Universidade do Minho
Escola de Medicina

Joana Vanessa Santos dos Reis

The role of functional connectivity in the treatment of depression: exploring novel neuroimaging techniques

maio de 2023

The role of functional connectivity in the treatment of depression: exploring novel neuroimaging techniques
Joana Vanessa Santos dos Reis
UMinho | 2023

Universidade do Minho
Escola de Medicina

Joana Vanessa Santos dos Reis

The role of functional connectivity in the treatment of depression: exploring novel neuroimaging techniques

Tese de Doutoramento
Doutoramento em Ciências da Saúde

Trabalho efetuado sob a orientação do
Professor Doutor João Miguel Seiça Bessa Peixoto
e do
Professor Doutor Nuno Sérgio Mendes Dias

maio de 2023

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

Este é um trabalho académico que pode ser utilizado por terceiros desde que respeitadas as regras e boas práticas internacionalmente aceites, no que concerne aos direitos de autor e direitos conexos.
Assim, o presente trabalho pode ser utilizado nos termos previstos na licença abaixo indicada.
Caso o utilizador necessite de permissão para poder fazer um uso do trabalho em condições não previstas no licenciamento indicado, deverá contactar o autor, através do RepositóriUM da Universidade do Minho.

Licença concedida aos utilizadores deste trabalho



Atribuição-NãoComercial-SemDerivações

CC BY-NC-ND

https://creativecommons.org/licenses/by-nc-nd/4.0/

ACKNOWLEDGEMENTS

I would like to acknowledge all the people that made this work possible.

I would like to thank my supervisors, Dr. João Miguel Bessa and Dr. Nuno Dias, for the patience, guidance and support. I would like to thank the colleagues and friends that shared the journey with me and made my days a little brighter. Carlos Portugal-Nunes thanks for always being there, you are a true friend. Rita Viera thanks for the friendship and support. Ricardo Magalhães, Pedro Moreira and Paulo Marques thanks for sharing all your knowledge and for giving me a hitchhike into the neuroimaging world. Sónia Ferreira. Madalena Esteves, Edward Ganz, Nuno Costa, Maria Picó-Perez. Ana Coelho, Inês Caetano, thanks for the good vibes and friendship. Thanks to all people with whom I crossed my path during my years at ICVS. Thanks to all my colleagues from CCA. A special thanks to Mónica Gonçalves, Prof. João Cerqueira and Prof. Nuno Sousa. I would like to give a special thanks to my family (Paula Penas, José Reis, Ana Reis e João Reis). Thank you for always supporting and believing in me, even in the days when I did not believe in myself. Thanks to my grandparents, aunts, uncles, and cousins. I would like to thank my dearest friends, Adália, Minervina, Joana Chaves and Joana Costa. Last but not least, thanks to my fiancé Tiago Conceição. Thanks for being my support and motivation. Thanks for the love and understanding. Without you it would not have been possible to complete this chapter of my life.

Thanks to all of you! I am forever grateful for having you in my life.

The work presented in this thesis was performed in the Life and Health Sciences Research Institute (ICVS), Minho University and the Clinical Academic Centre (2CA), Hospital of Braga. Financial support was provided by the Foundation for Science and Technology (FCT) through the PDE/BDE/113602/2015 from the PhD-iHES program. This work was also funded by the Portuguese Foundation for Science and Technology (FCT) under scope of project PTDC/DTP-PIC/6936/2014.

This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020 and by the project NORTE-01-0145-FEDER-000039, supported by Norte Portugal Regional Operational Program (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).



STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

O papel da conectividade funcional no tratamento da depressão: explorando novas abordagens

RESUMO

A perturbação depressiva maior (PDM) é uma perturbação de humor prevalente e incapacitante. A depressão pode ter um impacto importante na capacidade que os pacientes têm em lidar com os eventos e atividades do dia-a-dia. Apesar da eficácia das opções de tratamento atuais, como antidepressivos ou psicoterapias baseadas em evidência, ainda existe uma importante fração de pacientes que experimentam longos episódios de depressão com várias tentativas terapêuticas antes de encontrar um tratamento eficaz. De fato, apenas cerca de 30% dos pacientes conseguem atingir a remissão dos sintomas após o primeiro tratamento.

No trabalho apresentado nesta Tese exploramos os potenciais biomarcadores da depressão bem como da resposta ao tratamento numa amostra de doentes antes do início do tratamento. No capítulo 1, exploramos as diferenças entre pacientes com PDM e controlos saudáveis, mostrando que a depressão estava associada à conectividade funcional (CF) anormal, bem como a um pior desempenho cognitivo. No capítulo 2, investigamos possíveis biomarcadores em ressonância magnética funcional (fMRI) relacionados com o tratamento com paroxetina. Os resultados obtidos revelaram efeitos da paroxetina no cérebro em várias redes de conectividade funcional intra e interconectividade. Uma única dose de paroxetina foi capaz de alterar o conectoma funcional de pacientes com depressão e além disso, após tratamento sustentado por 6-12 semanas os efeitos conseguiram distinguir os doentes que responderam ou não ao tratamento. No capítulo final desta tese explorámos um importante sintoma da depressão, a ideação suicida, explorando os seus correlatos neuronais. Estes achados sugerem que alterações na estrutura e função das redes fronto-subcorticais estão implicadas na ideação suicida. Globalmente, o trabalho desenvolvido nesta Tese permitiu alargar o nosso conhecimento sobre a fisiopatologia da depressão, bem como o seu tratamento. Esperamos que no futuro, estes novos dados possam ser usados para melhorar o tratamento da depressão com abordagens personalizadas.

Palavras-chave: Antidepressivos, biomarcadores, depressão, neuroimagem, redes de repouso

The role of functional connectivity in the treatment of depression: exploring novel neuroimaging techniques

ABSTRACT

Major depression disorder (MDD) is a prevalent and disabling mood disorder that can have a great impact in the patient's ability to function and cope with daily activities and events. Despite the effectiveness of current treatment options, such as antidepressants or evidence-based psychotherapies, there is still an important fraction of patients that experience long episodes of depression with multiple therapeutic trials before finding an effective treatment regimen. Indeed, only around 30% of patients are able to achieve remission of symptoms after first treatment.

In the work presented in this Thesis we explored potential neuroimaging markers of depression and antidepressant treatment in a sample of MDD treatment-naïve patients. In chapter 1 we explored differences between MDD patients and healthy controls showing that depression was associated with abnormal functional connectivity (FC) as well as poorer cognitive performance. In chapter 2 we investigated possible functional magnetic resonance Imaging (fMRI) biomarkers related with treatment with paroxetine. The obtained results revealed effects of paroxetine in brain crossing several functional connectivity networks intra and inter connectivity. A single dose of paroxetine was able to change the functional connectome of MDD patients and additionally, after sustained treatment for 6-12 weeks, the effects could distinguish between responders and non-responder. In the final chapter of this thesis we explored an important symptom of depression, suicidal ideation, exploring its neuronal correlates. These findings suggest that alterations in structure and function of frontal-subcortical networks are implied in suicidal ideation in MDD.

Overall the work developed in this Thesis allowed to expand our knowledge about the physiopathology of depression as well as antidepressant treatment. We hope that in the future, this new data can be used to improve the treatment for depression with personalized approaches.

Key-words: Antidepressants, biomarkers, depression, neuroimaging, resting-state fMRI

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii
RESUMO v
ABSTRACT vi
TABLE OF CONTENTS vii
LIST OF ABBREVIATIONS ix
LIST OF FIGURES xii
LIST OF TABLES xiv

INTRODUCTION **1**

Depression 2
The major biological hypothesis for depression 4
The treatment of depression 6
Predictors of treatment outcomes 7
Sociodemographic predictors 8
Symptom profile predictors 9
Neuroimaging and electrophysiological predictors 9
Electroencephalography (EEG) 10
Magnetic Resonance Imaging (MRI) 10
CHAPTERS OVERVIEW 13
REFERENCES 14

CHAPTER I **27**

The functional connectome of treatment-naïve depressed patients is associated with different psychopathological domains of Depression. 27

Abstract 29
Introduction 30
Subjects and Methods 31
Statistics 34
Results 34
Discussion 37
Conflict of interest declaration 39
Data availability statement 39
Acknowledgments 39
References 41
Supplementary material 47

CHAPTER II **50**

Treatment response to paroxetine in drug-naïve MDD patients is associated with specific functional connectivity patterns. 50

Abstract....52
Introduction....52
Subjects and Methods....54
Statistics....58
Results....59
Discussion....64
Conflict of interest declaration....67
Data availability statement....67
Acknowledgments....67
References....69
Supplementary material....76

CHAPTER III **79**

Suicidal Ideation is associated with reduced functional connectivity and white matter integrity in drug-naïve patients with major depression 79

Abstract....81
Introduction....82
Subjects and Methods....84
Statistics....88
Results....90
Discussion....94
Conflict of interest declaration....97
Data Availability Statement....97
Acknowledgments....98
References....99
Supplementary material....106

DISCUSSION **110**

DISCUSSION....111
References....116

CONCLUSION AND FUTURE PERSPECTIVES **120**

CONCLUSION AND FUTURE PERSPECTIVES....121

LIST OF ABBREVIATIONS

A

AAL - Anatomical Automatic Labeling atlas

ACC – Anterior cingulate cortex

AD - Axial Diffusivity

AD - Antidepressant

APA - American Psychiatry Association

ATR - Anterior thalamic radiation

ACTH - Adrenocorticotropic hormone

B

BD - Bipolar disorder

BOLD – Blood-oxygen-level-dependent

BSSI - Beck Scale for Suicidal Ideation

C

CBT – Cognitive-behavioral therapy

CNS – Central nervous system

COVID-19 – Coronavirus disease 19

CR - Corona radiata

CSF - Cerebrospinal fluid

CLTR - Consistent long-term retrieval

CRH - Corticotropin-releasing hormone

D

dlPFC – Dorsolateral prefrontal cortex

DMN – Default mode network

dDMN - Dorsal DMN

DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, fifth edition

DTI - Diffusion Tensor Imaging

DWI - Diffusion-weighted imaging

dHb – Deoxyhemoglobin

DSST - Digit Symbol Substitution test

E

EPI – Echo-planar imaging

ECT - Electroconvulsive therapy

EEG - Electroencephalography

ERP – Event related potentials

F

FA – Flip angle

FA - fractional anisotropy

FC – Functional connectivity

fMRI – Functional magnetic resonance imaging

FoV – Field of view

FWE-R - Family-wise error rate

FN - Frontoparietal network

H

HAM-A – Hamilton Anxiety Rating Scale

HAM-D – Hamilton Depression Rating Scale

HB – Hospital de Braga

HPA - Hypothalamic-pituitary-adrenal

I

ICA – Independent component analysis

ICD-10 – International Classification of Diseases, tenth revision

L

L – Left

LECN – Left executive control network

LPFC - Lateral prefrontal cortex

LTS - long-term storage

M

MDD – Major depressive disorder

MELODIC – Multivariate Exploratory Linear Optimized Decomposition into Independent Components

mPFC – Medial prefrontal cortex

MPRAGE – Magnetization-prepared rapid acquisition with gradient echo

MRI – Magnetic resonance imaging

MMSE - Mini-Mental Sate Examination

MNI - Montreal Neurological Institute

MD - Mean Diffusivity

MAOI - Monoamine Oxidase Inhibitors

N

NBS - Network-based statistics

O

OCD – Obsessive-compulsive disorder

OFC – Orbitofrontal cortex

P

PFC – Prefrontal cortex

PSS-10 – Perceived Stress Scale-10

PTSD – Post-traumatic stress disorder

PICA - Probabilistic independent component analysis

PET - positron emission tomography

PALM - permutation analysis of linear models

R

R – Right

RECN – Right executive control network

rs-FC – Resting-state functional connectivity

rs-fMRI – Resting-state functional magnetic resonance imaging

RSN – Resting-state network

RD - Radial diffusivity

S

SSRI – Selective serotonin reuptake inhibitor

SCID - Structured clinical interview for DSM disorders

SNRI - Norepinephrine Reuptake Inhibitors

SRT - Selective Reminding test

T

TCA – Tricyclic antidepressant

TE – Echo-time

TFCE – Threshold-free cluster enhancement

TR – Repetition-time

TBSS - Tract-based Spatial Statistics

TMS - transcranial magnetic stimulation

TDCS - transcranial direct current stimulation

TRD - Treatment Resistant depression

v

vDMN - Ventral DMN

w

WHO – World Health Organization

WM – White matter

LIST OF FIGURES

INTRODUCTION

Figure 1. Currently available treatments for MDD.

CHAPTER I

Figure 1. Differences between MDD patients and matching healthy controls.

Figure 2. Neuroimaging differences at baseline between drug-naïve depressive subjects and healthy controls.

Supplementary Figure 1. Detailed information on subject recruitment and exclusion.

Supplementary Figure 2. Resting state networks identified through MELODIC.

CHAPTER II

Figure 1. Graphical representation of the implemented study design.

Figure 2. Resting state networks identified through MELODIC.

Figure 3. Differences between responders and non-responders at baseline.

Figure 4. Intra-Network connectivity.

Figure 5. Inter-Network connectivity.

Supplementary Figure 1. Detailed information on subject recruitment and exclusion.

CHAPTER III

Figure 1. Resting State Networks (RSNs) using ICA.

Figure 2. Network Based Statistics (NBS).

Figure 3. Tract-Based Spatial Statistics (TBSS).

Supplementary Figure 1. Detailed information on subject recruitment and exclusion.

Supplementary Figure 2. Resting State Networks.

LIST OF TABLES

CHAPTER I

Table 1. Pearson correlation coefficients and p-values

Supplementary Table 1: Descriptive statistics of the demographic, neuropsychological and cognitive data.

Supplementary Table 2: Significant clusters obtained between drug-naïve MDD patients and healthy controls in intra-network connectivity.

CHAPTER II

Supplementary Table 1. Descriptive statistics between non-responders and responders after 6-12 weeks of sustained treatment

Supplementary Table 2. Significant neuroimaging results obtained for the acute and long-term comparison

CHAPTER III

Table 1. Demographic and psychological characterization of the sample.

Supplementary Table 1: Results of the functional connectivity analysis using NBS.

Supplementary Table 2. Nodes of the networks with connecting edges that have a significant correlation with BSI.

