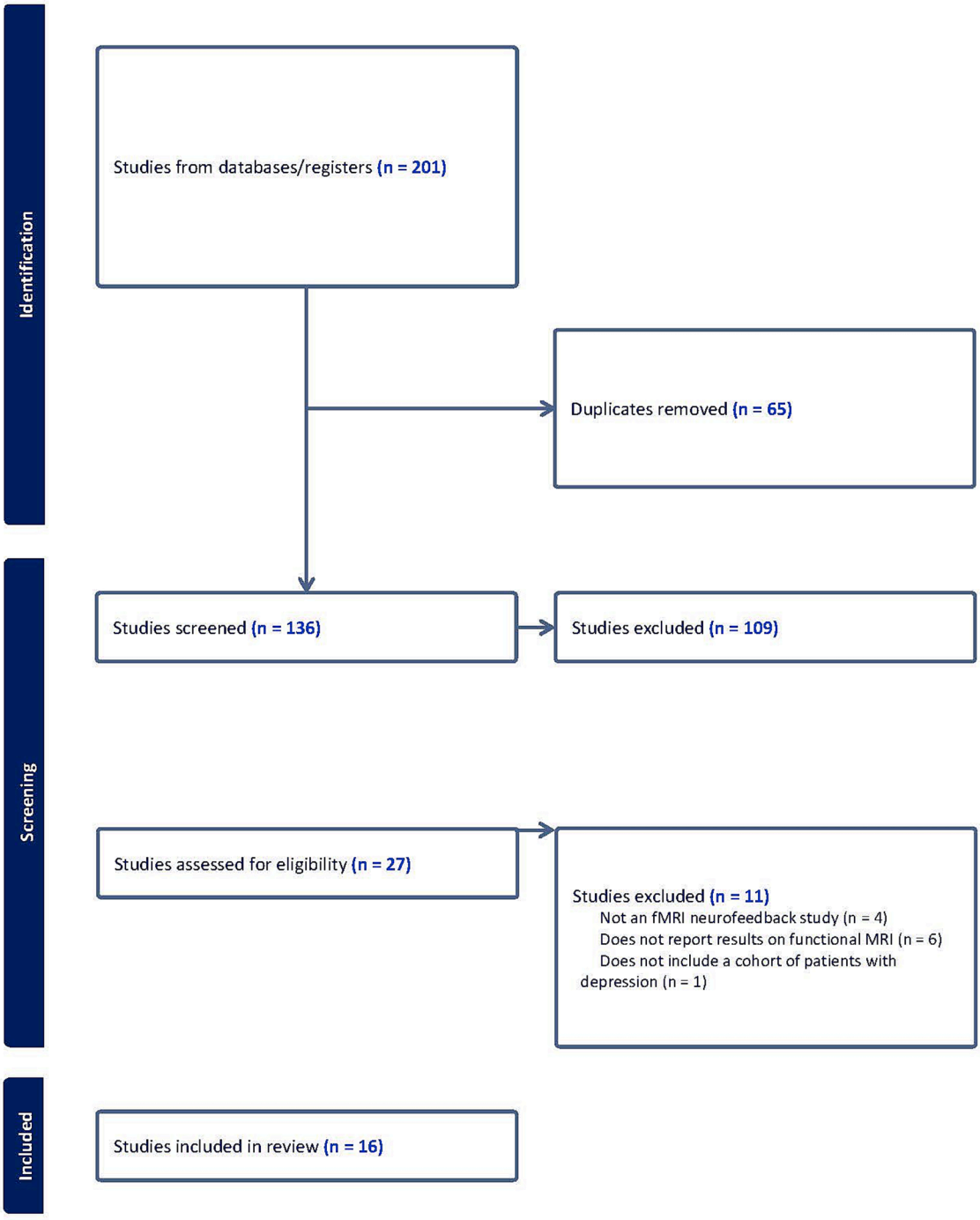

Fig. 1. Flow diagram depicting the literature search and selection process. The diagram illustrates the number of records identified through database searches, screened abstracts, full-text articles assessed for eligibility, and studies included in the final review, along with reasons for exclusions at each stage.

Table 1
Summary of demographic and clinical characteristics of the studies' populations.

Study	*Reference*	*Patient cohort*	*N*	*Gender (F,M)*	*Currently medicated*	*Clinical outcome measures*
1	Young et al., 2017	MDD	34	Not reported	No	Depression symptomatology (MADRS)
2	Young et al., 2014	MDD	21	Not reported	No	Depression (HDRS, MADRS) and anxiety (HARS) symptomatology
3	Zotev et al., 2016	MDD	24	(18,8)	No	n.a.
4	Young et al., 2018	MDD	36	Not reported	No	n.a.
5	Yuan et al., 2014	MDD	27	Not reported	No	Clinical ratings
6	Zotev et al., 2020	Recurrent MDD	24	(17,7)	No	n.a.
7	Young et al., 2017	MDD	36	Not reported	No	Depression symptomatology (MADRS)
8	Zahn et al., 2019	MDD	28	(21,07)	Yes	Clinical ratings
9	Jaeckle et al., 2021	Recurrent MDD	35	Not reported	Yes	Measures of symptoms, self-esteem and self-blame
10	Keller et al., 2021	MDD	39	(17,22)	Yes	Measures of mood, emotion regulation and depressive symptomatology
11	Hamilton et al., 2016	MDD	22	Not reported	Yes	n.a.
12	Bezmaternykh et al., 2021	Mild depression	6	(6,0)	No	n.a.
13	Melnikov et al., 2022	Mild depression	8	(8,0)	No	Depression symptomatology (MADRS)
14	Linden et al., 2012	Recurrent MDD	8	(0,8)	Yes	Depression symptomatology (HDRS)
15	Taylor et al., 2022	Mild depression	9	Not reported	Not reported	Depression (BDI) and trait anxiety (STAI2)
16	Mehler et al., 2018	MDD	43	(22,11)	Yes	n.a.

MDD – Major Depressive Disorder; M – mean, SD – standard deviation; F – female, M – Male.

3.3. Qualitative review of studies' characteristics

Our systematic review included 16 studies that investigated the effects of fMRI neurofeedback interventions on FC and BOLD activation patterns in individuals with depression. The methodologies and outcome measures varied significantly across the studies, complicating direct comparisons and precluding a quantitative *meta*-analysis. Despite this heterogeneity, a qualitative synthesis revealed several key themes and findings.

3.3.1. Categories of neuromodulatory effects

Efforts were undertaken to identify a consistent and comparable statistical measure to facilitate a *meta*-analysis of the primary effects. However, due to the heterogeneity in methodology across studies, a *meta*-analysis could not be conducted. A subset of the most representative outcome measures is presented in Supplementary Table 1. A broad spectrum of distinct statistical outcome measures was identified in the studies, encompassing > 13 outcome measures reported that gauged the impact of neurofeedback training. Consequently, a qualitative examination of the findings and protocol characteristics is reported.

For the purpose of this qualitative analysis, the different outcome measures extracted were synthesized into four main categories of neuromodulatory effects: effect of training, effect of session, effect of group and effect of condition. The effect of training refers to whether there was an effect of the neurofeedback intervention over the course of the session (pre vs post-neurofeedback session). The effect of session refers to the effect observed when comparing baseline or session 0 to follow-up or the last session of the intervention course. The effect of group refers to the effect observed when comparing the experimental group (EG) to the control group (CG) at the last session, last run, or transfer run. Finally, the effect of condition refers to the effect observed when comparing different conditions or tasks.

3.3.2. Experimental designs and neurofeedback protocols

Table 2 provides details on the experimental design and neurofeedback protocol used in each study.

Among the studies, 2 utilized backward masking tasks (tasks measuring responses to emotionally valenced stimuli, subliminally processed) and the Emotional Test Battery as the regulation strategy, 4 studies employed an autobiographical memory recall task, 5 studies employed emotion-evoking memories for regulation, 2 studies used a cognitive reappraisal task, and 4 studies were task-free. In terms of neurofeedback signal, 11 studies utilized BOLD activation analysis, while 5 studies focused on FC measures. Specifically, amongst the activation studies, 3 studies targeted the bilateral amygdala, 3 studies focused on the left amygdala, and 2 studies targeted the right ventrolateral prefrontal cortex (vlPFC). The feedback source varied, including ROI percent signal change, Pearson correlation coefficients, and corrected time series data. The duration of neurofeedback runs ranged from 160 s to 1500 s, and the number of neurofeedback runs varied from 1 to 6. Additionally, 6 studies conducted practice runs and transfer runs, and the number of neurofeedback sessions ranged from 1 to 8.

3.3.3. Factors contributing to neuromodulation effects

Several factors influence the effects of neurofeedback, including the presence of control groups, the conditions used, regulation strategies, neurofeedback signals and targets, and the duration and number of neurofeedback runs (see Fig. 2 for a comprehensive summary). Studies with a sham neurofeedback control group generally reported significant effects of session and training, while those without control groups showed varied results. The conditions employed (e.g., Happy/Sad vs Neutral) did not consistently predict outcomes. Different regulation strategies, such as autobiographical memory recall and emotion evocation, yielded mixed results, with significant training effects noted in some studies. Neurofeedback targeting specific regions like the amygdala and medial prefrontal cortex (mPFC) showed variable but notable effects, with the amygdala being a predominant target. Lastly, the duration and number of neurofeedback runs also played a role, with longer and multiple runs generally associated with significant training and session effects. To provide a comprehensive understanding of the neurofeedback tasks utilized in the studies, we have summarized the description of each task and their respective protocols:

Backward Masking Task (BMT): Participants responded to target faces by identifying subsequent faces based on identity (not emotion). Faces were rapidly shown (masked) for 26 ms, followed by a masking face for 107 ms, with inter-stimulus intervals of 10–13 s. The task aimed to differentially alter hemodynamic activity in response to happy and sad faces presented below conscious awareness (Young et al., 2017).

Autobiographical Memories Recall Task: Participants retrieved positive memories to increase hemodynamic activity in a target region. They maintained the recall strategy, focusing on the memory's positive aspects, its importance, and its relation to their self-concept, regardless of perceived effectiveness (Young et al., 2018).

Emotion Evoking Task (Indignation and Guilt): Participants recalled autobiographical events that evoked guilt or indignation. They used cue words to think about these events while trying to adjust the correlation of fMRI signals between specific brain regions, with different instructions for increasing or stabilizing the correlation (Zhan et al., 2019).

Emotion Evoking Task (Self-Regulation): Participants alternated between recalling happy memories, counting backward, and resting.

Table 2
Summary of neurofeedback intervention protocol characteristics and reported neuromodulation success.

	Experimental design			Neurofeedback protocol									Neuromodulation effects		
Study	*Control group*	*Condition*	*Regulation strategy*	*NF target*	*NF signal*	*Feedback source*	*Duration of NF run*	*# NF runs*	*Practice Run*	*Transfer Run*	*# sessions*	*Effect of training*[1]	*Effect of session*[2]	*Effect of group*[3]	*Effect of condition*[4]
1	Sham NF	Happy/Sad vs Neutral	Backward masking task and the Emotional Test Battery	Amygdala	BOLD activation	ROI percent signal change	Not reported	3	Yes	Yes	2	Not reported	Yes	Yes	Yes
2	Sham NF	Happy vs Count	Positive autobiographical memories recall	Amygdala	BOLD activation	ROI percent signal change	526 s	3	Yes	Yes	1	Yes	N/A	Yes	Not reported
3	Sham NF	Happy vs Count	Positive autobiographical memories recall	Left amygdala	BOLD activation	ROI percent signal change	526 s	3	Yes	Yes	1	Yes	N/A	No	Yes
4	Sham NF	Happy/Sad vs Counting	Positive autobiographical memories recall	Left amygdala	BOLD activation	ROI percent signal change relative to the average fMRI signal	Not reported	3	Yes	Yes	2	Not reported	Yes	No	Not reported
5	Sham NF	Happy vs Rest	Emotion evoking memories	Left amygdala	FC	ROI percent signal change	520 s	6	Yes	Yes	1	Yes	N/A	Yes	Not reported
6	Sham NF	Happy vs Rest	Emotion evoking memories	Left amygdala/ Left rACC	BOLD activation	ROI percent signal change	526 s	1	Yes	Yes	1	Yes	N/A	Yes, for FC, not for BOLD	Yes
7	Sham NF	Happy vs Count	Positive autobiographical memories recall	Left amygdala	BOLD activation	ROI percent signal change	520 s	3	Yes	Yes	1	Yes	N/A	Yes	Not reported
8	Other	Indignation vs Guilt	Emotion evoking memories	RATL/SCC	FC	Pearson correlation coefficient of the correlation of fMRI signal between ROIs	Not reported	2	Yes	Yes	1	Yes	N/A	Yes	No
9	No	Self-blame vs other-blame	Emotion evoking memories	RATL/SCC	FC	Pearson correlation coefficient of the correlation of fMRI signal between ROIs	424 s	2	Yes	Yes	3	Not reported	No	N/A	Yes
10	No	Cognitive Reappraisal vs View	Cognitive reappraisal	Right vlPFC	BOLD activation	ROI percent signal change	420 s	4	Yes	No	2	Not reported	Yes	N/A	No
11	Sham NF	N/A	Cognitive reappraisal	Salience Network	BOLD activation	Corrected time series data that correlated with a gamma-function-convolved boxcar covariate reflecting the onset and offset of negative IAPS pictures.	204 s	3	Yes	Yes	1	Yes	N/A	Yes	N/A
12	No	N/A	Strategy free	Left mPFC	FC	Not reported	1500 s	1	Yes	Yes	8	Not reported	Yes	N/A	N/A
13	No	N/A	Strategy free	Left mPFC	BOLD activation	ROI percentage signal change	900 s	2	Yes	Yes	8	Not reported	Not reported	No	N/A
14	No	N/A	Strategy free	vlPFC/insula	BOLD activation	ROI percent of signal change	420 s	3	Yes	Yes	4	Yes	No	N/A	N/A
15	No	Counting vs Rest	N-back	dlPFC/PCC	FC	FC between dfPFC and PCC	Not reported	1	Not reported	Not reported	4	Not reported	Yes	N/A	Yes
16	Sham NF	N/A	Strategy free	Brain areas involved in emotional processing	BOLD activation	ROI percent signal change	160 s	6	Yes	Yes	5	Yes	No	No	N/A

[1] reported significant effects of training (effect of time or pre vs post-NF).
[2] reported significant effects of session (effect of session or baseline/session 0 vs follow-up/last session).
[3] reported significant effects of group (effect of group or EG vs CG at the last session/last run/transfer run).
[4] reported significant effects of experimental condition (effect of condition/task or effect of ROI).

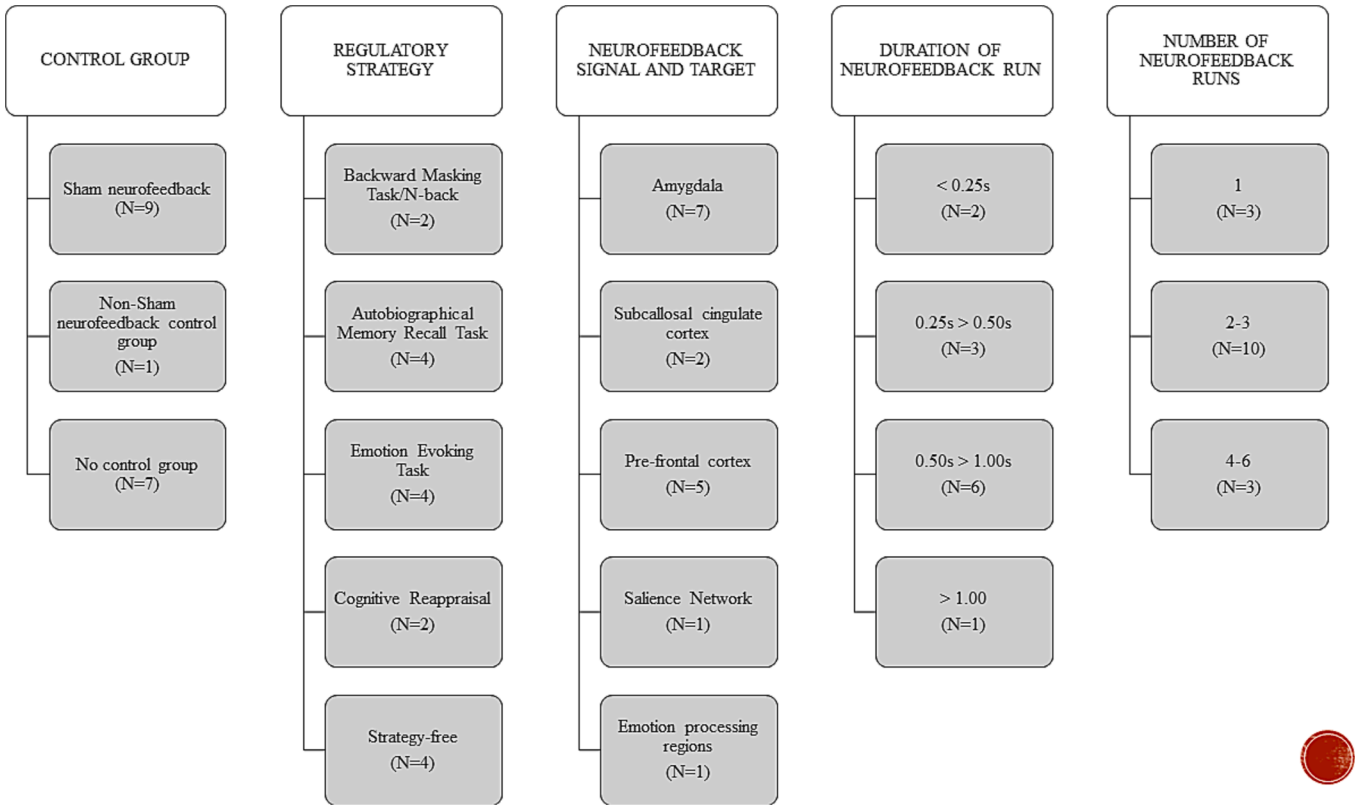

Fig. 2. Summary of factors influencing the effects of neurofeedback.

They aimed to induce happy emotions and raise neurofeedback bars while recalling happy memories. The task included both neurofeedback and transfer runs without neurofeedback to assess the learned ability to control brain activity (Zotev et al., 2020).

Emotion Regulation Task (Cognitive Reappraisal): Participants viewed negative pictures and used cognitive reappraisal strategies to modulate their emotional response. They upregulated the BOLD signal in the target region by reinterpreting the emotional content of the pictures. Feedback was given via a numerical value indicating success in the "reappraise" condition (Keller et al., 2021).

3.3.3.1. Studies with no significant modulatory effects reported. Study 13 reported no significant neuromodulation effects.

Participants from this study were currently mildly or moderately depressed and not on medication. This study did not include a control group. This was a "strategy free" study, meaning that it employed an approach that did not follow a predefined neurofeedback strategy in their protocol. The neurofeedback signal source used in this study was BOLD activation. Specifically, the study focused on the percentage BOLD signal change in the left mPFC. The intervention protocol included eight sessions with two long neurofeedback runs (900 ms), a practice run and transfer run. To note that this was a pilot study with only 8 participants.

4. Discussion

The primary objective of this systematic review was to examine the effects of fMRI neurofeedback interventions on FC and BOLD activation patterns in individuals with depression. Our review revealed a significant challenge stemming from the diversity in methodologies and outcome measures across the selected studies. This heterogeneity was such that we were unable to conduct a quantitative *meta*-analysis to assess the overall effect of fMRI neurofeedback on depression-related neural measures. Importantly, this indicates that the effect of fMRI neurofeedback on depression-related circuitry needs to be more systematically investigated and reported upon in future studies. Despite the methodological heterogeneity and gaps in reported measures, we conducted a qualitative analysis, grouping neuromodulatory effects into distinct categories to provide a comprehensive perspective.

4.1. Outcomes of neurofeedback interventions

The studies included in our review displayed a range of outcomes, with varying degrees of significance in terms of training, session, group, and condition effects. This highlights the complexity of the interaction between neurofeedback interventions and neuromodulatory effects. Importantly, most of the studies (13/14) reported significant effects of training or session, suggesting the potential of fMRI neurofeedback to induce modulatory changes in neural activity over time (even in a single session), despite the variety of protocols implemented.

4.2. Control conditions and regulation strategies

Factors such as the presence of a control group (i.e., effects relative to control), the choice of regulation strategy, the neurofeedback signal used, the neurofeedback target region, and the duration of neurofeedback runs had varied associations with neuromodulatory effects. However, the strength and specificity of this neuromodulatory effect were rarely measured with appropriate control conditions, making it difficult to ascertain which factors yield the best results in depression.

The inclusion of appropriate control conditions in neurofeedback (NF) protocols is essential to evaluate the specificity of the observed effects. By comparing NF training with a control condition, it becomes possible to disentangle the specific effects of NF from non-specific factors such as placebo effects or general cognitive improvements. All nine studies in our review that implemented a sham neurofeedback control group consistently showed neuromodulatory effects. Additionally, studies without a control group are unable to conclude whether changes in depression symptoms are specifically due to the fMRI neurofeedback.

However, not all studies with a control group reported between-group comparative results or the outcomes of contrasts within the control group, introducing a relevant reporting bias.

4.3. Neurofeedback tasks

The diverse nature of the neurofeedback tasks utilized in these studies likely contributes to the variability in outcomes observed. Tasks differed in several key aspects, including the type of emotion targeted, the nature of the regulation strategy, the complexity of the task structure, and the type of feedback provided to participants. For example, tasks focusing on personal, emotionally charged memories (such as the autobiographical memories recall task) may lead to stronger emotional engagement and, consequently, more significant modulation of neural activity. In contrast, tasks like the backward masking task, which involves rapid, subconscious face recognition, may engage different neural pathways and result in distinct outcomes.

The cognitive load required by the tasks also varies, potentially impacting participants' ability to successfully modulate their brain activity. Cognitive reappraisal tasks, which involve reinterpreting emotional stimuli, may present a higher cognitive demand compared to simpler recall tasks, thereby influencing the effectiveness of the neurofeedback. Additionally, the clarity and immediacy of feedback provided during the tasks play a crucial role in the participants' ability to adjust their strategies. Clear and immediate feedback (such as visual bars or numerical values) can enhance participants' understanding of their performance and facilitate more effective regulation of neural activity. These differences underscore the importance of carefully selecting and designing neurofeedback tasks to match the specific goals of the intervention and the characteristics of the target population. Future research should continue to explore how task design influences neurofeedback outcomes and identify optimal strategies for enhancing the efficacy of neurofeedback interventions.

4.4. Selection of neurofeedback targets

Neuromodulatory effects seem to be specific to the target region selected, as experimental and control groups differed in neurofeedback training effects on the BOLD signal based on the specific target region (Young et al., 2014; Keller et al., 2021). Moreover, there seems to be a hemispheric-specific NF effect, as demonstrated in one of the studies where feedback from the left ROI was advantageous over feedback from the right ROI (Keller et al., 2021).

The selection of some neurofeedback targets, such as the left medial prefrontal cortex (mPFC), is informed by their established roles in the pathology of depression and seems to have been carefully selected according to the clinical profile. For instance, mPFC was only chosen as a target in studies done on patients with mild depression. The amygdala is pivotal in emotional responses and has been found to be hyperactive in depression, while the vlPFC and mPFC are integral to cognitive aspects of emotional regulation, often showing altered activation patterns in MDD (Keller et al., 2021). However, different studies have targeted the same brain region for neurofeedback with different clinical populations: the subgenual cingulate cortex, the left amygdala, and the ventrolateral PFC were all used as the neurofeedback target in studies with patients diagnosed with MDD (Zahn et al., 2019; Yuan et al., 2014; Keller et al., 2021) and with recurrent MDD (Jaeckle et al., 2023; Zotev et al., 2020; Linden et al., 2012).

4.5. Neural changes beyond the target region

Our review also found that fMRI-based neurofeedback interventions have the potential to induce not only significant changes in the neural activation of the targeted region (Mel'nikov et al., 2023; Taylor et al., 2022; Young et al., 2017), but also changes in large-scale connectivity patterns (Taylor et al., 2022; Bezmaternykh et al., 2021). The amygdala is crucial for processing emotional responses, while the mPFC is involved in higher-order cognitive functions and emotion regulation. These regions are part of broader neural networks that are implicated in the pathophysiology of depression. For instance, targeting the amygdala has shown to modulate its overactivity (Yuan et al., 2014), as well as its FC in within-default mode network (DMN) (Yuan et al., 2014) and DMN to executive control network (ECN) connections (Hamilton et al., 2016). These changes in neural functioning are promising indicators of the potential for neurofeedback to rectify the neural changes underlying depression beyond the target region.

Furthermore, the neural changes observed in regions beyond the targeted areas highlight the interconnected nature of brain networks involved in depression. The triple network model, which includes the DMN, salience network (SN), and ECN, provides a framework for understanding these interconnections. Studies have shown that these networks are often dysregulated in depression (Yuan et al., 2014), with the DMN being associated with self-referential thoughts, the SN with detecting and filtering salient stimuli, and the ECN with cognitive control and executive function.

Our review suggests that modulating the salience network, which plays a critical role in switching between the DMN and ECN, might yield more consistent neuromodulatory effects. For example, studies targeting the amygdala, a key node within the SN, reported significant changes in both within-network and between-network connectivity, including alterations in DMN and ECN interactions.

Interestingly, while the modulation of the ECN was not as consistently observed, studies that targeted this network through working memory tasks did not show significant effects. This suggests that the effectiveness of neurofeedback may be influenced by the specific network and task engaged. Moreover, other neuromodulatory therapies have shown network-specific effects, highlighting the potential for technique-dependent outcomes. For instance, repetitive transcranial magnetic stimulation (rTMS) targeting the dorsolateral prefrontal cortex (DLPFC), part of the ECN, and deep brain stimulation (DBS) targeting the subgenual cingulate cortex (sgACC), part of the DMN, have demonstrated efficacy in treating depression (Fitzgerald et al., 2009; Lozano et al., 2008; Mayberg et al., 2005; Fox et al., 2012; Downar & Daskalakis, 2013).