INTRODUCTION

La tristesse durera toujours.

[*The sadness will last forever.*]"

— **Vincent van Gogh**

"Almost everyone feels down from time to time. Having a bad grade, losing a job, having an argument or even a rainy day can bring on feelings of sadness. Sometimes there is no trigger at all. Then circumstances change and those sad feelings disappear. Clinical depression is different. It's a medical disorder and it won´t go away just because you want it to. It lingers for a least two consecutive weeks and significantly interferes with one's ability to work, play or love. Because depression symptoms are intangible, it's hard to know who might look fine, but is struggling. According to the National Institute of Mental Health, it takes the average person over ten years to ask for help. But there are very effective treatments. If you know someone struggling with depression, encourage them gently, to seek out some of these options. To someone with depression the first steps can seem insurmountable. If they feel guilty or ashamed point out that depression is a medical condition just like asthma or diabetes. It's not a weakness or a personality trait, and they should not expect themselves to just get over it any more than they could will themselves to get over a broken arm. If you haven't experienced depression yourself, avoid comparing it to times you've felt down. Comparing depression to normal, temporary feelings of sadness can makes people feel guilty for struggling. Even just talking about depression openly can help. Research shows that asking someone about suicidal thoughts reduces their suicide risk. Open conversations about mental illness help erode stigma and make it easier for people to ask for help. And the more patients seek treatment, the more scientists will know about depression and the better the treatments will get."

Adapted from @tedtoks "what is depression?" (By Helen M. Farrell M.D.)

Depression

Depression or Major depressive disorder (MDD) is a common and serious mood disorder. MDD is a demanding mental health problem and was even ranked by the World Health Organization (WHO) as the single largest contributor for mental and physical disability worldwide (World Health Organization, 2017; World Health Organization (WHO), 2021a). The estimated number of people affected by depression exceeded 300 million (equivalent to 4.4% of the world's population) in 2019 (Abbafati et al., 2020), a number that is still increasing, particularly given the challenges imposed by the COVID-19 pandemic (Bueno-Notivol et al., 2021; Luo et al., 2020). The recurrence of depressive episodes after the initial one is also a problematic topic (Solomon et al., 2000). Indeed, The American Psychiatry Association (APA) estimates that a range of 75-90% of people experience of recurrent depressive episodes over the course of their life ('Diagnostic and Statistical Manual of Mental Disorders, 5th Edition', 2013; Monroe & Harkness, 2022).

Despite its increasing prevalence, relatively little is known about the neuro-biological mechanisms that lead to depression. Still, research has identified that the risk is mostly defined by a combination of genetic, psychological, demographical (such as sex, age or ethnicity) and environmental factors (such as sex, family history, poverty, unemployment, alcohol and drug abuse, adverse live events, and the existence of other physical illnesses) the interaction between these factors is, however still largely undetermined (Heim & Binder, 2012; Sullivan et al., 2000). Often depression can lead to difficulties in personal, social, and occupational areas of functioning that can ultimately have a negative impact on the person's life, that can result in the worsening of depression symptoms.

Depression is a heterogeneous disorder often described by a multifaceted interaction between psychological, behavioral, and biological factors. It can range in terms of severity (from mild to severe) depending on the number and gravity of symptoms combined with the impact on the individual's functioning and can have a duration that goes from months to years.

Currently, the criteria for the diagnosis of MDD (according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition - DSM-5 released in 2013) requires the presence of, at least, five well-known symptoms during the same 2-week period and at least one of the symptoms should be either depressed mood (feeling sad, irritable, or empty) or loss of interest or pleasure in daily activities. Other symptoms might range from affective, cognitive and somatic such as disturbed sleep or appetite patterns, tiredness (loss of energy), psycho-motor changes, feelings of guilt, worthlessness or low self-worth, diminished ability to concentrate and recurrent thoughts of death, suicidal ideation and/or attempt (American Psychiatric Association, 2013). In fact, depression is one of the most common diagnosis at the time of death by suicide (Ferrari et al., 2013; Nock et al., 2009), and depressed patients are 20 times more likely to die by suicide than the general population (Lépine & Briley, 2011; Ösby et al., 2001).

In short, depression is a disorder that deeply affects an individual's life impairing the capacity to function and cope with daily life activities and events. Furthermore, depression can be long-lasting and recurrent plus it is reported to be the major contributor to suicide deaths. Close to 703 000 people per year die by suicide (a number that reflects 1.3% of all deaths worldwide, a number higher than the deaths due malaria, HIV/AIDS, breast cancer, or war and homicide. causes of death in 2019) (Kessler, 2012; Naghavi, 2019; World Health Organization (WHO), 2021b).

MDD patients are often prone to other psychiatric comorbidities such as post-traumatic stress disorder (PTSD), anxiety disorders, obsessive compulsive disorder (OCD), substance abuse as well as other

medical conditions that include cardiovascular disease, diabetes and stroke that, together with the over-mentioned suicide risk results in an overall reduction in life expectancy (Elderon & Whooley, 2013).

Given the high prevalence of MDD, the implementation of effective strategies for prevention could have a huge impact on public health systems worldwide. Protective strategies such as better social support, better coping skills (such as strategies for emotional regulation and problem-solving) as well as acting on early stages (on reducing symptoms even before meeting the criteria for diagnosis) could have a great impact in reducing the burden of disease. A meta-analysis on 32 randomized controlled clinical trials concluded that preventive psychological interventions reduced 21% the incidence of MDD in experimental groups when compared with control groups (van Zoonen et al., 2014).

The major biological hypothesis for depression

The biological hypothesis for depression explores the biological susceptibilities that are linked to the predisposition of the onset or recurrence of MDD. Currently, there is not one theory for depression but rather a complex representation that involves multiple biological systems and can go from neurotransmitters to hormonal levels or abnormalities in the brain's structure and functioning. The study of the biological vulnerabilities that are linked to the predisposition for depression is critical, not only for the understanding of the disorder, but also for the development of new clinical approaches that can lead to improved diagnosis and treatment tools. There have been multiple biological factors hypothesized to be involved in the onset/relapse/recurrence of MDD. The lead biological factors studied are neuroimaging, gastrointestinal factors, immunology, neurotrophic factors, neurotransmitters, hormones, and oxidative stress (Kennis et al., 2020; Strawbridge et al., 2017).

There are four major hypotheses developed over the years for depression; (1) monoamine neurotransmission impairment (monoamine hypothesis), (2) hypothalamic–pituitary–adrenal (HPA) axis impaired control (stress hypothesis), (3) neuro-inflammation processes (Immune-inflammation hypothesis) and (4) neuroplasticity/ neurogenesis impairment (neuroplasticity hypothesis) (Jentsch et al., 2015). Theses hypotheses, however presented separately, probably represent different biological systems involved in MDD through a complex set of interactions.

According to the monoamine hypothesis, MDD is related with impairments in the monoamine neurotransmitter systems such as serotonin, noradrenaline and dopamine (Belmaker & Agam, 2008; Doris et al., 1999; Nutt, 2008). This theory is supported by the diminished concentrations of monoamine

metabolites in the cerebrospinal fluid (CSF), blood and postmortem brain tissues of patients diagnosed with MDD and also reports of dietary depletion of tryptophan being linked to decrease concentrations of serotonin inducting depressive symptoms in susceptible individuals. (Cheetham et al., 1989; Pryor & Sulser, 1991; Ruhé et al., 2007). However, limitations have been found with the evidence that supported this theory and some studies have been unsuccessfully trying to find conclusive evidence of a dysfunction of a specific monoamine system in MDD patients (Delgado, 2000; Ruhé et al., 2007). Although probably playing a part in the complex interaction of factors involved in MDD, such alterations alone are insufficient to explain the full etiology of MDD. In fact, the monoamine theory fails to explain the latency that MDD patients experience when initiating a treatment with an antidepressant, typically 2-4 weeks of sustained treatment are needed before therapeutic effects on depressive symptoms (Jentsch et al., 2015).

Another well-known hypothesis for depression is the activation of the hypothalamic-pituitary-adrenal (HPA) axis or the stress hypothesis (Holsboer, 2000). This hypothesis is one of the most replicated and enduring in biological psychiatry with several studies showing that patients with MDD present elevated cortisol, corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) in body fluids such as blood, saliva, CSF and also in postmortem brain tissue (Ehlert et al., 2001; Gold & Chrousos, 2002; Stetler & Miller, 2011). The causal effect between depression and the abnormal activation of the HPA is also suggested by research that shows the ability of a successful therapeutic to regulate the HPA (Carroll et al., 2007). Although such findings are relevant, the stress hypothesis for depression is still imperfect since only a fraction of patients present HPA dysregulation to begin with.

Another major hypothesis for depression that has been explored is the immune-inflammation hypothesis. The Immune systems plays an important role in brain function by modulating processes such as neuroplasticity and neurogenesis. This modulation happens thought a complex interaction between neuronal and non-neuronal cells and its mediated by neuro-transmitters, inflammatory cytokines and growth factors (Yirmiya & Goshen, 2011). It was then hypothesized that the neuro-inflammation processes were involved in the pathogenesis of MDD. Indeed, evidence suggested that inflammatory alterations such as increased levels of proinflammatory cytokines (IL-1, IL-6, TNF-α and IFN-γ) and decreased levels of anti-inflammatory cytokines (IL-4 and IL-10) as well as the activation of immune cells are related with MDD (Kim et al., 2007; Maes, 1995; Miller et al., 2009). Although dysregulation of immune activation is present in a variety of disorders, evidence suggests that this hypothesis could be relevant of a specific symptom profile of depression and thus suggesting a possible subtype (Duivis et al., 2013). Evidence is still not conclusive as more research is needed on the causal relationship between

immune processes and MDD and the potential contributes of both environmental and genetic factors (Jentsch et al., 2015).

Lastly, another major biological hypothesis for depression is the impairment in neuroplasticity/neurogenesis processes (Brunoni et al., 2008). On the core of this hypothesis is the relationship between decreased hippocampal neurogenesis and depression. There are several findings that support this hypothesis in the literature and studies that analyzed the levels of BDNF, a neurotrophic factor responsible for inducing proliferation and differentiation of neurons and the formation of new synapses, consistently show correlations between its levels and the depressive state of MDD patients (Bocchio-Chiavetto et al., 2010; Keith et al., 2007; Sen et al., 2008). Furthermore, studies have been demonstrating that AD treatments increase neurogenesis and neurotropic factors in rats further supporting this hypothesis (Malberg et al., 2000; Mateus-Pinheiro et al., 2013). However, there is still no definitive answer on the causal relationship between neurogenesis and depression.

The treatment of depression

Depression, as other mental disorders, is diverse in presentation and prognosis. However, there are effective treatments that clinicians can offer to patients depending on the severity and duration of depressive episodes. The available treatments include pharmacological options but also evidence-based psychotherapies such as behavioral activation, cognitive-behavioral therapy, or interpersonal psychotherapy (Cipriani et al., 2018; Cuijpers et al., 2020). Other modalities of treatment might include electroconvulsive therapy (ECT) (Elias et al., 2018) and neuromodulatory approaches such as transcranial magnetic stimulation (TMS) (Allan et al., 2011) or transcranial direct current stimulation (tDCS) (Borrione et al., 2018).

Within the pharmacological options there are different classes of antidepressants (AD) that have been proven to be effective when compared with a placebo in double-blind, randomized clinical trials (Cipriani et al., 2018). The most prescribed antidepressants are the Selective Serotonin Reuptake Inhibitors (SSRIs) such as citalopram, escitalopram, fluoxetine, paroxetine, and sertraline. Serotonin is a neuromodulator that plays an important role in balancing mood (Frazer & Hensler, 1999) and SSRIs work by blocking its reabsorption into neurons and thus increasing its available levels in the brain. SSRIs are typically the recommended first line of pharmacological treatment of depression due to tolerability, generally presenting fewer side effects comparing to other classes of antidepressants available (Gelenberg et al., 2009). Other classes of antidepressants include the Serotonin-Norepinephrine

Reuptake Inhibitors (SNRIs) such as venlafaxine, duloxetine and milnacipram, the Tricyclic Antidepressants (TCAs) such as the amitriptyline, nortryptiline, imipramine and clomipramine, the Monoamine Oxidase Inhibitors (MAOIs) such as phenelzine, selegiline and rasagiline, and antidepressants from other classes such as vortioxetine, mirtazapine, bupropion, and trazodone. Finally, NMDA receptor antagonists such as ketamine and its enantiomer esketamine have revealed rapid onset antidepressant effects in recent years, paving the way for new treatment strategies in treatment-resistant depression (Canuso et al., 2018; Marcantoni et al., 2020).

The effectiveness of the current available pharmacotherapies and psychotherapies is well-established in the literature; however, one fundamental aspect remains: how to best implement them (Cipriani et al., 2018; Gillett et al., 2020a). Although the evidence is not conclusive, meta-analyses have found that the combination of psychological and pharmacological interventions may be more effective than either one alone (Cuijpers et al., 2014, 2020; Tolin, 2017). Studies have suggested that, over long-term follow-ups, psychotherapy can produce enduring effects when medication is discontinued. Nevertheless, when prescribing a treatment, clinicians should consider not only the possible side effects associated with medication but also the availability of the interventions as well as the patients' individual preferences (Cuijpers et al., 2020).

Predictors of treatment outcomes

The largest trial of depression to this date, STAR*D (Sequenced Treatment Alternatives to Relieve Depression), found that only 36.8% of MDD patients remit after the first treatment and, only 67% of patients can achieve remission after trying all possible levels of treatment, leaving the remaining patients enduring long episodes of depression (Rush et al., 2006, 2008). Even for patients that respond to treatment, the prognosis of depression stills remains disadvantageous. Indeed psychosocial, and work-related symptoms can persist after symptomatic amelioration that co-exist with significant side effects of antidepressant treatment (such as gastro-intestinal disturbance, sexual dysfunction or weigh gain).

Nowadays, in regular clinical practice, there is no comprehensive, systematic, evidence-based, personalized tool to help clinicians choose the most appropriate treatment for their patients. So, for patients initializing pharmacological treatment the possible response to the prescribed treatment is hard to predict and often relies only on clinical judgement (Leuchter et al., 2010). This approach results in patients having to endure multiple trials of medication before finding an effective regimen thus increasing

suicide risk, disability, cognitive impairments as well as the risk for developing other co-morbidities (Ferrari et al., 2013) (Katon et al., 2010).