4.6. Enduring effects of neurofeedback

Although neurofeedback targeting BOLD activation might be more directly observable in session-by-session changes (7/11 reported significant effects of training within a session, versus 2/5 in FC studies), modulating FC could have more enduring effects on neural network patterns (as 2/5 FC studies reported an effect of session or significant interaction between session and condition, versus only 3/11 studies targeting ROI activation reporting significant effects of session). Interestingly, one of the studies reported that the more negative the FC became, the more participants' depressive symptoms were reduced (Taylor et al., 2022). Furthermore, there seem to be enduring neuromodulatory effects on the FC of neural networks even when the neurofeedback protocol targets activation of a single region. In three studies targeting the amygdala BOLD activation, with participants with MDD (Young et al., 2017; Yuan et al., 2014) and recurrent MDD (Zotev et al., 2020), neuromodulatory effects of group and session were found only on the FC of the amygdala, not on BOLD activation. In one of the studies (Yuan et al., 2014), amygdala FC with pgACC and with cuneus was no longer abnormal (compared to HC) in either the experimental group or the control group. Another study targeting the activation of the right vlPFC (Keller et al., 2021) with MDD participants found training-induced reduction of FC of the vlPFC with other brain regions (right anterior insula, left superior parietal cortex) over time. Collectively, these findings suggest that neurofeedback might be more effective in modulating FC than region-specific activity over a longer time frame, and this could mechanistically be related to clinical benefit.

4.7. Contradictory results

Our review also found some results that were contradictory across studies. While in some studies the BOLD response in the amygdala increased relative to their own baseline and to the control group (Young et al., 2017; Young et al., 2014; Zotev et al., 2020), other studies found no differences in the activation of the amygdala between experimental and control groups (Mehler et al., 2018; Yuan et al., 2014). One possible explanation for these contradictory results may be the experience of success of self-regulation and the positive reinforcement for task success that both groups receive, associated with an increase in self-efficacy, which is inherent to the neurofeedback experiment (Mehler et al., 2018). This explanation is supported by the results from Young and collaborators (2017), showing that amygdala connectivity during the neurofeedback task increased with widespread regions of the reward system.

4.8. Limitations

Our systematic review encountered some limitations. The diversity in methodologies and outcome measures, while reflective of the field's complexity, hindered the possibility of conducting a *meta*-analysis, and as such, only a qualitative synthesis was possible. Additionally, the limited number of studies and the variability in sample sizes and characteristics pose challenges in drawing definitive conclusions. The heterogeneity in the disorder MDD also suggests that better characterization of the clinical sample is required. It would be expected for there to be different effects in young treatment-naive participants as against elderly treatment-resistant participants. The lack of consistent reporting on training effects in some studies also adds to the complexity of the interpretation.

Despite these limitations, our review underscores the potential of fMRI neurofeedback as a modulatory technique for major depressive disorder. The consistent reports of significant effects of training and session, despite variety in protocols including condition, fMRI measure, and regulation strategy, provide encouraging evidence for the modulatory impact of fMRI neurofeedback.

Our findings emphasize the need for standardized methodologies and outcome measures in future research on fMRI neurofeedback for depression. Implementing rigorous control groups and well-defined regulation strategies can enhance the validity of study outcomes. Given the diverse manifestations of depression, including symptom severity and treatment resistance, the choice of neurofeedback targets must be strategically guided. The variability in neural circuitry among individuals with depression underscores the potential of fMRI neurofeedback to serve as a customizable treatment modality, capable of addressing specific neural dysfunctions unique to each patient's condition. This individualized targeting aligns with the emerging paradigm in interventional psychiatry, which advocates for treatments tailored to the unique neural profiles of patients, thereby addressing the heterogeneity inherent in depressive disorders.

5. Conclusions

In conclusion, this systematic review contributes insights into the emerging field of fMRI neurofeedback for depression. By synthesizing the available evidence, we shed light on the potential of fMRI neurofeedback to modulate neural networks and to provide a more targeted approach to depression treatment. Our systematic review offers evidence for the potential of fMRI neurofeedback in the treatment of major depressive disorder. The findings underscore the feasibility of modulating BOLD activity through fMRI neurofeedback, demonstrating its efficacy even in strategy-free protocols and within a single session.

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CRediT authorship contribution statement

Ana Rita Barreiros: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. **Isabella B. Breukelaar:** Conceptualization, Writing – review & editing. **Anthony W.F. Harris:** Conceptualization, Writing – review & editing. **Mayuresh S. Korgaonkar:** Conceptualization, Writing – review & editing.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2024.10.003.

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Supplementary Materials

Supplementary Table 1. Neuromodulation effects of neurofeedback training, experimental design (group and condition), and ROI

							Experimental Group						
	Training x Group x Condition	**Training x Group x ROI**	**Training x Group**	**Group x ROI**	**Group**	**Condition**	**Training x ROI**	**Baseline x Follow-up**	**Training x Condition**	**Condition**	**ROI**	**Run**	**Session**
Study	*F (p), d*	*F (p), d*	*F (p), d*	*F (p), d*	*t (p), d*	*t (p), d*	*F (p), d*	*t (p), d*	*F (p), d*	*t (p), d*	*t (p), d*	*F/t (p), d*	*F (p), d*
1	5.63 (0.02)				[3.35-3.89] (0.001-0.002), [0.52-1.13]			[3.13-3.44](0.002-0.003), [0.91-1.47]					
2		2.66 (0.045)					2.24 (0.05)				3.13 (0.025)	2.41 (0.02)	
3	4.96 (0.036)		**n.s.***			4.64 (0.005)	2.28 (0.074)				4.95 (0.046)	9.38 (0.01)	
4		[5.56-10.1] (<0.05)											
5								(<0.05)					
6					(0.04)					[3.21-2.49] (0.006-0.025), [0.62-0.80]			
7		2.37 (0.04)			[2.74-7.63] (0.001-0.04), [0.89-1.44]								
8	5.6 (0.03)				2.4 (0.03)				**0.11 (0.75)***	**1.9 (0.18)***			
9									6.4 (0.02)	**0.43 (0.52)***		**0.29 (0.87)***	
10					−6.69 (<0.05)	**n.s.***				**n.s.***			
11			**(0.06), 0.7***					(<0.05)					
12								[3-3.35] (0.02-0.03)					
13											**n.s.***		

14	6.88 (<0.034)							4.08 (0.04)	**n.s.***
15				− 2.31 (0.03)	(<0.05)				
16		**0.16 (0.095)***	**0.12 (0.73)***				(<0.05)	2.397 (0.023)	**1.965 (0.104)***

***n.s.** not significant

Data quality/Risk of bias assessment

To form a comprehensive assessment of the collective internal validity of the findings in the examined literature, we addressed significant methodological limitations and conducted an analysis of the inconsistencies within those findings. Biases can to influence the accurate estimation of intervention effects, either through underestimation or overestimation. To assess the risk of bias, we investigated the following categories: randomisation and selection bias, assignment of intervention bias, outcome data bias, measurement bias, and reporting bias (Higgins et al., 2019). Results are presented in Supplementary Table 2.

The signalling questions for each bias assessment category are as follows:

1. Randomisation and selection bias

1.1.Was the allocation sequence random?

1.2. Was the allocation sequence concealed until participants were enrolled and assigned to interventions?

1.3.Did baseline differences between intervention groups suggest a problem with the randomization process?

2. Assignment of intervention bias

2.1. Were participants aware of their assigned intervention during the trial?

2.2. Were carers and people delivering the interventions aware of participants assigned intervention during the trial?

2.3 Were there deviations from the intended intervention that arose because of the trial context?

2.4 Were these deviations likely to have affected the outcome?

2.5 Were these deviations from intended intervention balanced between groups?

2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?

2.7 Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?

3. Outcome data bias

3.1 Were data for this outcome available for all, or nearly all, participants randomized?

3.2 Is there evidence that the result was not biased by missing outcome data?

3.3 Could missingness in the outcome depend on its true value?

3.4 Is it likely that missingness in the outcome depended on its true value?

4. Measurement bias

4.1 Was the method of measuring the outcome inappropriate?

4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?

4.3 Were outcome assessors aware of the intervention received by study participants?

4.4 Could assessment of the outcome have been influenced by knowledge of intervention received?

4.5 Is it likely that assessment of the outcome was influenced by knowledge of intervention received?

5. Reporting bias

5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?

Is the numerical result being assessed likely to have been selected, on the basis of the

results, from...

5.2. multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?

5.3 multiple eligible analyses of the data?

Supplementary Table 2

	Domain 1: Randomisation and selection bias				*Domain 2: Assignment of intervention bias*								*Domain 3: Outcome data bias*					*Domain 4: Measurement bias*						*Domain 5: Reporting bias*				
Reference	***1.1***	***1.2***	***1.3***	***Risk***	***2.1***	***2.2***	***2.3***	***2.4***	***2.5***	***2.6***	***2.7***	***Risk***	***3.1***	***3.2***	***3.3***	***3.4***	***Risk***	***4.1***	***4.2***	***4.3***	***4.4***	***4.5***	***Risk***	***5.1***	***5.2***	***5.3***	***Risk***	***Notes***
Young et al., 2017	Y	Y	N	Low	N	N	Na	Na	Na	NI	Na	Low	Y	Na	Na	Na	Low	N	N	N	Na	Na	Low	NI	N	N	Low	
Young et al., 2018	Y	Y	N	Low	N	N	Na	Na	Na	NI	Na	Low	Y	Na	Na	Na	Low	N	N	N	Na	Na	Low	NI	N	N	Low	
Zahn et al., 2019	Y	Y	N	Low	N	N	Na	Na	Na	NI	Na	Low	Y	Na	Na	Na	Low	N	N	N	Na	Na	Low	Y	N	N	Low	
Jaeckle et al., 2021	Y	Y	N	Low	N	Y	NI	NI	NI	Y	Na	Some	PN	Y	Na	Na	Some	N	N	Y	PY	NI	Some	Y	N	N	Low	Single-blinded study; data not obtained from all randomised participants; Assessment of outcome could have been influenced by knowledge about intervention assigned Constrained longitudinal analysis model
Zotev et al., 2020	Y	Y	N	Low	N	Y	NI	NI	NI	NI	Na	Some	Y	Na	Na	Na	Low	N	N	NI	N	N	Low	NI	N	N	Low	
Yuan et al., 2014	NI	Y	N	Some	N	N	Na	Na	Na	NI	Na	Low	Y	Na	Na	Na	Low	N	N	N	Na	Na	Low	NI	N	N	Low	No evidence of randomisation
Keller et al., 2021	Y	Y	N	Low	N	N	Na	Na	Na	NI	Na	Low	PY	Na	Na	Na	Low	N	N	N	Na	Na	Low	NI	PY	N	Some	Analyzed data from selected sessions only
Hamilton et al., 2016	N	N	Na	Some	PN	PN	Na	Na	Na	NI	Na	Low	PY	Na	Na	Na	Low	N	N	N	Na	Na	Low	NI	N	N	Low	No evidence of randomisation;
Young et al., 2017	Y	Y	N	Low	N	N	N	Na	Na	Y	N	Low	Y	Na	Na	Na	Low	N	N	N	Na	Na	Low	Y	N	N	Low	
Bezmaternykh et al., 2021	NI	NI	N	Some	NI	NI	NI	NI	NI	N	NI	Some	NI	NI	Na	Na	Some	N	N	N	Na	Na	Low	Y	N	N	Low	No evidence of randomisation; No evidence on blinding; No information on missing data or participants excluded for analyses
Young et al., 2014	PN	PN	PY	Some	Y	Y	PN	Na	Na	N	NI	Some	N	N	PY	NI	Some	N	N	N	Na	Na	Low	NI	N	NI	Low	No evidence of randomisation; No evidence of blinding; There was a difference between groups in the proportion of comorbid diagnoses and a disproportion in sample size between the groups; 2 participants were excluded from analyses, with no information on the reason why.
Mehler et al., 2018	Y	Y	N	Low	N	N	N	Na	Na	Y	Na	Low	Y	Na	Na	Na	Low	N	N	N	Na	Na	Low	Y	N	N	Low	

Zotev et al., 2016	PN	PN	PY	Some	Y	Y	PN	Na	Na	N	NI	Some	N	N	PY	NI	Some	N	N	N	Na	Na	Low	NI	N	NI	Low	No evidence of randomisation; There was a difference between groups in the proportion of comorbid diagnoses and a disproportion in sample size between the groups; No evidence of blinding
Melnikov et al., 2022	N	N	PY	Some	Y	Y	NI	Na	Na	N	NI	Low	N	N	PN	NI	Some	PN	Na	Y	N	Na	Some	NI	N	NI	Low	No randomised assignment; significant differences in IQ between the groups; No analyses of effect of missing data; The small sample size did not allow for thresholding at a significance level of $p < 0.05$ FWE-corrected.
Linden et al., 2012	N	N	PN	Some	Y	Y	N	Na	Na	Y	PN	Low	Y	Na	Na	Na	Low	N	N	Y	N	Na	Low	NI	N	NI	Low	No evidence of randomisation.
Taylor et al., 2022	PN	PN	PN	Some	Y	Y	PN	Na	Na	N	Na	Low	N	N	PN	NI	Some	N	N	Y	N	Na	Low	NI	N	NI	Low	No evidence of randomisation; No analyses of effect of missing data.