Besides the growing body of research on biomarkers of treatment outcomes in depression, only a few made their way into the clinical practice and a lot of them remain to be replicated. Given the complex etymology of the disorder itself, it's very unlikely that a single biomarker or predictor could be sufficient to guide clinical decisions (Januar et al., 2015). Large-scale efforts have been in the last decade in order to produce biomarkers for the treatment outcomes of depression, specifically by the following trials; EMBARC (Trivedi et al., 2016), iSPOT-D (Williams et al., 2011), PReDICT (Dunlop et al., 2012) and CAN-BIND (Macqueen et al., 2019) .

A meta-review from 2019 analyzed the research on the topic of predictors associated with treatment outcomes in MDD patients and grouped them into the following categories: sociodemographic, symptom profile, genetic, neuroimaging, clinical comorbidities, and peripheral markers (such as biological compounds that can be measured in the blood, saliva, urine, or CSF) (Perlman et al., 2019). Building on that, a different meta-review from 2020 analyzed the body of research (systematic reviews) and studied the predictors of treatment response in the context of the existing treatment modalities (Gillett et al., 2020b). Next, we will briefly summarize a few of the sociodemographic, symptom profile and neuroimaging predictors reported in these studies.

Sociodemographic predictors

Regarding sociodemographic predictors of treatment outcomes, just a few have been associated with AD response, though the direction of these associations is not consistent throughout the literature (Perlman et al., 2019).

Older age is often associated with a poor response to antidepressant treatment, however TCAs were reported to produce a better response than SSRI in older individuals (De Carlo et al., 2016; Kemp et al., 2008).

Ethnicity, for instance, was found to have little direct effect on treatment outcomes, nonetheless, some research suggested that Caucasians respond better to citalopram and Asians to fluvoxamine (Duval et al., 2006). In an equivalent manner, there is no robust evidence of sex being a strong predictor of AD response, however, research has found that males responded better to TCA's and females to MAOIs and SSRIs (Berlim & Turecki, 2007).

A good social support is predictive of a better treatment outcome (Van et al., 2008). Whereas childhood trauma or abuse are associated with worse treatment outcomes and patients with family history of affective disorders have an increased risk for developing treatment-resistant depression (TRD) (Berlim & Turecki, 2007; Dodd & Berk, 2004; Uher, 2011). Other social aspects such as employment status, high income and high levels of education are associated with a more favorable prognosis in the treatment of depression (Van et al., 2008).

Symptom profile predictors

Symptom profile usually refers to the variables that describe the symptoms, patient clinical history, the nature and history of the depressive episodes as well as the depression subtype. Symptom profile predictors of treatment response are the most used in the clinical practice as they are often collected as a part of the clinical interview for the diagnosis of depression (Perlman et al., 2019).

When analyzing the symptoms present at time of diagnosis, guidelines recommend dopaminergic antidepressants to patients with symptoms like anhedonia or loss of motivation whereas SSRI would be more indicated for patients with comorbid obsessions and compulsions and SNRIs will be more suitable for patients with excessive fatigue or psychomotor retardation (Kennedy et al., 2016).

Other profile characteristics like the age of the patients at the time of first depressive episode onset is related with treatment prognosis, in fact, both early age and late age are related with worse treatment prognosis (Berlim & Turecki, 2007; Kemp et al., 2008; Van et al., 2008). The characteristics of the depressive episode can also contribute to predict the outcome of treatment since larger episode durations, recurrence and history of previous hospitalizations were associated with worst treatment outcomes (Berlim & Turecki, 2007; De Carlo et al., 2016; Kemp et al., 2008). Likewise, baseline symptoms severity, history of physical and emotional abuse, cognitive impairment, neuroticism, and anxiety symptoms have been associated with poorer treatment effects (De Carlo et al., 2016; Dodd & Berk, 2004).

Neuroimaging and electrophysiological predictors

The term neuroimaging is usually used to refer to an array of techniques that allow the direct observation of the structure and activity of the brain. Neuroimaging predictors of treatment response can be explored using various neuroimaging techniques probing different aspects of brain structure and function. The most used ones are the magnetic resonance imaging (MRI), positron emission tomography (PET) and

electroencephalography (EEG). Next, we will explore the current literature on the EEG and MRI predictors for treatment outcomes.

Electroencephalography (EEG)

EEG is a technique that reflects the electrical activity of the brain measured using electrodes at the scalp. EEG can capture the temporal dynamics (in milliseconds) of rapid neuronal processes such as transient responses to stimulus (ERP – event related potentials) and a time-varying composition of frequencies called brain rhythms. An EEG spectrogram could be divided in 5 different rhythms: delta (1-4 Hz), alpha (4 – 8 Hz), theta (8 – 13 Hz), beta (13 – 30 Hz) and gamma (30 – 50 Hz). These rhythms are attributed to regional synchronous oscillatory activity associated different cognitive processes (Buzsáki, 2006). Research has suggested that increased activity in the frontal theta was associated with a better antidepressant treatment prognosis (Breitenstein et al., 2014; Dodd & Berk, 2004; Papakostas & Fava, 2008). In a similar manner, increased alpha symmetry and delta activity were also found to be predictors of clinical improvement (Steiger & Kimura, 2010; Wichniak et al., 2013). Also, changes in the prefrontal cordance of the theta band in the first week of treatment were found to be related with the likelihood of symptom resolution after 9 weeks of antidepressant treatment but not placebo treatment (Hunter et al., 2006; Leuchter et al., 2002). Cordance is an EEG metric based on the absolute power (the power of a frequency band) and relative power (the percentage of power in a frequency band compared to the total power across all frequency bands) (Leuchter et al., 1994). Another EEG based metric is the antidepressant treatment response index (ATR) (Iosifescu JE; Greenwald DV; Devlin S.; Alpert, 2005). A study (yet to be replicated) found that the ATR patterns changed after 1 week of treatment and differentiated patients with clinical improvement during treatment with escitalopram or bupropion monotherapy, but not bupropion augmentation (Leuchter et al., 2009).

Magnetic Resonance Imaging (MRI)

MRI is a technique used to study brain's structure and function. Using structural MRI sequences, we can obtain measures of brain morphology or volume such as cortical thickness, grey matter volumetry as well as white matter integrity. Studies have reported such measures to be related with treatment outcomes in patients with depression (Chi et al., 2015; Dusi et al., 2015; Vieira et al., 2021). However, one of the most well replicated findings regarding brain structure connects the volume of the hippocampus to the remission of depression. Indeed, the hippocampal volume was found to be reduced in depressive patients in comparison to healthy controls and patients in remission from depression.

Moreover, the volume of the hippocampus was found to distinguish between responders and non-responders to pharmacological treatment (meaning that patients with smaller hippocampal volumes had poorer treatment outcomes) (Breitenstein et al., 2014; Chi et al., 2015; El-Hage et al., 2013; Leuchter et al., 2010). The reduction of volume in other brain areas have been also linked to depression, such as the anterior cingulate cortex (ACC), the basal ganglia, the medial prefrontal cortex (mPFC) or the lateral prefrontal cortex (LPFC) (Wise et al., 2014).

Functional magnetic resonance imaging (fMRI) is a technique based on the blood- oxygen-level-dependent (BOLD) signal. The BOLD signal is based on T2*, a contrast that is sensitive to the concentration of deoxyhemoglobin (dHb). Because dHB is a paramagnetic substance it causes distortions to magnetic field across the capillaries leading to a signal loss in the T2* images (Huettel et al., 2014). A local increase in neuronal activity will result in an increased metabolic rate of O_2 and cerebral blood flow and consequently a decrease in dHb that produces a positive BOLD response. Because this is a rather slow phenomenon (with a delay of several seconds) the temporal dynamics is poor. On the other hand, BOLD acquisitions can achieve sub-millimeter localization power which confers the technique great spatial resolution (Huettel et al., 2014).

FMRI is a particularly valuable technique in the study of MDD as it allows the exploration of abnormal brain function activations and patterns (Soares et al., 2016). There is a lot of literature in the neuroimaging field that explores the activation of particular cerebral regions in MDD patients as compared with healthy controls. Regarding brain activations in depression, the most consistent findings across literature are the abnormal activations in cortico-limbic structures as well as in the amygdala, anterior cingulate cortex (ACC) and the prefrontal cortex (PFC) (Ball et al., 2014; Cannon, 2010; Phillips et al., 2015).

However, brain structures do not work in isolation but rather as a complex form of integrated brain networks (Friston, 2011). There are consistent findings across literature that shown the impact of depression in the brain's connectome, specifically in the resting state networks (RSNs) (Damoiseaux et al., 2006; van den Heuvel & Hulshoff Pol, 2010). RSNs are anatomically separated brain areas that exhibit temporal correlated signal changes and are believed to describe the neuronal patterns of the resting brain.

Research has showed that MDD patients present a reduced connectivity in the frontoparietal network (FN) as well as an increased connectivity in the default mode network (DMN) that can underlie the deficits in cognitive control as well as the biases towards ruminative thoughts (Kaiser et al., 2015).

Studies have also used fMRI to study possible neuroimaging predictors for antidepressant treatment response. One of the most studied possible biomarkers is the neuronal activity of the ACC. This biomarker was studied using not only fMRI but also PET, and it was consistent across a range of antidepressant medication that includes SSRIs, SNRIs and TCAs, reporting that a decreased activity in the ACC was associated with a worse treatment response (Ball et al., 2014; Breitenstein et al., 2014; Dichter et al., 2015; Evans et al., 2006; Kemp et al., 2008; Lener & Iosifescu, 2015). Other possible biomarker of treatment response is the functional connectivity between frontal and limbic regions (Ball et al., 2014; Breitenstein et al., 2014; Dichter et al., 2015). And lastly, decreased connectivity within the DMN was also suggested as to be a possible biomarker of treatment response in MDD (Dichter et al., 2015). The connectivity within the DMN was found to be increased in patients diagnosed with depression and it was suggested that antidepressant medication may help normalize this hyperconnectivity (Chi et al., 2015).

CHAPTERS OVERVIEW

In this thesis, our purpose was to explore the potential neuroimaging biomarkers of depression and depression treatment in a cohort of drug-naïve patients. This document is organized in the following chapters:

- **Introduction**: In first chapter we introduced the main theme of this dissertation as well as a brief review of the literature exploring the current available treatments for depression and a set of predictors of treatment outcomes,

- **Chapter 1**: In this chapter we investigated the differences between drug naïve MDD patients and matching sex and age controls in cognition but also in the brain's resting-state functional connectivity using functional MRI,

- **Chapter 2**: In this chapter we described a set of possible RSN functional connectivity alterations related to treatment response in MDD patients by exploring the short- (hours) and long-term (weeks) response to the treatment with a 20mg daily dose of the SSRI paroxetine,

- **Chapter 3**: In this chapter we studied suicidal ideation, and its neuronal correlates in terms of brain function and white matter integrity,

- **Discussion**: The final chapter is a discussion of our main findings as well as the future directions for the research.

REFERENCES

Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., Adekanmbi, V., Adeoye, A. M., Adetokunboh, O. O., ... Wiangkham, T. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396(10258)*, 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

Allan, C. L., Herrmann, L. L., & Ebmeier, K. P. (2011). Transcranial magnetic stimulation in the management of mood disorders. In *Neuropsychobiology*. https://doi.org/10.1159/000328951

American Psychiatric Association. (2013). American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.books.9780890425596.744053

Ball, T. M., Stein, M. B., & Paulus, M. P. (2014). Toward the application of functional neuroimaging to individualized treatment for anxiety and depression. In *Depression and Anxiety*. https://doi.org/10.1002/da.22299

Belmaker, R. H., & Agam, G. (2008). Major Depressive Disorder. *New England Journal of Medicine*, *358*(1), 55–68. https://doi.org/10.1056/NEJMra073096

Berlim, M. T., & Turecki, G. (2007). Definition, assessment, and staging of treatment-resistant refractory major depression: A review of current concepts and methods. In *Canadian Journal of Psychiatry*. https://doi.org/10.1177/070674370705200108

Bocchio-Chiavetto, L., Bagnardi, V., Zanardini, R., Molteni, R., Gabriela Nielsen, M., Placentino, A., Giovannini, C., Rillosi, L., Ventriglia, M., Riva, M. A., & Gennarelli, M. (2010). Serum and plasma BDNF levels in major depression: A replication study and meta-analyses. *The World Journal of Biological Psychiatry*, *11*(6), 763–773. https://doi.org/10.3109/15622971003611319

Borrione, L., Moffa, A. H., Martin, D., Loo, C. K., & Brunoni, A. R. (2018). Transcranial Direct Current Stimulation in the Acute Depressive Episode: A Systematic Review of Current Knowledge. In *Journal of ECT*. https://doi.org/10.1097/YCT.0000000000000512

Breitenstein, B., Scheuer, S., & Holsboer, F. (2014). Are there meaningful biomarkers of treatment response for depression? In *Drug Discovery Today*. https://doi.org/10.1016/j.drudis.2014.02.002

Brunoni, A. R., Lopes, M., & Fregni, F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: Implications for the role of neuroplasticity in depression. In *International Journal of Neuropsychopharmacology* (Vol. 11, Issue 8, pp. 1169–1180). https://doi.org/10.1017/S1461145708009309

Bueno-Notivol, J., Gracia-García, P., Olaya, B., Lasheras, I., López-Antón, R., & Santabárbara, J. (2021). Prevalence of depression during the COVID-19 outbreak: A meta-analysis of community-based studies. *International Journal of Clinical and Health Psychology*, *21 (1)*. https://doi.org/10.1016/j.ijchp.2020.07.007

Buzsáki, G. (2006). *Rhythms of the Brain*. Oxford University Press.

Cannon, D. M. (2010). Neuroimaging and the pathophysiology and treatment of depression: Recent advances and future needs. In *Depression: From Psychopathology to Pharmacotherapy*. https://doi.org/10.1159/000319510

Canuso, C. M., Singh, J. B., Fedgchin, M., Alphs, L., Lane, R., Lim, P., Pinter, C., Hough, D., Sanacora, G., Manji, H., & Drevets, W. C. (2018). Efficacy and safety of intranasal esketamine for the rapid reduction of symptoms of depression and suicidality in patients at imminent risk for suicide: Results of a double-blind, randomized, placebo-controlled study. *American Journal of Psychiatry*, *175*(7). https://doi.org/10.1176/appi.ajp.2018.17060720

Carroll, B. J., Cassidy, F., Naftolowitz, D., Tatham, N. E., Wilson, W. H., Iranmanesh, A., Liu, P. Y., & Veldhuis, J. D. (2007). Pathophysiology of hypercortisolism in depression. *Acta Psychiatrica Scandinavica*, *115*(SUPPL. 433), 90–103. https://doi.org/10.1111/j.1600-0447.2007.00967.x

Cheetham, S. C., Crompton, M. R., Czudek, C., Horton, R. W., Katona, C. L. E., & Reynolds, G. P. (1989). Serotonin concentrations and turnover in brains of depressed suicides. *Brain Research*, *502*(2), 332–340.

Chi, K. F., Korgaonkar, M., & Grieve, S. M. (2015). Imaging predictors of remission to anti-depressant medications in major depressive disorder. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2015.07.002

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., Leucht, S., Ruhe, H. G., Turner, E. H., Higgins, J. P. T., Egger, M., Takeshima, N., Hayasaka, Y., Imai, H., Shinohara, K., Tajika, A., Ioannidis, J. P. A., & Geddes, J. R. (2018). Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *The Lancet*. https://doi.org/10.1016/S0140-6736(17)32802-7

Cuijpers, P., Noma, H., Karyotaki, E., Vinkers, C. H., Cipriani, Andrea, & Furukawa, T. A. (2020). A network meta-analysis of the effects of psychotherapies, pharmacotherapies and their combination in the treatment of adult depression. *World Psychiatry*. https://doi.org/10.1002/wps.20701

Cuijpers, P., Sijbrandij, M., Koole, S. L., Andersson, G., Beekman, A. T., & Reynolds, C. F. (2014). Adding psychotherapy to antidepressant medication in depression and anxiety disorders: A meta-analysis. *World Psychiatry*. https://doi.org/10.1002/wps.20089

Damoiseaux, J. S., Rombouts, S. A. R. B., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., & Beckmann, C. F. (2006). Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences*, *103*(37), 13848–13853. https://doi.org/10.1073/pnas.0601417103

De Carlo, V., Calati, R., & Serretti, A. (2016). Socio-demographic and clinical predictors of non-response/non-remission in treatment resistant depressed patients: A systematic review. In *Psychiatry Research*. https://doi.org/10.1016/j.psychres.2016.04.034

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *Journal of Clinical Psychiatry*, *61*(6), 7–11.

Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. (2013). In *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition*. https://doi.org/10.1176/appi.books.9780890425596.893619

Dichter, G. S., Gibbs, D., & Smoski, M. J. (2015). A systematic review of relations between resting-state functional-MRI and treatment response in major depressive disorder. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2014.09.028

Dodd, S., & Berk, M. (2004). Predictors of antidepressant response: A selective review. In *International Journal of Psychiatry in Clinical Practice*. https://doi.org/10.1080/13651500410005423

Doris, A., Ebmeier, K., & Shajahan, P. (1999). Depressive illness. *The Lancet*, *354*(9187), 1369–1375.

Duivis, H. E., Vogelzangs, N., Kupper, N., de Jonge, P., & Penninx, B. W. J. H. (2013). Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: Findings from the Netherlands Study of Depression and Anxiety (NESDA). *Psychoneuroendocrinology*, *38*(9), 1573–1585. https://doi.org/https://doi.org/10.1016/j.psyneuen.2013.01.002

Dunlop, B. W., Binder, E. B., Cubells, J. F., Goodman, M. M., Kelley, M. E., Kinkead, B., Kutner, M., Nemeroff, C. B., Newport, D. J., Owens, M. J., Pace, T. W. W., Ritchie, J. C., Rivera, V. A., Westen, D., Craighead, W. E., & Mayberg, H. S. (2012). Predictors of remission in depression to individual and combined treatments (PReDICT): Study protocol for a randomized controlled trial. *Trials*. https://doi.org/10.1186/1745-6215-13-106

Dusi, N., Barlati, S., Vita, A., & Brambilla, P. (2015). Brain Structural Effects of Antidepressant Treatment in Major Depression. *Current Neuropharmacology*. https://doi.org/10.2174/1570159x1304150831121909

Duval, F., Lebowitz, B. D., & Macher, J. P. (2006). Treatments in depression. In *Dialogues in Clinical Neuroscience*. https://doi.org/10.31887/dcns.2006.8.2/fduval

Ehlert, U., Gaab, J., & Heinrichs, M. (2001). Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder, and stress-related bodily disorders: the role of the hypothalamus–pituitary–adrenal axis. *Biological Psychology*, *57*(1), 141–152. https://doi.org/https://doi.org/10.1016/S0301-0511(01)00092-8

Elderon, L., & Whooley, M. A. (2013). Depression and cardiovascular disease. *Progress in Cardiovascular Diseases*. https://doi.org/10.1016/j.pcad.2013.03.010

El-Hage, W., Leman, S., Camus, V., & Belzung, C. (2013). Mechanisms of antidepressant resistance. In *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2013.00146

Elias, A., Phutane, V. H., Clarke, S., & Prudic, J. (2018). Electroconvulsive therapy in the continuation and maintenance treatment of depression: Systematic review and meta-analyses. *Australian and New Zealand Journal of Psychiatry*. https://doi.org/10.1177/0004867417743343

Evans, K., Dougherty, D., Pollack, M., & Rauch, S. (2006). Using neuroimaging to predict treatment response in mood and anxiety disorders. In *Annals of Clinical Psychiatry*. https://doi.org/10.1177/0143034306062814

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., Vos, T., & Whiteford, H. A. (2013). Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. *PLoS Medicine*. https://doi.org/10.1371/journal.pmed.1001547

Frazer, a, & Hensler, J. (1999). Serotonin Involvement in Physiological Function and Behavior. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*.

Friston, K. J. (2011). Functional and Effective Connectivity: A Review. *Brain Connectivity*. https://doi.org/10.1089/brain.2011.0008

Gelenberg, A.J., Freeman, M.P., Markowitz, J.C., Rosenbaum, J.F., Thase, M.E.,... Trivedi, M. H. (2010). Practice guideline for the treatment of patients with major depressive disorder. In *American Psychiatric Association*. https://doi.org/10.1176/appi.books.9780890423387.654001

Gillett, G., Tomlinson, A., Efthimiou, O., & Cipriani, A. (2020a). Predicting treatment effects in unipolar depression: A meta-review. In *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2020.107557

Gillett, G., Tomlinson, A., Efthimiou, O., & Cipriani, A. (2020b). Predicting treatment effects in unipolar depression: A meta-review. In *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2020.107557

Gold, P. W., & Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Molecular Psychiatry*, *7*(3), 254–275. https://doi.org/10.1038/sj.mp.4001032

Heim, C., & Binder, E. B. (2012). Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene-environment interactions, and epigenetics. In *Experimental Neurology*. https://doi.org/10.1016/j.expneurol.2011.10.032

Holsboer, F. (2000). The Corticosteroid Receptor Hypothesis of Depression Clinical Evidence. In *Neuropsychopharmacology* (Vol. 23, Issue 5).

Huettel, S. A., Song, A. W., & McCarthy, G. (2014). *Functional Magnetic Resonance Imaging* (Third Edit). Sinauer.

Hunter, A. M., Leuchter, A. F., Morgan, M. L., & Cook, I. A. (2006). Changes in brain function (quantitative EEG cordance) during placebo lead-in and treatment outcomes in clinical trials for major depression. *American Journal of Psychiatry*. https://doi.org/10.1176/ajp.2006.163.8.1426

Iosifescu JE; Greenwald DV; Devlin S.; Alpert. (2005). Frontal EEG at 1 Week Predicts Clinical Response to SSRIs in MDD. *Presented at the 158th Annual Meeting of the American Psychiatric Association*.

Januar, V., Saffery, R., & Ryan, J. (2015). Epigenetics and depressive disorders: A review of current progress and future directions. *International Journal of Epidemiology*. https://doi.org/10.1093/ije/dyu273

Jentsch, M. C., Van Buel, E. M., Bosker, F. J., Gladkevich, A. V., Klein, H. C., Oude Voshaar, R. C., Ruhé, E. G., Eisel, U. L. M., & Schoevers, R. A. (2015). Biomarker approaches in major depressive disorder evaluated in the context of current hypotheses. In *Biomarkers in Medicine* (Vol. 9, Issue 3, pp. 277–297). Future Medicine Ltd. https://doi.org/10.2217/bmm.14.114

Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D., & Pizzagalli, D. A. (2015). Large-Scale Network Dysfunction in Major Depressive Disorder. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2015.0071

Katon, W., Unützer, J., & Russo, J. (2010). Major depression: The importance of clinical characteristics and treatment response to prognosis. *Depression and Anxiety*. https://doi.org/10.1002/da.20613

Keith, T. Q., Newton, S. S., & Duman, R. S. (2007). Targeting Neurotrophic/Growth Factor Expression and Signaling for Antidepressant Drug Development. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, *6*(2), 151–160.

Kemp, A. H., Gordon, E., Rush, A. J., & Williams, L. M. (2008). Improving the prediction of treatment response in depression: Integration of clinical, cognitive, psychophysiological, neuroimaging, and genetic measures. In *CNS Spectrums*. https://doi.org/10.1017/S1092852900017120

Kennedy, S. H., Lam, R. W., McIntyre, R. S., Tourjman, S. V., Bhat, V., Blier, P., Hasnain, M., Jollant, F., Levitt, A. J., MacQueen, G. M., McInerney, S. J., McIntosh, D., Milev, R. V., Müller, D. J., Parikh, S. V., Pearson, N. L., Ravindran, A. V., & Uher, R. (2016). Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: Section 3. Pharmacological Treatments. In *Canadian Journal of Psychiatry*. https://doi.org/10.1177/0706743716659417

Kennis, M., Gerritsen, L., Marije Van Dalen, •, Williams, A., Cuijpers, P., & Claudi Bockting, •. (2020). Prospective biomarkers of major depressive disorder: a systematic review and meta-analysis. *Molecular Psychiatry*, *25*, 321–338. https://doi.org/10.1038/s41380-019-0585-z

Kessler, R. C. (2012). The costs of depression. In *Psychiatric Clinics of North America*. https://doi.org/10.1016/j.psc.2011.11.005

Kim, Y.-K., Na, K.-S., Shin, K.-H., Jung, H.-Y., Choi, S.-H., & Kim, J.-B. (2007). Cytokine imbalance in the pathophysiology of major depressive disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *31*(5), 1044–1053. https://doi.org/https://doi.org/10.1016/j.pnpbp.2007.03.004

Koenig, A. M., & Thase, M. E. (2009). First-line pharmacotherapies for depression - What is the best choice? In *Polskie Archiwum Medycyny Wewnetrznej*. https://doi.org/10.20452/pamw.743

Lener, M. S., & Iosifescu, D. V. (2015). In pursuit of neuroimaging biomarkers to guide treatment selection in major depressive disorder: A review of the literature. *Annals of the New York Academy of Sciences*. https://doi.org/10.1111/nyas.12759

Lépine, J. P., & Briley, M. (2011). The increasing burden of depression. *Neuropsychiatric Disease and Treatment*, *7 (SUPPL.)*, 3–7. https://doi.org/10.2147/NDT.S19617

Leuchter, A. F., Cook, I. A., Gilmer, W. S., Marangell, L. B., Burgoyne, K. S., Howland, R. H., Trivedi, M. H., Zisook, S., Jain, R., Fava, M., Iosifescu, D., & Greenwald, S. (2009). Effectiveness of a quantitative electroencephalographic biomarker for predicting differential response or remission with escitalopram and bupropion in major depressive disorder. *Psychiatry Research*. https://doi.org/10.1016/j.psychres.2009.04.004

Leuchter, A. F., Cook, I. A., Hamilton, S. P., Narr, K. L., Toga, A., Hunter, A. M., Faull, K., Whitelegge, J., Andrews, A. M., Loo, J., Way, B., Nelson, S. F., Horvath, S., & Lebowitz, B. D. (2010). Biomarkers to predict antidepressant response. In *Current Psychiatry Reports*. https://doi.org/10.1007/s11920-010-0160-4

Leuchter, A. F., Cook, I. A., Lufkin, R. B., Dunkin, J., Newton, T. F., Cummings, J. L., Mackey, J. K., & Walter, D. O. (1994). Cordance: A new method for assessment of cerebral perfusion and metabolism using quantitative electroencephalography. *NeuroImage*. https://doi.org/10.1006/nimg.1994.1006

Leuchter, A. F., Cook, I. A., Witte, E. A., Morgan, M., & Abrams, M. (2002). Changes in brain function of depressed subjects during treatment with placebo. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.159.1.122

Luo, M., Guo, L., Yu, M., & Wang, H. (2020). The psychological and mental impact of coronavirus disease 2019 (COVID-19) on medical staff and general public – A systematic review and meta-analysis. *Psychiatry Research*, *291*(June), 113190. https://doi.org/10.1016/j.psychres.2020.113190

Macqueen, G. M., Hassel, S., Arnott, S. R., Addington, J., Bowie, C. R., Bray, S. L., Davis, A. D., Downar, J., Foster, J. A., Frey, B. N., Goldstein, B. I., Hall, G. B., Harkness, K. L., Harris, J., Lam, R. W., Lebel, C., Milev, R., Müller, D. J., Parikh, S. V., ... Kennedy, S. H. (2019). The canadian biomarker

integration network in depression (CAN-BIND): Magnetic resonance imaging protocols. In *Journal of Psychiatry and Neuroscience*. https://doi.org/10.1503/jpn.180036

Maes, M. (1995). Evidence for an immune response in major depression: A review and hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *19*(1), 11–38. https://doi.org/https://doi.org/10.1016/0278-5846(94)00101-M

Malberg, J. E., Eisch, A. J., Nestler, E. J., & Duman, R. S. (2000). Chronic Antidepressant Treatment Increases Neurogenesis in Adult Rat Hippocampus. *Journal of Neuroscience*, *20*(24), 9104–9110.