Y – yes, N – no, na – not applicable, PY – potentially yes, PN – potentially no, NI – not identified

Supplementary references

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Appendix B

Rostral Anterior Cingulate Cortex Oscillatory Power Indexes Treatment-Resistance to Multiple Therapies in Major Depressive Disorder

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Rostral Anterior Cingulate Cortex Oscillatory Power Indexes Treatment-Resistance to Multiple Therapies in Major Depressive Disorder

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Keywords
Treatment-resistant depression · Rostral anterior cingulate cortex · Electroencephalograpy · Theta · Biomarker

Abstract

Introduction: High rostral anterior cingulate cortex (rACC) activity is proposed as a nonspecific prognostic marker for treatment response in major depressive disorder, independent of treatment modality. However, other studies report a negative association between baseline high rACC activation and treatment response. Interestingly, these contradictory findings were also found when focusing on oscillatory markers, specifically rACC-theta power. An explanation could be that rACC-theta activity dynamically changes according to number of previous treatment attempts and thus is mediated by level of treatment-resistance. ***Methods:*** Primarily, we analyzed differences in rACC- and frontal-theta activity in large national cross-sectional samples representing various levels of treatment-resistance and resistance to multimodal treatments in depressed patients (psychotherapy [$n = 175$], antidepressant medication [AD; $n = 106$], repetitive transcranial magnetic stimulation [rTMS; $n = 196$], and electroconvulsive therapy [ECT; $n = 41$]), and the respective difference between remitters and non-remitters. For exploratory purposes, we also investigated other frequency bands (delta, alpha, beta, gamma). ***Results:*** rACC-theta activity was higher ($p < 0.001$) in the more resistant rTMS and ECT patients relative to the less resistant psychotherapy and AD patients (psychotherapy-rTMS: $d = 0.315$; AD-rTMS: $d = 0.320$; psychotherapy-ECT: $d = 1.031$; AD-ECT: $d = 1.034$), with no difference between psychotherapy and AD patients. This association was even more pronounced after controlling for frontal-theta. Post hoc analyses also yielded effects for delta, beta, and gamma bands. ***Conclusion:*** Our findings suggest that by factoring in degree of treatment-resistance during interpretation of the rACC-theta biomarker, its usefulness in treatment selection and prognosis could potentially be improved substantially in future real-world practice. Future research should however also investigate specificity of the theta band.

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Introduction

Major depressive disorder (MDD) is the leading cause of disability in the world, with symptoms ranging from mild anhedonia to social and occupational malfunctioning and death by suicide [1]. It is believed to be characterized by a disruption in the frontal-limbic circuitry [2], in which there is a deficit in switching between the default mode network and the central executive network [3]. Various therapies aim to modify the pathological network activity in depression using different modi operandi [4] and are usually delivered in a stepped-care model. This means that patients start with less invasive treatments (e.g., psychotherapy and/or antidepressant medication [AD]) and progress to more intensive treatments accompanied with higher costs and side effects (e.g., intranasal or intravenous ketamine, repetitive transcranial magnetic stimulation [rTMS], electroconvulsive therapy [ECT]) once prior treatments have failed [5]. Remission rates for treatments in the early stages are around 35% [6–10] and decrease to around 13–14% after 2–3 failed treatments [11], after which a patient's depression is categorized as treatment-resistant depression (TRD), also named "difficult-to-treat depression" [11]. To help treat MDD more efficiently, current research aims to identify biomarkers (i.e., prognostic metrics based on the brain network activity at baseline) that can identify likely TRD cases at an early stage, and predict which treatments are likely to be more effective in such cases.

One brain region of interest for biomarker discovery in depression is the rostral anterior cingulate cortex (rACC). High rACC activity has been proposed to be a nonspecific prognostic marker for treatment response [2, 12]. Studies using EEG, fMRI, or PET have found that patients with high rACC activity are more likely to achieve a positive treatment outcome, independent of modality (e.g., AD, sleep deprivation, rTMS) [2]. However, other studies using PET/SPECT have reported the opposite finding of a negative association between baseline rACC activation and treatment response [2]. These contrary findings were reported for treatments with paroxetine, venlafaxine, cognitive behavioral therapy (CBT), ECT, and rTMS [2, 13–16].

A similar apparent contradiction is seen in this region with theta power on EEG. A recently published follow-up to the large EMBARC biomarker study reported that patients with high baseline rACC-theta activity were more likely to improve with treatment in general, whether on sertraline or on placebo [12]. However, in contrast, the iSPOT-D biomarker study reported the opposite finding that patients with high baseline rACC-theta showed poorer response in general, to escitalopram, sertraline, or venlafaxine [17].

One proposal to reconcile these findings is that the predictive value of rACC-theta may depend on the degree of treatment-resistance. For example, Hunter et al. [18] found that antidepressant-naïve patients with high rACC-theta had greatest treatment improvement and that for antidepressant-experienced patients it was those with low rACC-theta that showed greatest treatment improvement. In keeping with this pattern, EMBARC patients (who were required to be fairly treatment-naïve, with no failed medication trials in the current episode) with high rACC-theta showed the greatest treatment improvement [12], while iSPOT-D patients with more treatment failures showed the strongest relationship between high rACC-theta and poor treatment response [17].

Following these studies, we may question whether high rACC-theta should be considered a general (treatment nonspecific) marker of depressive symptom improvement (prognostic marker) [2], whether rACC-theta is a biological marker correlated to treatment-resistance (trait), or whether rACC-theta dynamically changes according to the number of previous treatment attempts and thus is mediated by the level of treatment-resistance [17, 18]. The aim of the present study was therefore to clarify this issue, by first determining whether the degree of treatment-resistance correlates with baseline rACC-theta activity, and second, whether rACC-theta activity can be used to predict treatment outcome more effectively when the degree of treatment-resistance is integrated in prediction analyses. We hypothesized that baseline rACC-theta activity would change with increasing levels of treatment-resistance and that patients with high treatment-resistance and co-occurring high baseline rACC-theta activity would show poorer treatment outcomes.

We set out to test this hypothesis in a new, large, naturalistic dataset comprising of four Dutch national open-label datasets, in which patients were allocated to treatment in a stepped-care model according to structured clinical guidelines [19–21]. The use of these datasets provided some assurance that patients were truly referred to treatments based on their level of depression severity and resistance level. The datasets covered a variety of treatment modalities: psychotherapy and AD for patients with low treatment-resistance, rTMS for patients with medium treatment-resistance, and ECT for patients with high treatment-resistance [22–24]. This study focused on rACC-theta and frontal-theta activity as this study was an a priori planned extension of the earlier 2015 iSPOT-D study [17]. Pretreatment EEG recordings and standardized clinical questionnaires obtained before and after treatment enabled assessment of rACC-theta at different

levels of treatment-resistance, as well as assessment of whether rACC-theta was associated with remission for any treatment modality.

Materials and Methods

Participants

Five hundred eighteen EEG recordings of participants from different clinics were collected for this study and were categorized into four datasets. These datasets were national open-label datasets, collected under naturalistic conditions, in which patients were allocated to treatment in a stepped-care-model according to structured clinical guidelines [19–21]. We also used the healthy control group from the iSPOT-D study [17] (n = 336) to visualize how rACC-theta activity compared to the following datasets in the remission section. All participants provided written informed consent.

Dataset 1: Psychotherapy

The psychotherapy dataset consisted of patients diagnosed with nonpsychotic MDD or dysthymia and a Beck Depression Inventory second edition (BDI-II) [25] score ≥14 at baseline, who received any form of psychotherapy as monotherapy (n = 175). Patients could further be divided in having received CBT (n = 94) or other types of psychotherapy (other; n = 81). BDI-II was recorded before and after treatment. Details about this sample are described elsewhere [26].

Dataset 2: Antidepressants

The AD dataset consisted of patients diagnosed with nonpsychotic MDD or dysthymia and a BDI-II score ≥14 at baseline, who received AD, either as monotherapy or in combination with psychotherapy (n = 106). This sample was taken from van der Vinne et al. [27]. BDI-II was recorded before and again after 8 weeks of medication (monotherapy) or at the end of psychotherapy if this preceded the 8 weeks of medication (combination).

Dataset 3: rTMS

The rTMS dataset consisted of 196 patients, diagnosed with nonpsychotic MDD or dysthymia and BDI-II ≥14 at baseline, who underwent protocolized rTMS treatment concurrent with psychotherapy. Patients received high-frequency TMS (10 Hz left dorsolateral prefrontal cortex, DLPFC), low-frequency TMS (1 Hz right DLPFC), or both 1 Hz and 10 Hz sequentially. All patients completed at least 10 sessions of treatment and filled in the BDI-II at baseline and at the last session (on average session 21). Details about this sample are described elsewhere [20, 26, 28], and data are part of the open access dataset TDBRAIN (https://brainclinics.com/resources/) [28].

Dataset 4: ECT

The fourth dataset consisted of 41 patients, treated with complete ECT-courses at the Rijnstate Hospital. Most patients suffered from TRD. Depression severity was scored with the Hamilton Rating Scale for Depression ($HRSD_{17}$), within 1 week before start of ECT and within 1 week after the course. ECT was administered according to Dutch standards, and right unilateral and bifrontotemporal electrode placements were applied according to the psychiatrists' discretion. The ECT course was terminated after reaching remission ($HRSD_{17}$ score ≤7), or when patients did not improve any further after 1 week, or if ten bilateral ECT-sessions did not show any change in depression.

EEG Data Collection and Preprocessing

EEG data were collected using a standardized methodology and platform (Brain Resource Ltd., Australia). The EEG platform and methodology used in this study were identical to the iSPOT-D study [17], and details and validations have been published elsewhere [29–31].

In summary, patients were seated in a sound and light attenuated room within a clinical setting. As a naturalistic dataset, recordings occurred either in the morning or in the afternoon based on patient and room availability. EEG data were acquired from 26 channels: Fp1, Fp2, F7, F3, Fz, F4, F8, FC3, FCz, FC4, T3, C3, Cz, C4, T4, CP3, CPz, CP4, T5, P3, Pz, P4, T6, O1, Oz, and O2 (ANT Waveguard-cap; NuAmps; 10–20 electrode international system). EEG was collected for 2 min with eyes open, with the patient asked to fixate on a red dot on the screen, and 2 min with eyes closed. The patient was instructed to remain relaxed for the duration of the recording. No intervention took place when drowsiness patterns were observed in the EEG. Data were referenced to averaged mastoids with a ground at AFz. Horizontal eye movements were recorded with electrodes placed 1.5 cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3 mm above the middle of the left eyebrow and 1.5 cm below the middle of the left bottom eyelid. Impedances were kept below 10 kΩ and the sampling rate was 500 Hz. A low pass filter with an attenuation of 40 dB per decade above 100 Hz was employed prior to digitization.

Data were filtered (0.3–100 Hz and notch of 50 Hz), EOG-corrected using a regression-based technique similar to that used by Gratton et al. [32], and automatic artifact-detection and -removal were performed. Artifact signals included EMG, sharp channel-jumps (up and down), kurtosis, extreme voltage swing, residual eyeblinks, electrode bridging, and extreme correlations. Automatic artifact rejection was performed using a custom-built Python package [28]. This package is based on the automated method employed in the iSPOT-D study [17], as it showed an agreement of 98.4% with "manual" artifact rejection by certified EEG expert [29] and was further validated by van Dijk et al. [28] [full python code available online (http://www.brainclinics.com/resources)].

Analysis

EEG eLORETA Analyses

EEG analysis was performed identical to the prior report by Arns and colleagues [17], but in short: based on the scalp-recorded electric potential distribution, the exact low-resolution brain electromagnetic tomography (eLORETA) software (http://www.uzh.ch/keyinst/loreta.htm) was used to compute the cortical three-dimensional (3D) distribution of current density (i.e., the amount of electrical current flowing through a solid; unit: amperes per square meter, $Å/m^2$).

Previous studies have demonstrated that eLORETA can outperform other linear inverse solutions (e.g., minimum norm estimate, dynamic statistical parametric mapping, weighted minimum norm, local autoregressive average, dynamic imaging of coherent sources, and linearly constrained minimum variance) under ideal, noise-free conditions [33–35]. It has also been reported that this linear inverse solution for the EEG has the unique

property of having exact (zero-error) localization for point test sources anywhere in the brain, albeit with low spatial resolution [33, 36–38].

Previous studies cross-validated the LORETA algorithm to ensure eLORETAs confidence estimates in the respective inverse solutions, using not only EEG but also independent techniques of higher anatomical precision such as fMRI and PET. These studies showed that the anatomical localization provided by eLORETAs intracortical EEG-source estimates showed good concordance with independent measures of neural activation obtained via BOLD signals on fMRI, and via glucose metabolism on PET imaging [39, 40]. eLORETA is an improvement over the original LORETA version [41] and the standardized version sLORETA [42]. The eLORETA method is described in detail by Pascual-Marqui [36].