Marcantoni, W. S., Akoumba, B. S., Wassef, M., Mayrand, J., Lai, H., Richard-Devantoy, S., & Beauchamp, S. (2020). A systematic review and meta-analysis of the efficacy of intravenous ketamine infusion for treatment resistant depression: January 2009 – January 2019. In *Journal of Affective Disorders* (Vol. 277). https://doi.org/10.1016/j.jad.2020.09.007

Mateus-Pinheiro, A., Pinto, L., Bessa, J. M., Morais, M., Alves, N. D., Monteiro, S., Patrício, P., Almeida, O. F. X., & Sousa, N. (2013). Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Translational Psychiatry*, *3*(1), e210–e210. https://doi.org/10.1038/tp.2012.141

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biological Psychiatry*, *65*(9), 732–741. https://doi.org/https://doi.org/10.1016/j.biopsych.2008.11.029

Monroe, S. M., & Harkness, K. L. (2022). Major Depression and Its Recurrences: Life Course Matters. *Annual Review of Clinical Psychology*, *18*(1). https://doi.org/10.1146/annurev-clinpsy-072220-021440

Naghavi, M. (2019). Global, regional, and national burden of suicide mortality 1990 to 2016: systematic analysis for the Global Burden of Disease Study 2016. *BMJ (Clinical Research Ed.)*. https://doi.org/10.1136/bmj.l94

Nock, M. K., Hwang, I., Sampson, N., Kessler, R. C., Angermeyer, M., Beautrais, A., Borges, G., Bromet, E., Bruffaerts, R., De Girolamo, G., De Graaf, R., Florescu, S., Gureje, O., Haro, J. M., Hu, C.,

Huang, Y., Karam, E. G., Kawakami, N., Kovess, V., ... Williams, D. R. (2009). Cross-national analysis of the associations among mental disorders and suicidal behavior: Findings from the WHO World Mental Health Surveys. *PLoS Medicine*, *6 (8)*. https://doi.org/10.1371/journal.pmed.1000123

Nutt, D. J. (2008). Relationship of Neurotransmitters to the Symptoms of Major Depressive Disorder. In *J Clin Psychiatry* (Vol. 69).

Ösby, U., Brandt, L., Correia, N., Ekbom, A., & Sparén, P. (2001). Excess mortality in bipolar and unipolar disorder in Sweden. *Archives of General Psychiatry*, *58 (9)*, 844–850. https://doi.org/10.1001/archpsyc.58.9.844

Papakostas, G. I., & Fava, M. (2008). Predictors, moderators, and mediators (correlates) of treatment outcome in major depressive disorder. *Dialogues in Clinical Neuroscience*. https://doi.org/10.31887/dcns.2008.10.4/gipapakostas

Perlman, K., Benrimoh, D., Israel, S., Rollins, C., Brown, E., Tunteng, J. F., You, R., You, E., Tanguay-Sela, M., Snook, E., Miresco, M., & Berlim, M. T. (2019). A systematic meta-review of predictors of antidepressant treatment outcome in major depressive disorder. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2018.09.067

Phillips, M. L., Chase, H. W., Sheline, Y. I., Etkin, A., Almeida, J. R. C., Deckersbach, T., & Trivedi, M. H. (2015). Identifying predictors, moderators, and mediators of antidepressant response in major depressive disorder: Neuroimaging approaches. In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2014.14010076

Pryor, J. C., & Sulser, F. (1991). CHAPTER 3 - EVOLUTION OF THE MONOAMINE HYPOTHESES OF DEPRESSION. In R. W. Horton & C. L. E. Katona (Eds.), *Biological Aspects of Affective Disorders* (pp. 77–94). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-356510-5.50009-1

Ruhé, H. G., Mason, N. S., & Schene, A. H. (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. *Molecular Psychiatry* , *12*(4), 331–359.

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., Niederehe, G., Thase, M. E., Lavori, P. W., Lebowitz, B. D., McGrath, P. J., Rosenbaum, J. F., Sackeim, H. A., Kupfer, D. J., Luther, J., & Fava, M. (2008). Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. *FOCUS*. https://doi.org/10.1176/foc.6.1.foc128

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Stewart, J. W., Nierenberg, A. A., Thase, M. E., Ritz, L., Biggs, M. M., Warden, D., Luther, J. F., Shores-Wilson, K., Niederehe, G., & Fava, M. (2006). Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *The New England Journal of Medicine*, *354*(12), 1231–1242.

Sen, S., Duman, R., & Sanacora, G. (2008). Serum Brain-Derived Neurotrophic Factor, Depression, and Antidepressant Medications: Meta-Analyses and Implications. *Biological Psychiatry*, *64*(6), 527–532. https://doi.org/https://doi.org/10.1016/j.biopsych.2008.05.005

Soares, J. M., Magalhães, R., Moreira, P. S., Sousa, A., Ganz, E., Sampaio, A., Alves, V., Marques, P., & Sousa, N. (2016). A Hitchhiker's guide to functional magnetic resonance imaging. *Frontiers in Neuroscience*, *10*, 515. https://doi.org/10.3389/fnins.2016.00515

Solomon, D. A., Keller, M. B., Leon, A. C., Mueller, T. I., Lavori, P. W., Shea, M. T., Coryell, W., Warshaw, M., Turvey, C., Maser, J. D., & Endicott, J. (2000). Multiple recurrences of major depressive disorder. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.157.2.229

Steiger, A., & Kimura, M. (2010). Wake and sleep EEG provide biomarkers in depression. In *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2009.08.013

Stetler, C., & Miller, G. E. (2011). Depression and Hypothalamic-Pituitary-Adrenal Activation: A Quantitative Summary of Four Decades of Research. *Psychosomatic Medicine*, *73*(2). https://journals.lww.com/psychosomaticmedicine/Fulltext/2011/02000/Depression_and_Hypothalamic_Pituitary_Adrenal.2.aspx

Strawbridge, R., Young, A. H., & Cleare, A. J. (2017). Biomarkers for depression: Recent insights, current challenges and future prospects. *Neuropsychiatric Disease and Treatment*, *13*, 1245–1262. https://doi.org/10.2147/NDT.S114542

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: Review and meta-analysis. In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.157.10.1552

Tolin, D. F. (2017). Can Cognitive Behavioral Therapy for Anxiety and Depression Be Improved with Pharmacotherapy? A Meta-analysis. In *Psychiatric Clinics of North America*. https://doi.org/10.1016/j.psc.2017.08.007

Trivedi, M. H., McGrath, P. J., Fava, M., Parsey, R. V., Kurian, B. T., Phillips, M. L., Oquendo, M. A., Bruder, G., Pizzagalli, D., Toups, M., Cooper, C., Adams, P., Weyandt, S., Morris, D. W., Grannemann, B. D., Ogden, R. T., Buckner, R., McInnis, M., Kraemer, H. C., ... Weissman, M. M. (2016). Establishing moderators and biosignatures of antidepressant response in clinical care (EMBARC): Rationale and design. In *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2016.03.001

Uher, R. (2011). Genes, environment, and individual differences in responding to treatment for depression. In *Harvard Review of Psychiatry*. https://doi.org/10.3109/10673229.2011.586551

van den Heuvel, M. P., & Hulshoff Pol, H. E. (2010). Exploring the brain network: A review on resting-state fMRI functional connectivity. *European Neuropsychopharmacology*, *20*(8), 519–534. https://doi.org/10.1016/j.euroneuro.2010.03.008

Van, H. L., Schoevers, R. A., & Dekker, J. (2008). Predicting the outcome of antidepressants and psychotherapy for depression: A qualitative, systematic review. In *Harvard Review of Psychiatry*. https://doi.org/10.1080/10673220802277938

van Zoonen, K., Buntrock, C., Ebert, D. D., Smit, F., Reynolds, C. F., Beekman, A. T. F., & Cuijpers, P. (2014). Preventing the onset of major depressive disorder: A meta-analytic review of psychological interventions. *International Journal of Epidemiology*. https://doi.org/10.1093/ije/dyt175

Vieira, R., Coelho, A., Reis, J., Portugal-Nunes, C., Magalhães, R., Ferreira, S., Moreira, P. S., Sousa, N., & Bessa, J. M. (2021). White Matter Microstructure Alterations Associated With Paroxetine

Treatment Response in Major Depression. *Frontiers in Behavioral Neuroscience*. https://doi.org/10.3389/fnbeh.2021.693109

Wichniak, A., Wierzbicka, A., & Jernajczyk, W. (2013). Sleep as a biomarker for depression. *International Review of Psychiatry*. https://doi.org/10.3109/09540261.2013.812067

Williams, L. M., Rush, A. J., Koslow, S. H., Wisniewski, S. R., Cooper, N. J., Nemeroff, C. B., Schatzberg, A. F., & Gordon, E. (2011). International Study to Predict Optimized Treatment for Depression (iSPOT-D), a randomized clinical trial: Rationale and protocol. *Trials*. https://doi.org/10.1186/1745-6215-12-4

Wise, T., Cleare, A. J., Herane, A., Young, A. H., & Arnone, D. (2014). Diagnostic and therapeutic utility of neuroimaging in depression: An overview. In *Neuropsychiatric Disease and Treatment*. https://doi.org/10.2147/NDT.S50156

World Health Organization. (2017). Depression and other common mental disorders: global health estimates. *World Health Organization*. https://doi.org/CC BY-NC-SA 3.0 IGO

World Health Organization (WHO). (2021a). *Depression. Fact Sheet*.

World Health Organization (WHO). (2021b). *Suicide worldwide in 2019: global health estimates*.

Yirmiya, R., & Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain, Behavior, and Immunity*, *25*(2), 181–213. https://doi.org/10.1016/j.bbi.2010.10.015

CHAPTER I

The functional connectome of treatment-naïve depressed patients is associated with different psychopathological domains of Depression.

Joana Reis, Rita Vieira, Carlos Portugal-Nunes, Ana Coelho, Ricardo Magalhães, Pedro Silva Moreira, Sónia Ferreira, Maria Pico-Pérez, Nuno Sousa, Nuno Dias, João Miguel Bessa

Manuscript under preparation.

The functional connectome of treatment-naïve depressed patients is associated with different psychopathological domains of Depression.

Joana Reis[1,2,3], Rita Vieira[1,2,3], Carlos Portugal-Nunes[1,2,3], Ana Coelho[1,2,3], Ricardo Magalhães[1,2,3], Pedro Silva Moreira[1,2,3], Sónia Ferreira[1,2,3], Maria Pico-Pérez[1,2,3], Nuno Sousa[1,2,3], Nuno Dias[5], João Miguel Bessa[1,2,]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

[2]ICVS-3Bs PT Government Associate Laboratory, Braga/Guimarães, Portugal.

[3]Clinical Academic Center – Braga, Braga, Portugal

[4]Psychology Research Centre (CIPsi), School of Psychology, University of Minho, Braga, Portugal

[5]2Ai—School of Technology, Instituto Politécnico do Cávado e do Ave (IPCA), Barcelos, Portugal

***Corresponding author:** João Miguel Bessa, MD/PhD; Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal e-mail: joaobessa@med.uminho.pt

Abstract

Major depression disorder (MDD) is a highly prevalent mood disorder. Depression is often associated with complications in social and cognitive functioning with patients reporting difficulties in personal and occupational areas that limit the ability to cope with life activities and events.

The main goal of this study was to explore the cognitive and neuro-biological markers of depression, before pharmacological intervention using neuroimaging techniques.

A sample of treatment-naïve MDD patients as well as matching controls underwent a magnetic resonance imaging (MRI) session before first treatment administration as well as a neuropsychological evaluation to access depression, anxiety, perceived stress, suicidal ideation as well as memory and cognitive functioning. Our results showed that, as expected, MDD patients differed from healthy controls in depression, anxiety, perceived stress and suicidal ideation symptoms. MDD patients also tended to score lower than healthy controls in all cognitive tests, with this tendency being significant specifically for the LTSS (Selective Remining Test, long-term storage) and the DSST (Digit Substitution test). Using functional MRI, we were able to measure differences in the functional connectivity within the typical resting state networks. The obtained results showed an increased connectivity within the ventral DMN and the RECN to the angular gyrus in MDD patients compared with healthy controls. Also, the connectivity within the RECN (but not within the DMN) correlated negatively with the scores in the Stroop and the DSST.

The present study explored differences, not only in cognition, but also in the functional connectivity within resting state networks often related with executive functioning and goal directed behavior (ECN) or internal mind wondering processes (DMN).

Keywords: Depression, fMRI, functional connectivity, RSN, cognition

Introduction

Major depression disorder (MDD) is a high prevalent and disabling mood disorder ranked by the World Health Organization (WHO) as the largest single contributor for disability worldwide (World Health Organization (WHO), 2021). MDD is characterized by the experience of affective, cognitive, and somatic symptoms (Fried & Nesse, 2015). According to the DSM-5, the criteria for the diagnosis of depression requires the existence of five or more symptoms during the same 2-week period. Depression symptoms should include depressed mood or loss of interest/pleasure in activities but also changes in body weight, sleep disturbances, psychomotor retardation, fatigue, feelings of worthlessness, diminished ability to concentrate, recurrent thoughts of death and suicidal ideation (American Psychiatric Association, 2013). These criteria allow for a vast range of combination of different symptom profiles that reflect the remarkable heterogeneity of depression. Such heterogeneity is often associated with the difficulty of understanding the pathophysiology of depression as well as the difficulty to determine the best treatment course as the diagnostic tools often assigns a label to a disorder that could be caused by distinct pathological processes, which would require different treatments.

Depression leads to difficulties in social functioning with patients reporting difficulties in personal, social, and occupational areas of that limit the ability to cope with life activities and events creating a profound psychosocial impairment and disability that culminates in a reduced overall quality of life (Atique-Ur-Rehman & Neill, 2019; Clark et al., 2016). Cognitive deficits, such as difficulties in attention, working and visuospatial memory, executive functioning or processing speed, are an important feature of depression that might be reflected in the experience of symptoms such as diminished ability to concentrate or indecisiveness as well as anhedonia and psychomotor retardation (Regier et al., 2013). Despite the evidence suggestion that a large number of patients experience cognitive impairments during depressive episodes and remission, only a small number of psychiatrists use cognitive instruments in patient screening (Belgaied et al., 2014; Mattingly et al., 2016; Seeberg et al., 2018). However, the severity of depression has been negatively associated with cognitive performance particularly in episodic memory, executive functioning and processing speed (Mattingly et al., 2016). Unlike other psychiatric disorders such as schizophrenia, cognitive impairments in depression are still poorly understood and the impact of treatment is yet to be established.

In biological psychiatry there is not one theory for depression but rather a complex representation that involves multiple biological systems and can go from neurotransmitters to hormonal levels or

abnormalities in the brain's structure and functioning. There have been multiple biological factors hypothesized to be involved in the onset/relapse/recurrence of MDD. The lead biological factors studied are neuroimaging, gastrointestinal factors, immunology, neurotrophic factors, neurotransmitters, hormones, and oxidative stress (Kennis et al., 2020; Strawbridge et al., 2017). From the neuroimaging perspective, several studies have explored the underlying pathophysiology, as well as the characterization of the structural (Hellewell et al., 2019) (Liao et al., 2013) and functional neurobiological correlates of MDD using neuroimaging techniques such as functional magnetic resonance imaging (fMRI) (Kerestes et al., 2014; Maglanoc et al., 2019; Mulders et al., 2015; Müller et al., 2017; Wang et al., 2012; Zou et al., 2016), namely in resting-state fMRI (RS-fMRI). Several studies have suggested that depression is a disorder associated with changes in FC crossing several large-scale neural networks considered to be involved in emotional processing, executive function and reward processing (Kaiser et al., 2015a; Kerestes et al., 2014; Zou et al., 2016b). These changes included a hyper-connectivity between the areas of the default mode network (DMN), hypoconnectivity within areas of the frontoparietal executive networks and between brain regions involved in emotional processing and salience. Such alterations in brain connectivity patterns can underlie deficits in cognitive control in MDD patients as well as reflect the internal bias towards ruminative thoughts and impairments in emotional regulation and salience monitoring (Kaiser et al., 2015).

With the present study the main goal was to explore the cognitive and neural differences between MDD patients (early in the course of the pharmacological treatment) using the fMRI. For that purpose, probabilistic independent component analysis (PICA) was applied to resting-state fMRI data. Our main hypothesis was that MDD patients would present abnormal patterns of FC in the networks previously linked to depression such as the DMN and the FN, executive networks.

Subjects and Methods

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and received approvals from local review boards, namely University of Minho and Hospital de Braga (Braga, Portugal). Participants received an explanation about study goals, procedures and assessments and had enough time to consider participating in the study. All participants gave a written informed consent to be included in the study. Anonymity and confidentiality were guaranteed during research activities.

Characterization of participants

A sample of patients diagnosed with a major depressive episode were recruited between January 2016 and January 2020 in the context of emergency psychiatric evaluations or the outpatient unit of the Psychiatry Department of the Hospital de Braga. Patients were recruited by an experienced psychiatrist and diagnosis was confirmed with the SCID (structured clinical interview for DSM disorders) according to DSM-IV-TR (First et al., 2008).

The inclusion criteria were being diagnosed with the first depressive episode and without prior exposure to antidepressant treatment. The exclusion criteria were: age inferior to 18 years or superior to 65 years, previous medical history of neurological disorders or traumatic brain injury, medical history of addictive disorders, family history of early onset dementia, cognitive impairments as defined by scores below or equal to 24 in the Mini-Mental Sate Examination (MMSE) test (Folstein et al., 1975). In accordance with the established criteria, 32 patients were selected for evaluation. From these, 2 were unable to perform the MRI due to claustrophobia, 2 were excluded due to brain abnormalities and 7 dropped out of the study before completion. Therefore, a total of 22 MDD subjects (8 males, 14 females) were included in the final analysis. Seventeen healthy controls matching for age, sex and education, were also included. Healthy controls were recruited in the community.

Participants performed neuropsychological evaluations in order to assess mood (Hamilton Depression scale – HAM-D) (M. Hamilton, 1960), anxiety (Hamilton Anxiety scale – HAM-A) (M. Hamilton, 1959), perceived stress (Perceived Stress Scale 10 – PSS-10) (Cohen, 1988), suicidal ideation (Beck Suicidal Ideation Scale - BSI) (Beck et al., 1979) and cognition (Selective Reminding test – SRT (both long-term storage - LTS and consistent long-term retrieval - CLTR) (Buschke, 1973), Stroop test (Scarpina & Tagini, 2017), and Digit Symbol Substitution test - DSST) (Jaeger, 2018a; Wechsler, 1997).

Study design

MRI data acquisitions

The MRI acquisitions were performed using a 1.5 T (Siemens Medical Solutions, Erlangen, Germany) with a 12-channel receive-only head coil. All MRI acquisitions were performed at Hospital de Braga. The MRI session included morphological assessments and a resting state functional paradigm were patients were instructed to remain awake, with their eyes closed.

Functional MRI images were obtained using a BOLD sensitive echo-planar imaging (EPI) with the following parameters: 30 interleaved axial slices, repetition time (TR) = 2000 ms, echo time (TE) = 50 ms, flip angle (FA) = 90°, slice thickness = 3.5 mm, slice gap = 0.48 mm, in-plane resolution = 3.5 x 3.5 mm^2, field of view (FoV) = 224 mm and 190 volumes. A T1-weighted structural image for anatomical reference was obtained using a magnetization-prepared rapid acquisition (MPRAGE) by gradient echo sequence with voxel resolution 1.0×1.0×1.0mm, FoV 234×234mm2, FA of 7°, 176 slices and TETR of 3.48/2730ms.

MRI data pre-processing

Custom scripts were written in order to preprocess the fMRI data using FMRIB Software Library (FSL v6.01) tools (Smith et al., 2004).

To exclude possible magnetic field inhomogeneities at the beginning of the acquisition, the first step of preprocessing was the removal of the first 5 volumes. In order to correct temporal offsets between volumes, slice timing correction was performed with a temporal data interpolation that used the first acquired volume as reference. Head motion was corrected using a function from FSL that searches motion parameters and uses them to correct movement in the time-series. In order to further reduce contamination on functional connectivity due to motion, motion scrubbing was also performed to identify and exclude time-points where head motion could be critical. A non-linear spatial normalization to Montreal Neurological Institute (MNI) standard space was performed to register the set of brains to a common space/template. The procedure involved: i) skull stripping of the mean image of the functional acquisition; ii) rigid-body registration of the mean functional image to the skull stripped structural scan; iii) affine registration of the structural scan to the MNI T1 template; iv) non-linear registration of the structural scan to the MNI T1 template using the affine transformation estimated previously as the initial alignment; v) non-linear transformation of the functional acquisition to MNI standard space through the sequential application of the rigid-body transformation, followed by non-linear warp and resampling to 2x2x2 mm^3 voxel size. A linear regression using the mean of white matter and cerebrospinal fluid signals as well as motion outliers was performed, and the residuals of the regression were smoothed using a Gaussian kernel smoother with a full width half maximum of 6 mm (σ = 2.55 mm), band-pass temporal filtered (0.01–0.08Hz) and used for the subsequent analysis.

Resting-state Networks (RSNs)

Probabilistic independent component analysis (PICA) was performed with MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components), distributed with FSL v6.01. PICA is a data driven analysis that isolates components or non-overlapping spatial maps corresponding to regions evidencing coherent time-courses (Beckmann & Smith, 2004; Soares et al., 2016). The software estimates group-wise spatial maps that correspond mainly to Resting State Networks (RSNs). A total number of 30 independent components were used. In order to study subject-specific components, a dual regression analysis was performed as well. The RSNs of interest were identified by visual inspection in combination with spatial correlations between the obtained components and RSN templates available from a public dataset from the Functional Imaging in Neuropsychiatric Disorders (FIND) Lab at Stanford University (Shirer et al., 2012).

Statistics

Psychometric and demographic data

Psychometric and demographic data were analyzed using the software IBM SPSS Statistics V27. Normality was assessed using the Shapiro-Wilk Test. All demographic and psychometric variables followed a normal distribution except for the BSI scale. Comparisons between groups were done using independent samples t-test. Differences were considered statistically significant for a p-value below 0.05.

Neuroimaging data

Intra-RSN FC was compared between groups using independent samples t-tests with the non-parametric permutation procedure implemented in the *randomise* tool from FSL (Winkler et al., 2014). Threshold-free cluster enhancement (TFCE) was used to detect widespread significant differences and control the family-wise error rate (FWE-R) at $\alpha = 0.05$. 5000 permutations were performed for each contrast.

Results

Demographic data and Psychometric data

From the sample of 38 participants, 22 were diagnosed drug naïve MDD patients and 17 were matching healthy controls. Details about demographics and neuropsychological scales can be found in Supplementary table 1. There was no significant difference in demographic variables (age and education) between MDD and controls (age: $t(37)=1.996$, $p>0.05$; education: $t(37)=-1.251$, $p>0.05$).

Participants were evaluated in the terms of their depression (HAM-D) and anxiety (HAM-A) as well as suicidal ideation (BSI) and perceived stress (PSS-10) scores. As expected MDD patients differed significantly from controls and presented higher HAM-D ($t(35)=-11.631$, $p<0.05$), HAM-A ($t(35)=-10.409$, $p<0.05$), PSS-10 ($t(34)=-8.926$, $p<0.05$), and BSI ($t()=x$, $p=x$) scores (Figure 1).

Participants were also evaluated in the cognitive dimension using the SRT (long-term storage – LTS and consistent long-term retrieval – CLTR), Stroop, and DSST tests. MDD patients presented significantly lower scores than controls in the LTS ($t(34)=-2.138$, $p<0.05$) and DSST ($t(35)=2,632$, $p<0.05$) but not the CLTR ($t(34)=1.971$, $p>0.05$) and Stroop ($t(35)=1.163$, $p>0.05$) tests (Figure 3a).

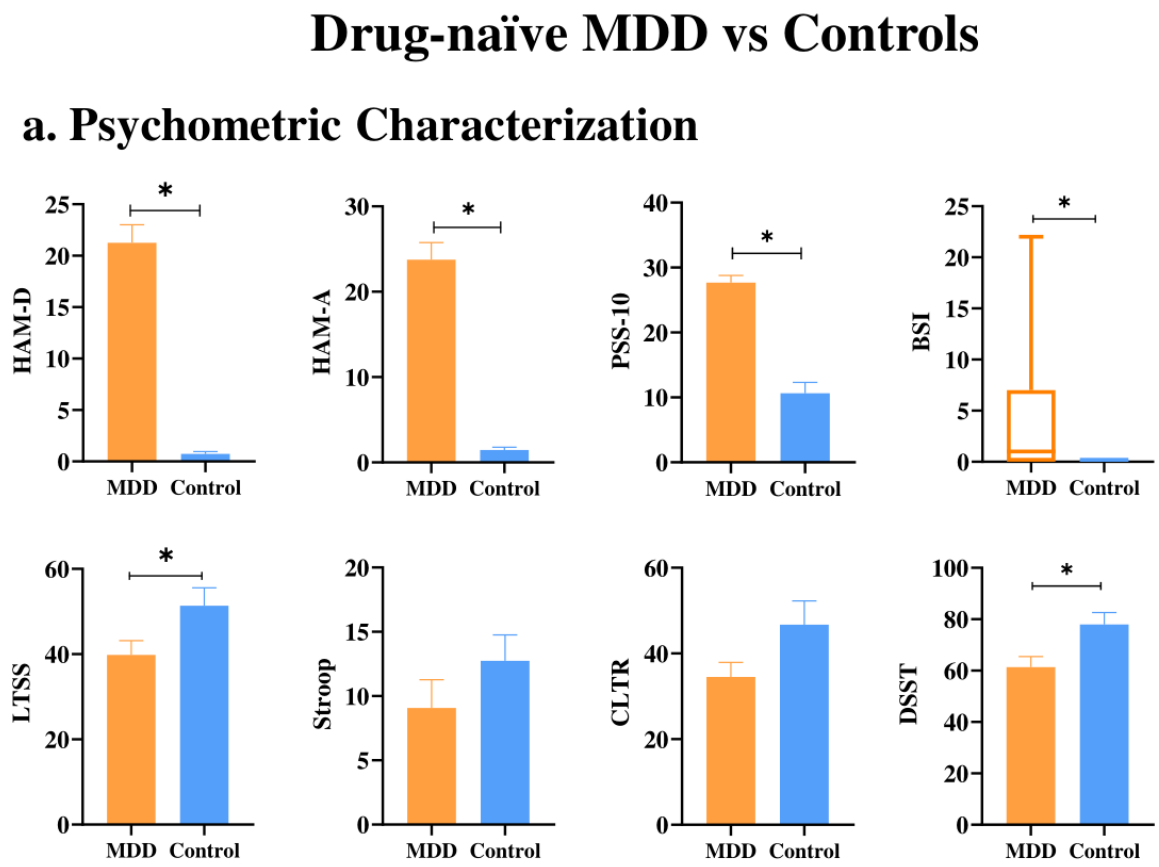
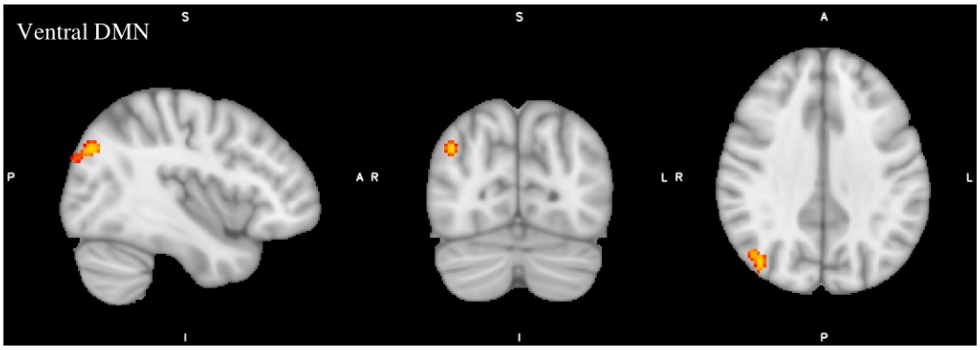


Figure 1: Differences between MDD patients and matching healthy controls in the psychometric characterization. As expected MDD patients differed from healthy controls on depression (HAM-D), anxiety (HAM-A), perceived stress (PSS-10) and suicidal ideation (BSSI) scales. In terms of cognition (measured using the Selective Reminding test – SRT; long-term storage - LTSS and consistent long-term retrieval - CLTR, Stroop test, and Digit Symbol Substitution test – DSST) healthy controls tended to score higher

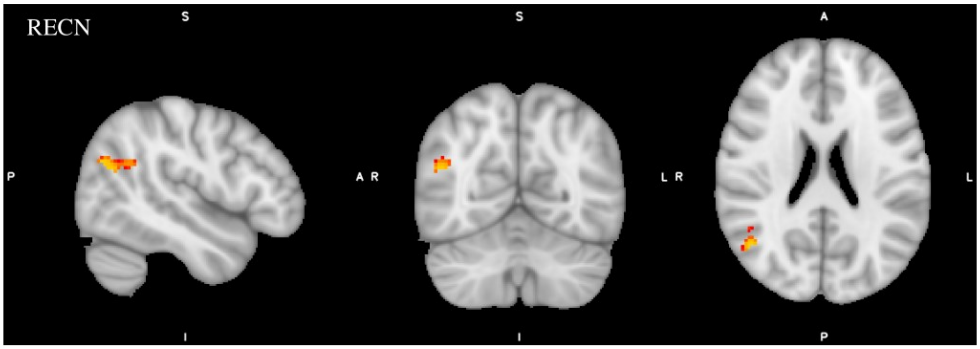
than MDD patients in all measurements with that tendency being statistically significant for the LTSS and the DSST tests.