As eLORETA is deemed a valid way of analyzing rACC-theta activity [43], we used it to extract EEG current source density from the rACC (using the voxels reported by Pizzagalli et al. [43]) and frontal cortex during resting state conditions with eyes closed. The regions of interest (ROI) did not overlap. The employed frequency band for theta was 6.5–8 Hz (for exploratory analyses also delta [1.5–3.5 Hz], alpha [8–13 Hz], beta [14.5–30 Hz], and gamma [31–49 Hz] bands were assessed). Participants were excluded if extraction was not possible.

Statistics

SPSS version 28 was used for statistical analyses. Remission was defined as a score ≤12 on the BDI-II posttreatment and ≤7 on the $HRSD_{17}$ (see Participants).

In accordance with the iSPOT-D study [17], the primary analysis consisted of assessing whether there was a significant difference in the ROI (rACC and frontal) in the theta frequency band between treatments (psychotherapy, AD, rTMS, ECT) at baseline. Normal distribution of EEG measures was inspected, and theta measures were log transformed before statistical analysis. Differences in age, sex, and baseline depressive severity were tested using One-Way ANOVA or nonparametric tests (sex). In case of group differences in one of these measures, these variables were added as a covariate. To determine whether there is a difference in activity between rACC- and frontal-theta and the level of TRD, a repeated measures ANOVA was conducted with dependent variable rACC-theta and frontal-theta during EC; fixed factors treatment and sex; and covariate age. When significant interactions were found, we followed up with univariate ANOVA-analyses.

For exploratory purposes, we repeated the analyses performed for theta band on the delta, alpha, beta, and gamma bands, and reported these in online supplementary Materials (for all online suppl. material, see https://doi.org/10.1159/000533853). As we are investigating an a priori defined analysis based on the 2015 iSPOT-D study [17], these exploratory analyses should be considered as post hoc and secondary to the main analysis, and therefore more strict corrections were used for multiple testing ($p = 0.05/61$).

The secondary analysis consisted of comparing rACC-theta activity between remitters and non-remitters. A univariate ANOVA was conducted with dependent variable rACC-theta during EC; fixed factors treatment (CBT, other, AD, rTMS, ECT), sex, and remission; and covariates age and baseline severity. When significant interactions were found, we followed up with univariate ANOVA-analyses. To test for protocol specific effects, we also performed a univariate ANOVA analysis comparing rACC-theta activity between remitters versus non-remitters across the 1 Hz versus 10 Hz protocol groups, with sex, age, and baseline BDI as covariates of noninterest (demographic features are presented in online suppl. Materials).

For main effects, significance level was set at $\alpha = 0.05$, and for post hoc comparisons a Bonferroni-corrected p value was used (determined by the number of comparisons). Effect sizes of main effects are reported in Cohen's d.

Results

The final demographic features and depression severity of participants included in the analyses following exclusion criteria (e.g., no EEG data) are presented in Table 1.

Primary Analyses: ROI Activity and TRD Levels

No significant correlation was found between baseline BDI-II and rACC-theta and frontal-theta activity when grouping the psychotherapy, AD and rTMS datasets ($p = 0.826$ and $p = 0.929$), nor between baseline $HRSD_{17}$ and rACC-theta and frontal-theta activity for the ECT dataset ($p = 0.231$ and $p = 0.160$). As one dataset used $HRSD_{17}$ and the other three datasets used BDI-II, we removed baseline severity as covariate from further analyses in this section.

Repeated measures ANOVA, using age as a covariate, yielded for frontal and rACC-theta a significant effect of treatment ($F(3, 449) = 7.607$; $p < 0.001$), ROI ($F(1, 449) = 53.809$; $p < 0.001$), age ($F(1, 449) = 17.437$; $p < 0.001$), an interaction effect of ROI X treatment ($F(3, 449) = 4.949$; $p = 0.002$), and of ROI X sex ($F(1, 449) = 6.298$; $p = 0.012$). Post hoc analyses revealed a significant difference between the two ROIs across treatments ($p < 0.001$).

Following up on the interaction ROI X treatment, a univariate ANOVA, using age as covariate, was conducted for rACC-theta and yielded a significant main effect of treatment ($F(3, 449) = 10.186$; $p < 0.001$) and of age ($F(1, 449) = 16.382$; $p < 0.001$). Pairwise comparisons revealed a significant difference in rACC-theta activity between all types of treatments [ECT patients had higher rACC-theta activity than psychotherapy, AD, and rTMS patients (psychotherapy-ECT: $p < 0.001$, $d = 1.031$; AD-ECT: $p < 0.001$, $d = 1.034$; rTMS-ECT: $p = 0.005$, $d = 0.480$); rTMS patients had higher rACC-theta activity than psychotherapy and AD patients (psychotherapy-rTMS: $p < 0.001$, $d = 0.315$; AD-rTMS: $p = 0.004$, $d = 0.320$)], except between psychotherapy and AD ($p = 726$, $d = 0.008$; shown in Fig. 1a).

Table 1. Key demographic features and depression severity of the patient sample

	Datasets				
	psychotherapy		AD	rTMS	ECT
	CBT	other			
Full datasets					
Sample size, *n*	175 94	 81	106	196	41
Included in analysis					
Sample size, *n*	140 74	 66	97	193	28
Males, *n* (%)	50 (36) 32 (43)	 18 (27)	41 (42)	95 (49)	11 (39)
Age, mean (SD), years	37 (14.1) 35 (14.1)	 40 (14.1)	40 (14.2)	43 (13.4)	50 (14.2)
Baseline BDI-II/$HRSD_{17}$	31.6 30.2	 33.0	32.5	31.2	20.9
Posttreatment BDI-II/$HRSD_{17}$	20.1 20.1	 20.1	22.9	14.1	13.28

Sample size included in analysis reflects the number of people with complete baseline data who finished treatment and with successful eLORETA extraction. Dataset psychotherapy was divided into participants having received cognitive behavioral therapy (CBT) and other types of psychotherapy (other). AD, antidepressant medication; rTMS, repetitive transcranial magnetic stimulation; ECT, electroconvulsive therapy; CBT, cognitive behavioral therapy; SD, standard deviation; BDI-II, Beck Depression Inventory; $HRSD_{17}$, Hamilton Rating Scale for Depression.

Furthermore, a univariate ANOVA for frontal-theta, using age as covariate, yielded a significant main effect of treatment ($F(3, 449) = 5.071$; $p = 0.002$), and of age ($F(1, 449) = 15.783$; $p < 0.001$). Pairwise comparisons revealed only a significant difference in frontal-theta activity between ECT and the other types of treatments [ECT patients had higher rACC-theta activity than psychotherapy, AD, and rTMS patients (psychotherapy-ECT: $p < 0.001$, $d = 1.769$; AD-ECT: $p < 0.001$, $d = 0.913$; rTMS-ECT: $p = 0.003$, $d = 546$; shown in Fig. 1b)].

Due to the ROI X treatment interaction found in the repeated measures ANOVA, a mediation analysis was performed to rule out that rACC-theta effects were mediated by frontal-theta [44]. Univariate ANOVA, using age and frontal-theta as covariate, yielded for rACC-theta a significant main effect of treatment ($F(3, 448) = 8.901$; $p < 0.001$), frontal-theta ($F(1, 448) = 1{,}095.624$; $p < 0.001$), and of sex ($F(1, 448) = 5.128$; $p = 0.024$). Pairwise comparisons revealed rACC-theta activity to be significantly higher in rTMS and ECT patients compared to psychotherapy patients (psychotherapy – rTMS: $p < 0.001$, $d = 0.541$; psychotherapy – ECT: $p = 0.003$, $d = 0.628$) and in rTMS patients compared to AD patients ($p = 0.003$, $d = 0.374$; shown in Fig. 1c).

Finally, univariate ANOVA, using age and rACC-theta as covariate, yielded for frontal-theta a significant main effect of treatment ($F(3, 448) = 3.837$; $p = 0.010$), rACC-theta ($F(1, 448) = 1{,}095.624$; $p < 0.001$), and of sex ($F(1, 448) = 6.179$; $p = 0.013$). Pairwise comparisons only revealed frontal-theta activity to be significantly lower in rTMS patients compared to psychotherapy patients ($p < 0.001$, $d = 0.380$; shown in Fig. 1d).

For the other frequency bands, an exploratory analysis found that a significant interaction between ROIs and treatment groups was evident for delta (rACC), beta and gamma (rACC and frontal) bands, but not for the alpha band. The results remained significant when mediated by the respective frequency band in the frontal region (online suppl. Materials).

Secondary Analyses: rACC-Theta and Remission

When investigating whether rACC-theta activity was associated with remission, we divided the data further into five treatments: CBT, other psychotherapy forms (Other), AD, rTMS, and ECT. A one-way ANOVA with remission as factor determined that baseline severity was significant for each treatment, except for ECT (CBT $p = 0.006$; other $p = 0.006$; AD $p < 0.001$; rTMS $p < 0.001$;

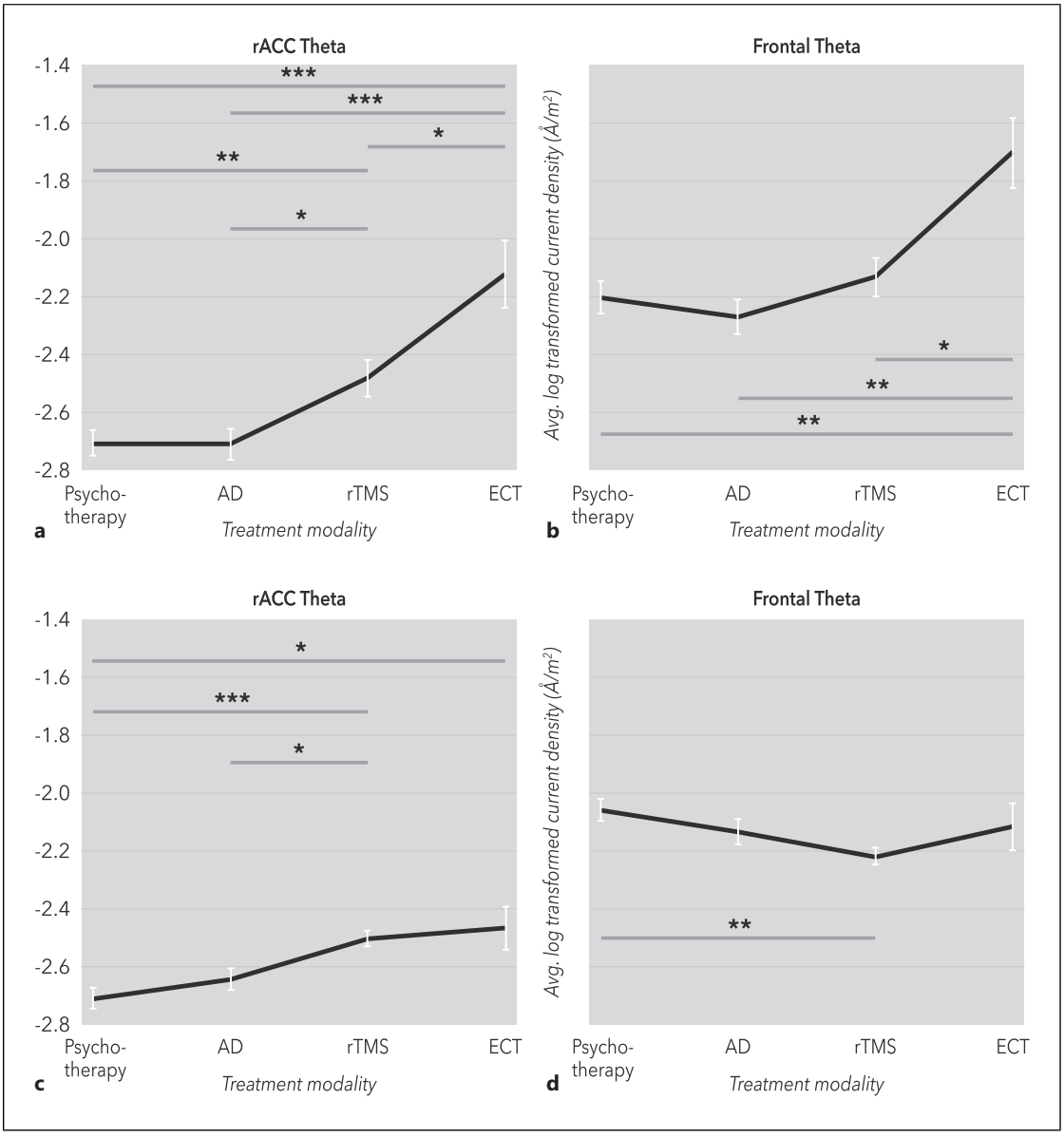

Fig. 1. rACC-theta and frontal-theta power levels across treatment modalities. **a** Illustrates rACC-theta activity being higher as treatment-resistance level increases, with no significant difference found between psychotherapy and AD. **b** Illustrates frontal-theta activity being higher in ECT compared to the other treatments. **c** Illustrates rACC-theta activity covaried by frontal-theta activity, and shows higher rACC-theta activity in rTMS compared to psychotherapy and AD, and in ECT compared to psychotherapy. **d** Illustrates frontal-theta activity covaried by rACC-theta activity, and shows lower frontal-theta activity in rTMS compared to psychotherapy. Significance level was Bonferroni-corrected. Error bars represent standard error of the mean. *$p < 0.008$, **$p < 0.0017$, ***$p < 0.00017$.