RS-FC differences between drug-naive MDD patients and healthy controls

The comparison between drug naïve MDD patients and healthy controls revealed differences within the ventral DMN and RECN connectivity patterns (Figure 2). In fact, MDD patients presented an increased connectivity to the angular gyrus (with the other regions of the ventral DMN) and the middle occipital gyrus (within the RECN) when compared to healthy controls (Table S2).



MDD>Controls -Angular Gyrus R



MDD>Controls - Middle Occipital Gyrus R

Figure 2: Differences at baseline between drug-naïve depressive subjects and healthy controls in the intra network resting state functional connectivity. The group differences within the ventral DMN and the RECN show an increased connectivity of the MDD subjects to the angular gyrus in comparison with the healthy controls (supplementary table 2).

Correlations between FC and cognitive scales

The clusters of functional connectivity that showed an increased connectivity within the RECN and the vDMN were further correlated with the scores in all cognitive tests applied. The results showed that the connectivity with the RECN (but not the FC within the DMN) was negatively correlated with the Stroop and the DSST tests.

Table 1: Pearson correlation coefficients and p-values between the scores obtained in the cognitive tests (Selective Reminding test – SRT; long-term storage - LTSS and consistent long-term retrieval - CLTR, Stroop test, and Digit Symbol Substitution test – DSST) and the significant cluster of FC within the vDMN and the RECN.

	LTS	**CLTR**	**Stroop**	**DSST**
vDMN	-0.270	-0.257	-0.232	-0.262
P-value	0.110	0.130	0.166	0.117
RECN	-0.255	-0.221	**-0.346***	**-0.471***
P-value	0.133	0.194	**0.036**	**0.003**

Discussion

The main goal of this study was to explore the differences between patients diagnosed with depression and healthy controls early in the course of pharmacological intervention, using resting state fMRI techniques. To do so, a sample of 22 patients and 16 controls (matching for sex and education) were recruited and evaluated in terms of depression (Hamilton Depression scale – HAM-D), anxiety (Hamilton Anxiety scale – HAM-A), perceived stress (Perceived Stress Scale 10 – PSS-10) and suicidal ideation (Beck Suicidal Ideation Scale - BSI) symptoms. As expected, MDD patients scored significantly higher in all scales compared with healthy controls (Figure 1).

Participants were also evaluated in terms of their memory and cognitive functioning using the Selective Reminding test – SRT (both long-term storage - LTS and consistent long-term retrieval - CLTR), Stroop test and the and Digit Symbol Substitution test - DSST). A trend in all applied tests revealed that MDD patients scored lower, in mean, than healthy controls (Figure 1). Statistically significant differences,

however, were only found in the LTS and DSST tests. Such results indicate that in our sample, in line with the literature, MDD patients presented poorer performance than healthy controls.(Bruder et al., 2014; Taylor et al., 2006)

The DSST is a test that evaluates cognitive functioning being sensitive to changes in processing speed, executive functioning and working memory (Jaeger, 2018b). Evidence suggested that the performance in the DSST test correlates with real-world outcomes as the ability to carry on everyday tasks, a measure useful to evaluate functional disability in MDD patients. In the context of psychiatric evaluations this test, in combination with other clinically relevant metrics, could be useful for therapeutically guidance.

These results show that, in line with the available literature, MDD patients present impaired cognitive function when compared to healthy subjects. We hypothesize that, in patients with mood disorders (specifically depression), the existence of difficulties in cognitive functioning could be related to common depression symptoms such as anhedonia, psychomotor retardation or inability to concentrate. An effective treatment regime would alleviate these symptoms producing an overall benefit in cognitive functioning with direct impact in everyday tasks and thus increasing patient's the quality of life.

Regarding neuroimaging results, the resting-state networks have proved to be a useful tool to obtain information about brain function independent of stimulus-based responses (Fox et al., 2006). MDD subjects showed an increased connectivity within the ventral DMN to the angular gyrus when compared with matching healthy controls. In fact, increased connectivity in anterior medial regions of the DMN in MDD patients was associated with introspective rumination (Zhu et al., 2012). The DMN, also known as task-negative network, is a network that typically involves regions of the medial prefrontal cortex (mPFC), the posterior cingulate/precuneus, superior temporal cortex, hippocampus, and the inferior parietal cortex (Raichle & Raichle, 2001; Smith et al., 2009). The DMN is more active in the absence of a task and is associated with introspective rumination, low-level arousal, homeostatic- and self-regulation, such as during "day-dreaming," and supporting autobiographical, internally focused, declarative, episodic memory retrieval (Andrews-Hanna et al., 2014). The DMN is reported to be activated in an antagonistic fashion with the fronto-parietal executive control networks (RECN and LECN), also known as task-positive networks. The ECNs are constituted typically by a frontoparietal system involving the dorsolateral prefrontal cortex (dlPFC) and inferior parietal cortex. ECNs are associated with executive control and goal-oriented behavior and impulse control (Fassbender et al., 2006). The "triple network model" for depression (Menon, 2011) suggests a dysregulation in large-scale brain networks in MDD, mainly

reporting increased activity within the DMN and decreased activity within ECN and salience network, suggesting increased activity in networks important for directing attention to introspective thoughts and decreased attention to outside world and goal-directed actions and impulse control, respectively (Greicius, 2008; J. P. Hamilton et al., 2015; Kaiser et al., 2015). Interestingly, in our sample of MDD patients we found an increased connectivity of the RECN to the middle occipital gyrus compared to healthy controls. Our hypothesis is that the inverse relationship between the DMN and ECN was reported during switch from rest to task fMRI and so during resting periods the controls maintained a lower connectivity, whereas the drug naïve MDD patients were unable to do so. In fact, in our cohort the connectivity within the RECN was found be negatively correlated with the Stroop and the DSST tests meaning that people with higher scores showed less FC within the RECN.

In conclusion, our study reinforced the idea that depression is associated with a worse cognitive function. Our results showed differences not only in cognition but also in the functional connectivity within networks of functional connectivity often related with executive functioning and goal directed behavior (ECN) or internal mind wondering processes (DMN). Importantly, our sample is exclusively drug-naïve MDD patients and that allows us to exclude medication effects. This study presents however some limitations. Our sample size is relatively small, however due to the restrictive inclusion and exclusion criteria the recruitment was difficulted and only a small number of patients were able to be included in the final analysis.

Conflict of interest declaration

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments

This work was funded by the Portuguese Foundation for Science and Technology (FCT) under scope of project PTDC/DTP-PIC/6936/2014.

This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020 and by the project NORTE-01-0145-FEDER-000039, supported by Norte Portugal Regional Operational Program (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

J.R. was supported by the FCT fellowship grant with the reference PDE/BDE/113602/2015 from the PhD-iHES program. R.V. was supported by the FCT fellowship grant with the reference PD/BDE/150619/2020 from the PhD-iHES program and UMINHO/BI/340/2018. C.P.N. was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Program in Ageing and Chronic Diseases. P.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113601/2015 from the PhD-iHES program. R.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113604/2015 from the PhD-iHES program. S.F. was supported by the FCT fellowship grant with the reference PD/BDE/127839/2016 from the PhD-iHES program.

References

American Psychiatric Association. (2013). American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.books.9780890425596.744053

Andrews-Hanna, J. R., Smallwood, J., & Spreng, R. N. (2014). The default network and self-generated thought: Component processes, dynamic control, and clinical relevance. *Annals of the New York Academy of Sciences*. https://doi.org/10.1111/nyas.12360

Atique-Ur-Rehman, H., & Neill, J. C. (2019). Cognitive dysfunction in major depression: From assessment to novel therapies. In *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2019.05.013

Beck, A. T., Kovacs, M., & Weissman, A. (1979). Assessment of suicidal intention: The Scale for Suicide Ideation. *Journal of Consulting and Clinical Psychology*. https://doi.org/10.1037/0022-006X.47.2.343

Beckmann, C. F., & Smith, S. M. (2004). Probabilistic Independent Component Analysis for Functional Magnetic Resonance Imaging. *IEEE Transactions on Medical Imaging*, *23(2)*, 137-152. https://doi.org/10.1109/TMI.2003.822821

Belgaied, W., Samp, J., Vimont, A., Rémuzat, C., Aballéa, S., El Hammi, E., Kooli, A., Toumi, M., & Akhras, K. (2014). Routine clinical assessment of cognitive functioning in schizophrenia, major depressive disorder, and bipolar disorder. *European Neuropsychopharmacology*. https://doi.org/10.1016/j.euroneuro.2013.11.001

Bruder, G. E., Alvarenga, J. E., Alschuler, D., Abraham, K., Keilp, J. G., Hellerstein, D. J., Stewart, J. W., & McGrath, P. J. (2014). Neurocognitive predictors of antidepressant clinical response. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2014.04.057

Buschke, H. (1973). Selective reminding for analysis of memory and learning. *Journal of Verbal Learning and Verbal Behavior*. https://doi.org/10.1016/S0022-5371(73)80034-9

Clark, M., DiBenedetti, D., & Perez, V. (2016). Cognitive dysfunction and work productivity in major depressive disorder. In *Expert Review of Pharmacoeconomics and Outcomes Research*. https://doi.org/10.1080/14737167.2016.1195688

Cohen, S. (1988). Perceived stress in a probability sample of the United States. In *The social psychology of health.*

Damoiseaux, J. S., Rombouts, S. A. R. B., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., & Beckmann, C. F. (2006). Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences*, *103*(37), 13848–13853. https://doi.org/10.1073/pnas.0601417103

Fassbender, C., Simoes-Franklin, C., Murphy, K., Hester, R., Meaney, J., Robertson, I. H., & Garavan, H. (2006). The Role of a Right Fronto-Parietal Network in Cognitive Control. *Journal of Psychophysiology*. https://doi.org/10.1027/0269-8803.20.4.286

First, M. B., Williams, J. B. W., Karg, R. S., Spitzer, R. L., & Gibbon, M. (2008). Structured Clinical interview for DSM-IV Axis I Disorders (SCID-I). *Handbook of Psychiatric Measures*.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*. https://doi.org/10.1016/0022-3956(75)90026-6

Fox, M. D., Corbetta, M., Snyder, A. Z., Vincent, J. L., & Raichle, M. E. (2006). Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0604187103

Fried, E. I., & Nesse, R. M. (2015). Depression is not a consistent syndrome: An investigation of unique symptom patterns in the STAR*D study. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2014.10.010

Greicius, M. (2008). Resting-state functional connectivity in neuropsychiatric disorders. *Current Opinion in Neurology*, *21*(4), 424–430. https://doi.org/10.1097/WCO.0b013e328306f2c5

Hamilton, J. P., Farmer, M., Fogelman, P., & Gotlib, I. H. (2015). Depressive Rumination, the Default-Mode Network, and the Dark Matter of Clinical Neuroscience. In *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2015.02.020

Hamilton, M. (1959). The assessment of anxiety states by rating. *British Journal of Medical Psychology*. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry*, *23*, 56–62.

Hellewell, S. C., Welton, T., Maller, J. J., Lyon, M., Korgaonkar, M. S., Koslow, S. H., Williams, L. M., Rush, A. J., Gordon, E., Grieve, S. M., & Gordon, E. (2019). Profound and reproducible patterns of reduced regional gray matter characterize major depressive disorder. *Translational Psychiatry*. https://doi.org/10.1038/s41398-019-0512-8

Jaeger, J. (2018a). Digit symbol substitution test. *Journal of Clinical Psychopharmacology*. https://doi.org/10.1097/JCP.0000000000000941

Jaeger, J. (2018b). Digit Symbol Substitution Test: The Case for Sensitivity Over Specificity in Neuropsychological Testing. *Journal of Clinical Psychopharmacology*, *38*(5), 513–519. https://doi.org/10.1097/JCP.0000000000000941

Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D., & Pizzagalli, D. A. (2015). Large-Scale Network Dysfunction in Major Depressive Disorder. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2015.0071

Kennis, M., Gerritsen, L., Marije Van Dalen, •, Williams, A., Cuijpers, P., & Claudi Bockting, •. (2020). Prospective biomarkers of major depressive disorder: a systematic review and meta-analysis. *Molecular Psychiatry*, *25*, 321–338. https://doi.org/10.1038/s41380-019-0585-z

Kerestes, R., Davey, C. G., Stephanou, K., Whittle, S., & Harrison, B. J. (2014). Functional brain imaging studies of youth depression: A systematic review. In *NeuroImage: Clinical*. https://doi.org/10.1016/j.nicl.2013.11.009

Liao, Y., Huang, X., Wu, Q., Yang, C., Kuang, W., Du, M., Lui, S., Yue, Q., Chan, R. C. K., Kemp, G. J., & Gong, Q. (2013). Is depression a disconnection syndrome? Meta- analysis of diffusion tensor

imaging studies in patients with MDD. *Journal of Psychiatry and Neuroscience*. https://doi.org/10.1503/jpn.110180

Maglanoc, L. A., Landrø, N. I., Jonassen, R., Kaufmann, T., Córdova-Palomera, A., Hilland, E., & Westlye, L. T. (2019). Data-Driven Clustering Reveals a Link Between Symptoms and Functional Brain Connectivity in Depression. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. https://doi.org/10.1016/j.bpsc.2018.05.005