ECT $p = 0.568$). We therefore took baseline severity into account for the following analyses of this section. We accounted for the difference between questionnaires (BDI-II and $HRSD_{17}$) by performing a Z-score transform.

Univariate ANOVA, using age and baseline severity as covariates, yielded for rACC-theta in relation to remission a significant main effect of treatment ($F(4, 432) = 5.793$; $p < 0.001$), age ($F(1, 432) = 17.456$; $p < 0.001$), and an interaction effect of treatment X remission ($F(4, 432) = 4.591$; $p = 0.001$). Repeating the analysis for every treatment independently only revealed a significant difference in rACC-theta activity

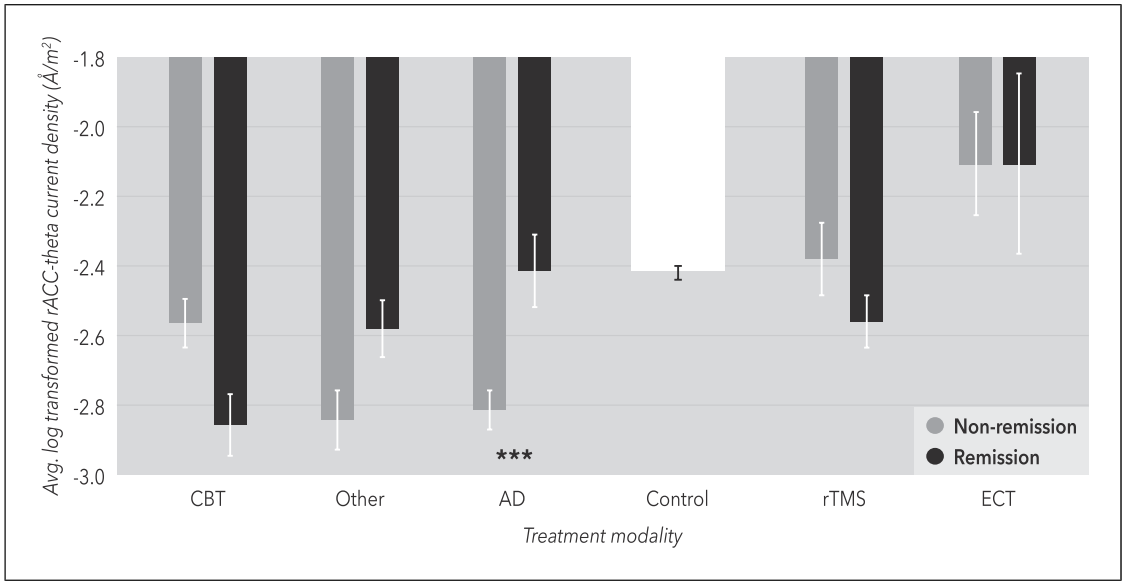

Fig. 2. rACC-theta and frontal-theta power levels across treatment modalities, and healthy controls (n = 336, from the iSPOT-D study [17]), in relation to remission. Graph shows higher rACC-theta activity in rTMS and ECT compared to psychotherapy and AD, with healthy controls in between these two levels of treatment-resistance. A significant difference in rACC-theta activity between remitters and non-remitters was only found in the AD dataset, with rACC-theta activity being higher in remitters. Significance level was Bonferroni-corrected. Error bars represent standard error of the mean. *p < 0.01, **p < 0.002, ***p < 0.0002.

between remitters and non-remitters for AD (remitters had higher rACC-theta activity than non-remitters: p < 0.001, d = 0.781), but not for CBT, other, rTMS or for ECT (p = 0.014; p = 0.019; p = 0.089; p = 0.820; respectively, shown in Fig. 2, in which the reader can also visually compare the results to a healthy control group extracted from the iSPOT-D study [17]). The exploratory analysis, testing for protocol specific effects, revealed no significant interaction between remission and rTMS protocol groups (p = 0.900).

Discussion

Following up on the iSPOT-D study [17], we investigated whether there are differences in pretreatment frontal- and rACC-theta activity across treatments with various levels of TRD. We found that rACC-theta activity differed between the first-line care (i.e., psychotherapy and AD; low activity) and the second/third line care (i.e., rTMS and ECT; high activity), and these effects were not mediated by frontal-theta activity. Therefore, the data suggest that these results are specifically associated with TRD level and rACC-theta, which is in favor of our hypothesis that baseline rACC-theta activity changes with increasing levels of treatment-resistance.

Furthermore, we only found a significant difference in rACC-theta between remitters and non-remitters for the patients treated with AD, with higher rACC-theta found in remitters compared to non-remitters. We believe that this result shows an interesting consistency with the previously reported findings in the fairly treatment-naïve patients of the EMBARC study: those with high rACC-theta activity likewise showed greater improvement on ADs than those with lower rACC-theta activity [12]. Intriguingly, resemblance to the iSPOT-D study finding that patients with high rACC-theta activity and higher levels of treatment-resistance showed poorer response to treatment [17], can be found in the current rTMS group. Close inspection of Figure 2 reveals a similar trend in the rTMS group (who had relatively higher levels of treatment-resistance): higher rACC-theta activity in non-remitters compared to remitters. However, this finding is not significant. Summarized, the present findings offer some suggestive evidence towards a reconciliation of the EMBARC and the iSPOT-D findings regarding rACC-theta and remission.

We also found that rACC-theta for healthy controls is situated in between the first-line care (lower rACC-theta) and the second/third-line care (higher rACC-theta). Based on these large sample sizes (n = 518 MDD, n = 336 healthy controls), it is interesting to find this scattered pattern as it further demonstrates that high rACC-theta activity is not a

nonspecific prognostic biomarker for patients with depression [2], with overall more variable effects and lower effect sizes relative to the primary analysis focused on treatment-resistance level, lending more support for an association with treatment-resistance. It is recommended that studies do follow-up EEGs in depressed patients (longitudinal studies) using a within-subject design.

Our finding that rACC-theta differed across TRD levels lends support to the suggestion that rACC-theta is not simply mediated by placebo response (since lower placebo response is expected with higher TRD level). Furthermore, as the EMBARC study determined that rACC-theta activity remained stable between baseline and week 1 for patients given either sertraline or placebo, rACC-theta is most likely not mediated by acute pharmacological effects nor placebo effects [12]. We must therefore question whether the difference in rACC-theta across TRD levels is a preexisting biomarker for resistance to first-line treatments, or whether it is instead associated with prior (pharmacological) treatment exposures. The results however are in line with our earlier iSPOT-D report [17] where the MDD population as a whole had higher rACC-theta compared to the control group – a finding which we can now interpret as a consequence of enrolling an MDD population with a fairly high TRD level, relative to our psychotherapy and AD samples.

It is also possible that the baseline differences in rACC-theta between the patient groups in our study reflect a process of neuroplasticity in response to illness progression and successive treatment failures. rACC-theta has been found to change in an individual over time within a single episode of MDD, meaning rACC-theta may capture some state-related aspect of brain functioning that is associated with subsequent response to medication [17, 18]. An MRI study reported that MDD patients with larger ACC volumes had fewer previous hospitalizations than patients with smaller ACC volumes [45]. Duman et al. [46] further associated the extent of ACC volume reduction with severity of depression, duration of illness, and time length of treatment. The process of neuroplasticity in response to illness progression and successive treatment failures would be an interesting issue for further studies to pursue.

The understanding of the pathophysiology of MDD has shifted to a model based on dysfunctional connectivity between neural networks, rather than abnormalities in the activity of a single neuroanatomical location [47]. In this view, we should consider the activity of the rACC as a node integrated in more complex neural brain networks with different patterns of connectivity and predictive capacities between different levels of response or resistance to treatments. We suggest an enhancement of our current analyses by incorporating connectivity with rACC and its association with treatments with levels of TRD, and also by comparing connectivity pre- and posttreatment.

Of note, the exploratory analyses for the other frequency bands in the ROIs also revealed significant differences between treatment groups in the delta (rACC), beta, and gamma (rACC and frontal) bands. The relatively treatment-resistant ECT patients had significantly higher power in beta and gamma bands in both rACC and frontal regions compared to the other, less treatment-resistant, patient samples. For the delta band, the rTMS patients had significantly higher power in the rACC compared to the psychotherapy and AD patients. In addition, when mediated by the respective frontal band, rTMS patients had significantly higher (delta, beta, gamma) power in the rACC compared to psychotherapy patients. These results could suggest that the effects of treatment-resistance are more specific to the theta band. However, since the literature on the role of these frequency bands in treatment-resistance is currently limited, and the focus of this paper is on rACC-theta, it falls beyond the scope of the present work to examine these findings in comprehensive detail. However, based on our results, we do suggest further exploration of frequency bands other than theta, and whether they may also play an important role in treatment selection or outcome prediction in TRD, or instead, whether they reflect other potential cofounding factors such as medication effects, sleep disturbance, or other factors yet to be determined.

The use of a large naturalistic dataset in this study offers benefits in terms of generalization to real-world practice. However, it also engenders a number of limitations that should be acknowledged. Demographic differences (e.g., premorbid IQ, social and economic status, ethnicity, smoking and drinking status, the history of drug abuse, and other psychiatric and neurological diseases) may affect a person's resistance to treatment, and therefore may have influenced our results. However, such data were not systematically collected for all datasets. Furthermore, in selecting confounds for our model, we were as comprehensive as possible given the available data in this large naturalistic community sample. Future research could consider collecting a wider array of variables to address other potential confounding factors. Regarding ROI, as the present work was an a priori planned follow-up study drawing upon the findings of the earlier 2015 iSPOT-D study [17], in which the effects

of interest were seen specifically in theta activity of the rACC and frontal regions, we here exclusively focused on these regions as a measure to limit type-I error. Exploring other regions may be of interest to future studies. In this study, the datasets were collected with the intention to identify predictors of treatment response; thus, posttreatment EEG was not systematically collected. Future studies could investigate the longitudinal development of the rACC-TRD association through within-subject designs, by comparing pre- and posttreatment EEGs. Since this dataset was collected under naturalistic conditions, standardized therapy regimens were sometimes influenced by clinical judgement. Although the lack of treatment standardization conform RCTs is a limitation to be acknowledged, it also offers the advantage of representing real-world clinical practice. As such, the findings are likewise more likely to translate successfully to real-world settings. While it would have been interesting to analyze the differences between specific ADs in relation to rACC-theta and levels of treatment-resistance, the number of participants for each AD was too small to perform a meaningful statistical analysis (there were a total of 24 different AD combinations, comprising of seven major group classes, for 106 subjects), reflecting the naturalistic research setting. Future research could focus on AD-specific markers. Although it is theoretically possible that MDD patients starting with different ADs have different levels of baseline rACC-theta activity, we consider this less likely due to previously reported results from the EMBARC study showing no differences in rACC-theta between unmedicated (baseline) and medicated (week 1) patients [12].

In conclusion, our study found that rACC-theta differs across treatments for MDD, with higher rACC-theta activity found in the second/third-line care (i.e., rTMS and ECT), and lower activity in the first-line care (i.e., psychotherapy and AD). Furthermore, our study found AD remitters to have higher rACC-theta activity compared with AD non-remitters, which is consistent with the EMBARC findings [12]. Our study also found suggestive evidence that other frequency bands outside of theta could potentially be useful in treatment selection or outcome prediction – an interesting topic for future study. Overall, the findings of the present study provide a new perspective on a known biomarker, by refining upon the older theory that high rACC activity is a nonspecific prognostic biomarker for patients with depression [2]. By factoring in the degree of treatment-resistance during interpretation of the rACC-theta biomarker, its usefulness in treatment selection and prognosis could potentially be improved substantially in future real-world practice.

Statement of Ethics

All subjects provided written informed consent that their data could be used for research purposes. Samples used all underwent open labeled treatment as usual and were treated in various clinics. Ethical approval is not required for this study in accordance with local or national guidelines.

Conflict of Interest Statement

M.A. holds equity/stock in neurocare, serves as consultant to Synaeda and Sama Therapeutics, and is named inventor on several patents related to EEG, ECG, and TMS, but receives no royalties; Research Institute Brainclinics received equipment support from neuroConn and Deymed. All the other authors have no conflict of interests to declare. A.T.S. is chief scientific advisor of PlatoScience Medical, scientific advisor of Alpha Brain Technologies, Founder and CEO of Neurowear Medical, Director of the International Clinical TMS Certification Course (www.tmscourse.eu), and he received equipment support from MagVenture, MagStim, and Deymed.

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Author Contributions

Amourie Prentice and Martijn Arns designed the study. Nikita van der Vinne, Sven Stuiver, and Jeroen Antonius van Waarde contributed to the acquisition of data. Amourie Prentice, Nikita van der Vinne, Hanneke van Dijk, and Martijn Arns conducted the data analysis and interpretation. Amourie Prentice wrote the first draft of the manuscript. Amourie Prentice, Ana Rita Barreiros, Nikita van der Vinne, Sven Stuiver, Hanneke van Dijk, Jeroen Antonius van Waarde, Mayuresh Korgaonkar, Alexander T. Sack, and Martijn Arns revised and approved the final version.

Data Availability Statement

Data from the TMS sample can be obtained from the TDBRAIN open access EEG database at: https://brainclinics.com/resources/ and other data are available from Martijn Arns upon reasonable request.

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Appendix C

Chapter 3 – Supplementary Material

Supplementary Methods

Imaging Data Analyses

Additionally, to the ANOVA tests of variance for three groups (TRD, TSD, HC), independent-samples Student's t-tests (for comparisons between each pair of groups) were also applied as complementary tests to further investigate the functional connectivity between the seeds BOLD timeseries and each voxel of the whole brain BOLD timeseries. A GLM approach was used, and the statistical parametric maps were thresholded at the cluster-level false discovery rate (FDR)-corrected for multiple comparisons $p < 0.05$, for voxel-wise $p < 0.001$. Mean beta resting-state FC (rsFC) values were extracted from significant clusters found in the seed-to-voxel whole brain analyses, and a one-way ANOVA was applied to investigate differences between the three groups. Significant group main effects were parsed with post-hoc paired contrasts.

Demographic and clinical data analysis

FC values were tested for correlations with clinical and demographic variables in the TRD group. The effect of history of suicidal ideation, history of suicidal attempt, and ECT treatment in explaining the variance of the neural measures were also explored, using independent samples t-tests to compare differences in the FC measures between those with and without history of suicidal ideation, suicidal attempt and ECT treatment.

Medication usage was also analysed for past and current use of mood stabilizers, anticonvulsants, antipsychotics and different classes of antidepressants (selective serotonin reuptake inhibitors - SSRI, serotonin and norepinephrine reuptake inhibitors - SNRI, other), by comparing FC measures in patients that were on these medications and patients that were not, using independent sample t-tests.

Finally, hierarchical multiple regression analyses were used to develop a model for predicting variance in the significant cluster (left habenula with the postcentral gyrus, precentral gyrus, and left and right precuneus cortex) in the two clinical groups (TRD and TSD participants pooled together), based on Age, HAMD-17 score, SOFAS score, number of previous depressive episodes, length of time on antidepressant medication, Age, and suicidal ideation. To assess the relative importance of each potential predictor variable (HAMD-17 score, SOFAS score, number of previous depressive episodes, length of time on antidepressant medication, Age, and suicidal ideation) in explaining the neural measures, each predictor was added one by one, and the subsequent increase in R-square was assessed. For each cluster, HAMD-17 was entered as step 1 (Model 1), SOFAS score was entered as step 2 (Model 2), number of previous depressive episodes was entered as step 3 (Model 3), length of time on antidepressant medication was entered as step 4 (Model 4), Age was entered as step 5 (Model 5), and suicidal ideation was entered as step 6 (Model 6). The increase in R-square resulting from the entry of a subsequent predictor variable indicates the amount of unique information in the neural measures that is accounted for by that variable, above and beyond what has already been accounted for by the other predictor variables in the equation.

Motion parameters

To determine if the MRI data from the groups differed significantly for motion-related BOLD variability, we compared the groups for maximum motion, average motion, and number of motion outliers through a one-way ANOVA. The largest and average motion observed across timepoints were registered for each subject (maximum motion and mean motion), and finally, potential outlier scans (framewise displacement above 0.9 or global signal changes above 5 s.d.) were identified and accounted for, for each participant.

Supplementary Results

Functional connectivity

Independent-sample Student's t-test revealed that the precuneus cortex (left/right) and the right precentral gyrus showed increased rsFC in the TRD patient group, when compared to the TSD patient group, whilst the right postcentral gyrus showed increased rsFC in the TRD patient group when compared to the HC group (Supplementary Table 1, Supplementary Figure 1). Higher rsFC in the right habenula with the frontal orbital cortex right in TRD patients compared to TSD patients was also found (Supplementary Table 1, Supplementary Figure 2).

The overall ANOVA model applied to the beta values extracted from the significant clusters resulting from the whole-brain analyses was significant in explaining the variance of the rsFC in the following clusters: left habenula with right postcentral gyrus [$F(2,107) = 11.646$, $p = 0.000$], and right habenula with right frontal orbital cortex [$F(2,107) = 10.157$, $p = 0.006$)]. For the left habenula with right postcentral gyrus cluster, post-hoc tests revealed significant hyperconnectivity in TRD when compared to both HC and TSD groups, but no significant differences between the HC and TSD groups (Supplementary Figure 1). For the right habenula with right frontal orbital cortex cluster, post-hoc tests showed hyperconnectivity in the TRD group in relation to both HC and TSD groups, but no significant differences between the HC and the TSD groups (Supplementary Figure 2).

Correlations between FC and demographic and clinical measures

Neither age, age at first episode, length of time on ADMs, HAMD-17 score nor SOFAS score were found to explain variance in the FC measures beyond the effect of group (Age: $F = 0.975$, $p = 0.414$; Age at first episode: $F = 1.298$, $p = 0.734$; length of time on ADMs: $F = 1.618$, $p = 0.106$; HAMD-17 score: $F = 0.118$, $p = 0.949$; SOFAS score: $F = 0.261$, $p = 0.853$).

TRD patients with a history of suicidal ideation were shown to have higher FC in the left habenula – left and right precuneus cortex – right precentral gyrus clusters, when compared to patients without history of suicidal ideation ($t = 2.407$, $p = 0.038$). There was no significant effect of history of ECT treatment, history of suicidal attempt, or current or past medications in explaining the variance of the neural measures in the TRD group. There were also no

significant correlations between the other clinical and demographic measures and FC in the TRD group. Multiple regression analyses showed no significant prediction equations for any of the clusters, indicating that none of the clinical and demographical variables significantly contributes for the prediction of differences in the variance of the neural measures between the two clinical groups.

Motion parameters

There are no significant differences between groups for maximum motion, average motion, and number of motion outliers.

Supplementary Tables

Supplementary Table 1. Summary of findings from the whole brain seed-based functional connectivity analyses through independent samples Student's t-tests comparisons between the groups.

Source seeds	Brain regions	Side	Cluster size (voxels)	Brain network*	F-value	post-hoc	Peak MNI coordinates (mm)		
							x	y	z
Habenula		L							
	Postcentral Gyrus	R	158	Somatomotor	11.646	TRD > HC, TSD	28	-32	72
Habenula		R							
	Frontal Orbital Cortex	R	99	Default Mode	10.157	TRD > HC, TSD	46	28	-8

*Brain networks were based on the Local-Global Parcellation of the Human Cerebral Cortex (Schaefer, 2017)

Supplementary Table 2. Information on current medications* from participants from the two clinical groups (TRD and TSD).

	TRD (21)	**TSD (24)**
ADM - SSRI	*5 (23.8)*	13 (54.2)
Citalopram, N (%)	n.a.	1 (4.2)
Escitalopram, N (%)	1 (4.8)	6 (25)
Zoloft, N (%)	1 (4.8)	4 (16.7)
Prozac, N (%)	3 (14.3)	n.a.
ADM - SNRI	*6 (28.6)*	7 (29.2)
Effexor, N (%)	4 (19)	1 (4.2)
Duloxetine, N (%)	n.a.	4 (16.7)
Pristiq, N (%)	1 (4.8)	2 (8.3)
ADM - MAOI	3 (14.3)	1 (4.2)
Aurorix, N (%)	2 (9.5)	1 (4.2)
Parnate, N (%)	1 (4.8)	n.a.
ADM – Tricyclic	3 (14.3)	n.a.
Imapramine, N (%)	1 (4.8)	n.a.
Amitriptyline, N (%)	2 (9.5)	n.a.
ADM - Atypical	3 (14.3)	5 (20.8)
Mirtazapine, N (%)	3 (14.3)	2 (8.3)
Agomelatine, N (%)	n.a.	3 (12.5)
Length of time on ADM (Years), Mean ± SD [Min-Max]	3.97 ± 4.03 [0.12 - 17]	4.44 ± 3.71 [0.17-12]
Antipsychotic Medication	8 (38.1)	1 (4.2)
Seroquel, N (%)	7 (33.3)	n.a.
Abilify, N (%)	n.a.	1 (4.2)
Zypreza, N (%)	2 (9.5)	n.a.
Length of time on Antipsychotic Medication (Years), Mean ± SD [Min-Max]	n.a.	1.13 (1.89)
Mood Stabilizers	6 (28.6)	2 (8.3)
Lithium, N (%)	3 (14.3)	2 (8.3)
Lamotrigine, N (%)	4 (19)	n.a.

Length of time on Mood stabilizers (Years), Mean ± SD [Min-Max]	6.5 (4.95)	1.11 (1.77)

ADM – antidepressant medication, SD – Standard Deviation

*Only considered participants who reported being currently on medications.

Supplementary Figures

Supplementary Figure 1

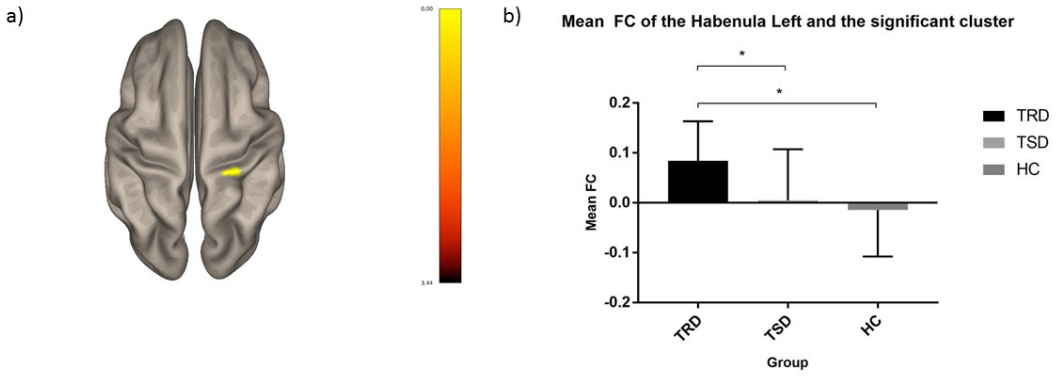


Figure 2. Illustration of the functional connectivity (FC) patterns of the left habenula in the three groups: (a) right postcentral gyrus FC with the left habenula showing differences between the TRD group and the HC group (TRD > HC) (superior view); (b) means of FC of the left habenula to the cluster described at (a) for TRD, TSD, and HC.

Supplementary Figure 2

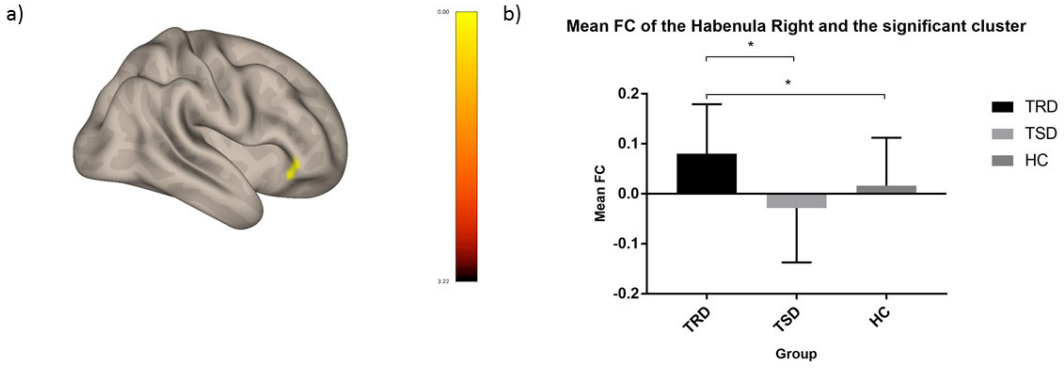


Figure 3. Illustration of the functional connectivity (FC) patterns of the right habenula in the three groups: (a) right frontal orbital cortex FC with the right habenula showed differences between the TRD group and the TSD group (TRD > TSD) (right side view); (b) means of FC of the right habenula to the cluster described at (a) for TRD, TSD, and HC.

Supplementary references

Schaefer, A., Kong, R., Gordon, E. M., Laumann, T. O., Zuo, X.-N., Holmes, A. J., et al. (2017). Local-global parcellation of the human cerebral cortex from intrinsic functional connectivity MRI. *Cerebral Cortex*, 1-20.

Appendix D

Chapter 4 – Supplementary Materials

Additional analyses 1) Head Motion Analysis

In our study, we utilized the Artifact Detection Tools (ART) within the CONN toolbox to identify motion outlier volumes in the fMRI data. The scrubbing procedure in ART detects volumes with a framewise displacement (FD) greater than 1mm. Our exclusion criterion was based on the number of outlier volumes: participants with more than 25% of their total volumes flagged as outliers were excluded from the analysis.

We performed additional analyses to evaluate the maximum motion, average motion, number of outliers, and maximum realignment for each participant. The number of outlier volumes was calculated for each participant and compared across the three groups: treatment-resistant depression (TRD), treatment-sensitive depression (TSD), and healthy controls (HC).

Supplementary Table 1. Descriptive statistics for head motion parameters and analyses of variance between the three groups.

	TRD	TSD	HC	F/t/X^2	sig
Head motion parameters					
Maximum Motion, Mean ± SD [Min-Max]	0.566 ± 0.437 [0.216-2.783]	0.684 ± 0.649 [0.217-3.363]	0.775 ± 0.899 [0.258-5.619]	n.s.	n.s.
Average Motion, Mean ± SD [Min-Max]	0.169 ± 0.075 [0.082-0.374]	0.148 ± 0.055 [0.092-0.335]	0.148 ± 0.054 [0.090-0.379]	n.s.	n.s.
Number of outliers, Mean ± SD [Min-Max]	2.028 ± 3.823 [0-16]	0.885 ± 1.622 [0-5]	2.815 ± 5.825 [0-27]	n.s.	n.s.
Maximum realignment, Mean ± SD [Min-Max]	0.838 ± 0.551 [0.233-2.541]	0.742 ± 0.419 [0.211-1.759]	0.839 ± 0.843 [0.224-5.338]	n.s.	n.s.

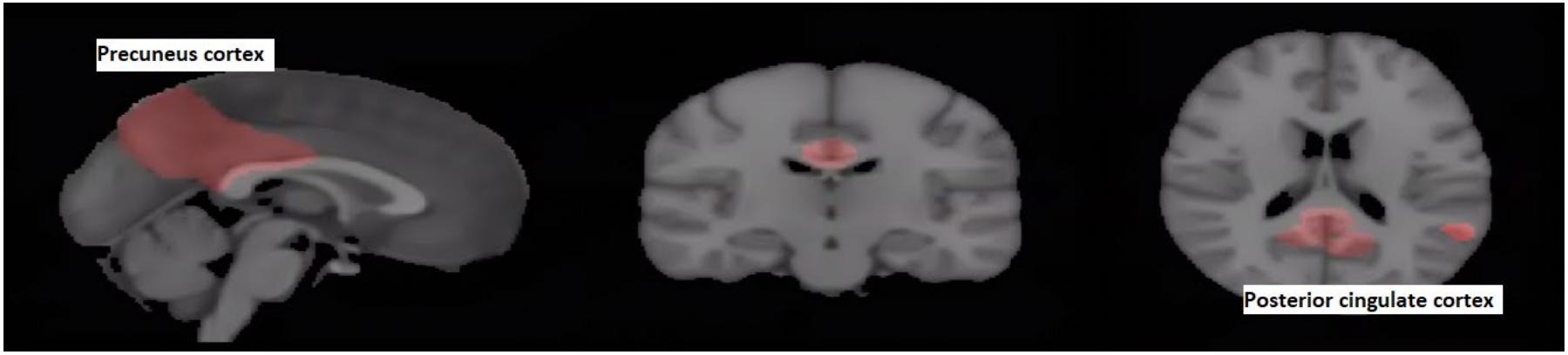
n.s. – not significant; SD – Standard Deviation;

Results:

No significant differences were found between the groups in terms of the mean percentage or number of scrubbed volumes. The head motion analysis indicated that there were no disproportionate differences in the number of scrubbed volumes between the TRD, TSD, and HC groups. These results suggest that head motion was adequately controlled and did not differentially impact the functional connectivity results reported in the main analyses.

Supplementary Figure 1.

This figure displays the spatial map corresponding to Independent Component Analysis (ICA) component #15, identified as the best-matching component to the default-mode network (DMN) in our study. The map illustrates the spatial distribution of brain regions that contribute to this DMN component.



Additional analyses 2) Given the evidence of functional connectivity changes following ECT and the fact that nearly 29% of the TRD group had previously received ECT, we conducted additional analyses excluding participants with a history of ECT treatment to account for the potential effect of prior ECT. The patterns of functional connectivity differences between the groups remained consistent with the original findings.

Supplementary Table 2. Summary of the main findings from the seed-DMN functional connectivity analyses, excluding participants who underwent ECT treatment in the past.

Source seed	DMN region	Contrast	Cluster size (voxels)*	Cluster P value**	Peak voxel P value (unc.)***	Cluster analyses			Post-hoc
						F/t	p	η2/d	
sgACC	PCC	ANOVA	81	ns	0.009	4.385	0.015	0.077	TRD, HC > TSD
rACC	PCC	ANOVA	116	ns	0.001	4.710	0.011	0.090	TRD, HC > TSD

*considered only k>10

**p value threshold < 0.05, Family-wise error (FWE) corrected

***uncorrected p values - did not survive family-wise error correction for multiple comparisons

TRD – treatment-resistant depressive patients, TSD – treatment-sensitive depressive patients, HC – healthy controls

ns – not significant

Supplementary Table 3. Summary of the main findings from Independent Component Analyses (ICA), for differences between the three groups on functional connectivity between the best matching DMN component (ICA#13) and other brain networks, excluding participants who underwent ECT treatment in the past.

Contrast	MNI coordinates (x y z)	Voxels	Region	Neural network	F value	P value	Post-hoc
TSD > TRD	-10 -92 26		Left Cuneus Cortex	Visual	7.252	0.001	TSD > TRD, HC
	10 -84 20		Right Cuneus Cortex	Visual	5.715	0.005	TSD > TRD, HC
TRD > TSD	-44 -72 38	117	Left Angular Gyrus	Default Mode Network	9.795	< 0.001	TRD > TSD, HC
TRD > HC	-	-	-	-	ns	ns	-
HC > TRD	-	-	-	-	ns	ns	-
TSD > HC	-	-	-	-	ns	ns	-
HC > TSD	-	-	-	-	ns	ns	-

Effects significant at vertex-level False Discovery Rate $p < 0.01$; cluster mass > 100.

TRD – treatment-resistant depression group, TSD – treatment-sensitive depression group, HC – healthy controls group

Additional analyses 3) To further investigate the connectivity between the dorsolateral prefrontal cortex (DLPFC) and the subgenual anterior cingulate cortex (sgACC), we conducted an additional analysis using a bilateral DLPFC mask based on the coordinates from Fox et al. (2013). The coordinates for the bilateral DLPFC were derived from a 10mm sphere centered at the following locations: [-38, 44, 26] for the left DLPFC and [40, 46, 26] for the right DLPFC. This analysis was performed in response to the suggestion that DLPFC-sgACC connectivity is a potential treatment hotspot in personalized TMS treatment (Fox et al., 2013), and to explore potential overlaps in functional signatures between different treatment modalities.

Methodology:

We used a region of interest (ROI) analysis focusing on the functional connectivity between the DLPFC and sgACC. The connectivity analysis was conducted using the same preprocessed data and statistical thresholds applied in the primary analyses. Specifically, we set an initial voxel-wise threshold of $p < 0.001$, followed by correction for multiple comparisons.

Results:

The connectivity analysis between the DLPFC and sgACC did not yield any significant clusters after correcting for multiple comparisons. At an uncorrected threshold, we observed two clusters with $k < 5$, which did not reach statistical significance.

Supplementary References:

Fox, M. D., Liu, H., & Pascual-Leone, A. (2013). Identification of reproducible individualized targets for treatment of depression with TMS based on intrinsic connectivity. *NeuroImage, 66*, 151-160. doi: 10.1016/j.neuroimage.2012.10.082

Appendix E

Chapter 5 – Supplementary Materials

Supplementary Methods

ROI to ROI analyses – Emotion Processing Network:

While the seed-to-voxel analyses were conducted to investigate whole-brain connectivity patterns emanating from the rACC, exploratory ROI-to-ROI analyses were also conducted. The aim of theses secondary analyses is to investigate how the connectivity of other regions of the affective network (Korgaonkar et al., 2019), beyond the rACC, differentiates between the groups. The emotion processing network is involved in the measurement of the emotional tone of the facial expression, and includes the amygdala, the insula, the hippocampus, and the subregions of the anterior cingulate cortex (Adolphs, 2022). These regions do not operate in isolation but are part of a dynamic network that processes emotional stimuli, integrating sensory, cognitive, and memory-related aspects to generate appropriate emotional and behavioural responses (Adolphs, 2022).

This dual approach allowed us to construct detailed connectivity maps and matrices, providing a nuanced understanding of the interactions between these critical brain regions in each subject under positive and negative emotion processing.

The regions of the affective network used in the ROI-to-ROI analyses are bilateral amygdala, bilateral insula, bilateral hippocampus, rACC and sgACC. The ROI masks for the right and left amygdala, right and left hippocampus, right and left insula regions were extracted from the Gordon atlas (Gordon et al., 2016). The selection of sgACC ROI anatomic landmark for Brodmann Area 25 (BA25) (Broadmann, 2005). The ROI for BA25 was generated from the AAL atlas (Tzourio-Mazover et al., 2002). The rACC ROI was the same as the one used for the seed-based analyses.

For the ROI-to-ROI analyses, an analyses-level corrected $p < 0.05$ threshold was considered (to control the likelihood of making any Type I errors across the entire set of pre-defined ROI

comparisons), as the connectivity is assessed between pairs of predefined ROIs rather than multiple voxels.

Whole brain seed-based functional connectivity analyses – Emotion vs Rest:

We performed exploratory functional connectivity analyses that complement the main findings described in the manuscript. These analyses were conducted to explore additional connectivity patterns associated with the rostral anterior cingulate cortex (rACC) and the affective network, contrasting specific emotional states and the brain at rest.

Investigating the contrast between specific emotional states and a baseline resting state provides critical insights into the neural dynamics underlying emotional experiences. This approach is particularly pertinent given the propensity for individuals with treatment-resistant depression (TRD) to exhibit a negative processing bias, wherein even neutral stimuli can be interpreted negatively (Gotlib and Joormann, 2010). This bias underscores the importance of contrasting emotional conditions with rest to elucidate the distinct neural activations associated with emotional processing and to differentiate them from the brain's baseline activity. Given their exploratory nature, the results should be interpreted as preliminary, guiding future hypothesis-driven research to further elucidate the role of these connectivity patterns in emotional processing and mood dysregulation.

Supplementary Results

Whole-brain seed-based functional connectivity analyses – Emotion vs Rest:

Supraliminal processing:

No significant differences between the groups were identified for the functional connectivity of the rACC during this task, neither for the processing of positive nor negative emotions.

Subliminal processing:

The whole-brain analysis indicated significant differences in the functional connectivity of the rACC with the bilateral frontal orbital cortex during the processing of positive emotion, when comparing with resting-state (see Supplementary Table 1). Post hoc tests revealed a

pattern of hyperconnectivity in the TRD group for the right frontal orbital cortex during the processing of positive emotions, and a pattern of hypoconnectivity in the TRD group for the left frontal orbital cortex during the processing of negative emotions. Reversely, a pattern of hyperconnectivity in TRD was found for the left frontal orbital cortex during the processing of negative emotions, and a pattern of hypoconnectivity in the TRD group for the right frontal orbital cortex during the processing of positive emotions.

ROI to ROI – Affective network

Supraliminal processing:

No significant differences in the functional connectivity within the affective network emerged for the processing of happy versus neutral faces, or the processing of negative emotions versus neutral faces for the supraliminal task.

Subliminal processing:

Significant differences in the functional connectivity of the right hippocampus with the sgACC were found during the processing of positive emotions (versus neutral faces), specifically between the TRD and the HC groups ($p=0.03$, FDR corrected; HC>TRD).

No significant differences were found for the functional connectivity for Negative vs neutral condition within the affective network during the subliminal task.

ROI to ROI analyses – Emotion vs Rest:

No significant differences between the groups were founds for the functional connectivity of the affective network, for any of the tasks or contrasts, when contrasted with rest.

Discussion

Additionally, our ROI-to-ROI analyses during subliminal emotion processing revealed hypoconnectivity between the sgACC and the right hippocampus in TRD, consistent with the hypoconnectivity observed between the rACC and the hippocampus during supraliminal

emotion processing. This overlap emphasizes the disrupted connectivity involving the hippocampus within the affective network in individuals with treatment-resistant depression, specific to positive emotion processing, even during subliminal level of emotion processing.

Supplementary Tables

Supplementary Table 1. Whole brain functional connectivity of the rACC compared between the three groups, for the supraliminal and subliminal processing of positive and negative emotions contrasting with rest.

Task	Contrast	Brain region (aal)	P (FDR corrected)	Cluster size (k)	Peak voxel coordinates (x y z)	Post-hoc
Faces Conscious	Positive vs Rest	-	-	-	-	-
	Negative vs Rest	-	-	-	-	-
Faces Non-Conscious	Positive vs Rest	Frontal orbital cortex L	0.009	59	-46 32 -14	HC, TSD > TRD
		Frontal orbital cortex R	0.026	41	18 12 -28	TRD > HC, TSD
	Negative vs Rest	Frontal orbital cortex R	<0.001	100	18 12 -28	HC, TSD > TRD
		Frontal orbital cortex L	0.01	63	-46 32 -14	TRD > HC, TSD

Supplementary references:

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