Manoliu, A., Meng, C., Brandl, F., Doll, A., Tahmasian, M., Scherr, M., Schwerthöffer, D., Zimmer, C., Förstl, H., Bäuml, J., Riedl, V., Wohlschläger, A. M., & Sorg, C. (2014). Insular dysfunction within the salience network is associated with severity of symptoms and aberrant inter-network connectivity in major depressive disorder. *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2013.00930

Mattingly, G., Anderson, R. H., Mattingly, S. G., & Anderson, E. Q. (2016). The impact of cognitive challenges in major depression: the role of the primary care physician. In *Postgraduate Medicine*. https://doi.org/10.1080/00325481.2016.1221318

Menon, V. (2011). Large-scale brain networks and psychopathology: A unifying triple network model. In *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2011.08.003

Mulders, P. C., van Eijndhoven, P. F., Schene, A. H., Beckmann, C. F., & Tendolkar, I. (2015). Resting-state functional connectivity in major depressive disorder: A review. In *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2015.07.014

Müller, V. I., Cieslik, E. C., Serbanescu, I., Laird, A. R., Fox, P. T., & Eickhoff, S. B. (2017). Altered Brain Activity in Unipolar Depression Revisited. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2016.2783

Raichle, M. E., & Raichle, M. E. (2001). Searching for a baseline: Functional imaging and the resting human brain. *Nature Reviews Neuroscience*. https://doi.org/10.1038/35094500

Regier, D. A., Kuhl, E. A., & Kupfer, D. J. (2013). The DSM-5: Classification and criteria changes. *World Psychiatry*. https://doi.org/10.1002/wps.20050

Scarpina, F., & Tagini, S. (2017). The stroop color and word test. In *Frontiers in Psychology*. https://doi.org/10.3389/fpsyg.2017.00557

Seeberg, I., Kjaerstad, H. L., & Miskowiak, K. W. (2018). Neural and behavioral predictors of treatment efficacy on mood symptoms and cognition in mood disorders: A systematic review. In *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2018.00337

Sheline, Y. I., Price, J. L., Yan, Z., & Mintun, M. A. (2010). Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1000446107

Shirer, W. R., Ryali, S., Rykhlevskaia, E., Menon, V., & Greicius, M. D. (2012). Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cerebral Cortex*, *22 (1)*, 158–165. https://doi.org/10.1093/cercor/bhr099

Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., Filippini, N., Watkins, K. E., Toro, R., Laird, A. R., & Beckmann, C. F. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0905267106

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*(SUPPL. 1), S208–S219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Soares, J. M., Magalhães, R., Moreira, P. S., Sousa, A., Ganz, E., Sampaio, A., Alves, V., Marques, P., & Sousa, N. (2016). A Hitchhiker's guide to functional magnetic resonance imaging. *Frontiers in Neuroscience*, *10*, 515. https://doi.org/10.3389/fnins.2016.00515

Strawbridge, R., Young, A. H., & Cleare, A. J. (2017). Biomarkers for depression: Recent insights, current challenges and future prospects. *Neuropsychiatric Disease and Treatment*, *13*, 1245–1262. https://doi.org/10.2147/NDT.S114542

Taylor, B. P., Bruder, G. E., Stewart, J. W., McGrath, P. J., Halperin, J., Ehrlichman, H., & Quitkin, F. M. (2006). Psychomotor slowing as a predictor of fluoxetine nonresponse in depressed outpatients. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.163.1.73

Wang, L., Hermens, D. F., Hickie, I. B., & Lagopoulos, J. (2012). A systematic review of resting-state functional-MRI studies in major depression. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2012.04.013

Wechsler, D. (1997). Wechsler adult intelligence scale - Third Edition (WAIS-III). *San Antonio*.

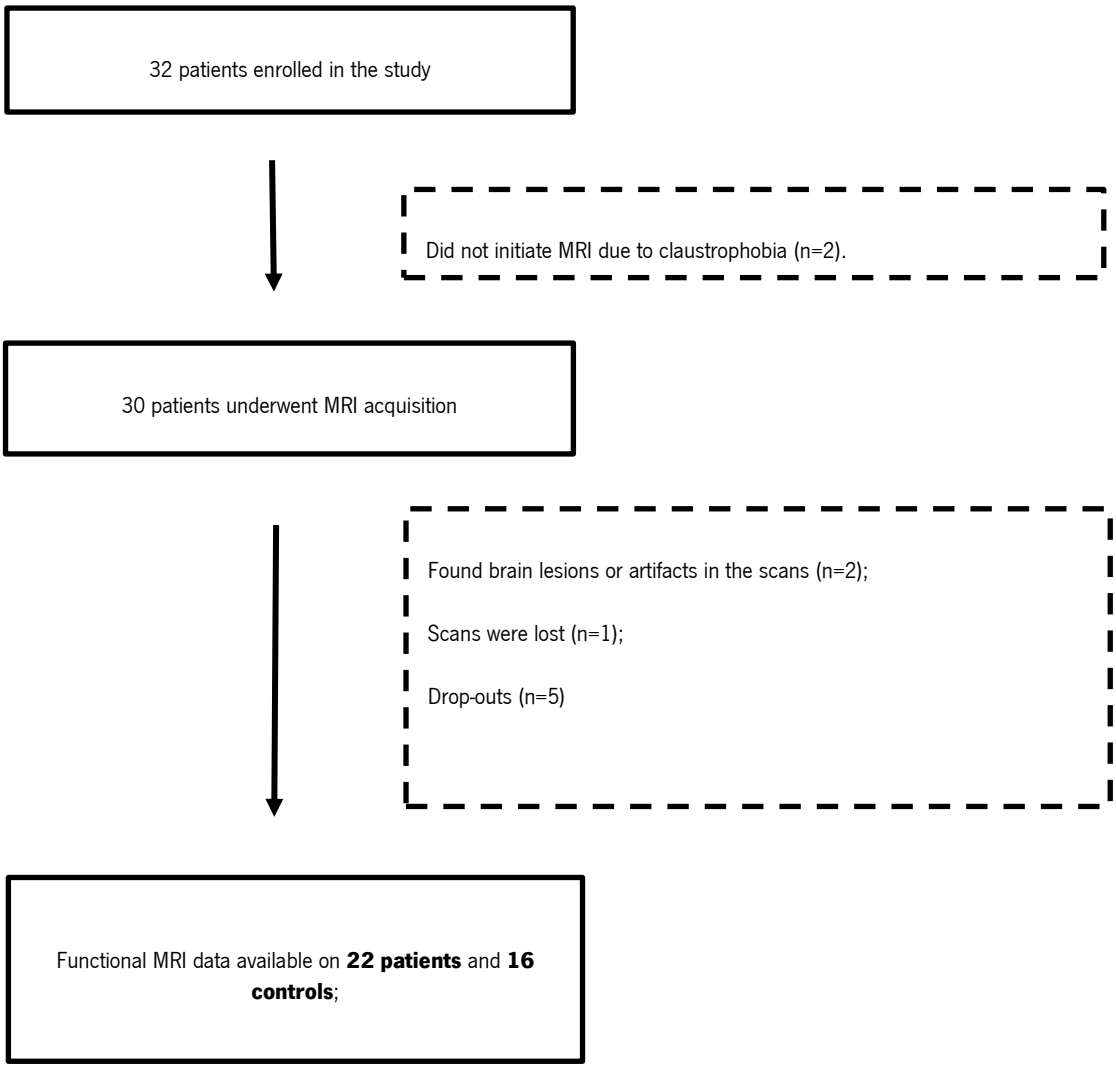
Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, *92*, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060

World Health Organization (WHO). (2021). *Depression. Fact Sheet*.

Zhu, X., Wang, X., Xiao, J., Liao, J., Zhong, M., Wang, W., & Yao, S. (2012). Evidence of a dissociation pattern in resting-state default mode network connectivity in first-episode, treatment-naive major depression patients. *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2011.10.035

Zou, K., Gao, Q., Long, Z., Xu, F., Sun, X., Chen, H., & Sun, X. (2016). Abnormal functional connectivity density in first-episode, drug-naive adult patients with major depressive disorder. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2015.12.081

Supplementary material



Supplementary Figure 1. Detailed information on subject recruitment and exclusion.

Supplementary Table 2: Descriptive statistics of the demographic, neuropsychological and cognitive data.

	Group	N	Mean	Std. Error Mean
Education (years)	MDD	22	11.5	1.09
	Control	17	14.53	0.99
Age	MDD	22	37	2,58
	Control	17	32	3.09
HAM-D	MDD	22	21.27	1.75
	Control	15	0.73	0.25
HAM-A	MDD	22	23.77	2.12
	Control	15	1.47	0.32
BSI	MDD	21	4.57	1.48
	Control	14	0	0
PSS-10	MDD	22	27.68	1.09
	Control	14	10.64	1.67
LTSS	MDD	22	39.82	3.36
	Control	14	51.36	4.24
Stroop	MDD	22	9.08	2.20
	Control	15	12.74	2.02
DSST	MDD	22	61.36	4.10
	Control	15	77.93	4.68
CLTR	MDD	22	34.5	3.45
	Control	14	46.71	5.59

Supplementary Table 3: Significant clusters obtained between drug-naïve MDD patients and healthy controls in intra-network connectivity.

	Network	*T* value	*P* value (corrected)	Cluster size	MNI Coordinates	Label
MDD>CTR	Ventral DMN	4.42	0.008	111	48 -50 24	Angular Gyrus R
	RECN	4.41	0.010	120	40 -74 32	Middle Occipital Gyrus R

CHAPTER II

Treatment response to paroxetine in drug-naïve MDD patients is associated with specific functional connectivity patterns.

Joana Reis, Carlos Portugal-Nunes, Rita Vieira, Ana Coelho, Ricardo Magalhães, Pedro Silva Moreira, Paulo Marques, Sónia Ferreira, Maria Pico-Pérez, Nuno Sousa, Nuno Dias, João Miguel Bessa

Manuscript under preparation.

Treatment response to paroxetine in drug-naïve MDD patients is associated with specific functional connectivity patterns.

Joana Reis[1,2,3], Rita Vieira[1,2,3], Carlos Portugal-Nunes[1,2,3], Ana Coelho[1,2,3], Ricardo Magalhães[1,2,3], Pedro Silva Moreira[1,2,3], Paulo Marques[1,2,3], Sónia Ferreira[1,2,3], Maria Pico-Pérez[1,2,3], Nuno Sousa[1,2,3], Nuno Dias[5], João Miguel Bessa[1,2,]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

[2]ICVS-3Bs PT Government Associate Laboratory, Braga/Guimarães, Portugal.

[3]Clinical Academic Center – Braga, Braga, Portugal

[4]Psychology Research Centre (CIPsi), School of Psychology, University of Minho, Braga, Portugal

[5]2Ai—School of Technology, Instituto Politécnico do Cávado e do Ave (IPCA), Barcelos, Portugal

***Corresponding author:** João Miguel Bessa, MD/PhD; Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal e-mail: joaobessa@med.uminho.pt

Abstract

Major depression disorder (MDD) was ranked by the WHO as the single largest contributor for disability worldwide. Despite the important role of antidepressant treatment, only 30-45% of MDD patients achieve remission of the symptoms. Indeed, for patients initializing pharmacological treatment, the search for the most effective antidepressant relies on a "watch and wait" approach that can lead to multiple trials of medication before finding an effective regimen.

In this study we investigated the short-term (first day of treatment) and long-term (6-12 weeks after treatment initiation) effects of paroxetine, a selective serotonin-reuptake inhibitor (SSRI), using functional magnetic resonance imaging (fMRI). Twenty-two treatment-naïve MDD patients were recruited and underwent an MRI session in the first day of pharmacological treatment and also 6 to 12 weeks after sustained treatment with a 20 mg daily dose of paroxetine. The results revealed effects of paroxetine in brain crossing several functional connectivity networks. In our cohort of MDD patients, poorer clinical improvement seems to be associated with higher FC within the executive and visuospatial networks as well as a lower connectivity within the ventral DMN measured before treatment initiation. Also, connectivity within the ventral DMN to the middle frontal gyrus was increased in non-responders prior to paroxetine medication, after 6-12 weeks of sustained treatment the connectivity was overall reduced. Paroxetine also reduced connectivity within the ventral DMN and Basal Ganglia networks hours after its intake. Long-term effects were able to distinguish responders and non-responders, namely the connectivity within regions of the LECN and sensorimotor networks. Other patterns of inter-network connectivity seem to indicate significant changes in network dynamics related to paroxetine treatment such as the connectivity between right ECN and the ventral DMN. This study showed alterations produced by paroxetine in the rs-FC, early in the course of the pharmacological treatment, that can lead to a better understanding of paroxetine treatment response in MDD.

Keywords: MDD, fMRI, Resting-state fMRI, SSRI, Paroxetine, functional connectivity.

Introduction

Major depression disorder (MDD) was ranked by the World Health Organization (WHO) as the single largest contributor for disability worldwide (World Health Organization, 2017). Depression is a complex

and heterogeneous disorder that is characterized by affective, cognitive, and somatic symptoms (Fried & Nesse, 2015). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria, the diagnosis of depression requires the experience of five or more symptoms during a 2-week period. Symptoms must include depressed mood or loss of interest/pleasure in activities as well as changes in body weight, sleep disturbances, psychomotor retardation, fatigue, feelings of worthlessness, diminished ability to concentrate, recurrent thoughts of death and suicidal ideation (American Psychiatric Association, 2013). Besides having a huge impact in public health systems, depression is a disorder that deeply affects an individual's life and it's reported to be the major contributor to suicide deaths, close to 800 000 per year (Kessler, 2012; World Health Organization, 2017).

The recommended first line of pharmacological treatment is the use of selective serotonin-reuptake inhibitors (SSRIs) (Gelenberg et al 2010)·(Koenig & Thase, 2009). However, after initial treatment only 30-45% of patients are able to achieve remission of the symptoms (Carvalho et al., 2009). For patients initializing pharmacological treatment, future response to antidepressants is hard to predict and often relies only on clinical judgement, leading to multiple trials of medication before finding an effective regimen. This failure to find an effective treatment increases the patient's suicide risk, disability, cognitive impairments as well as the risk for developing other co-morbidities (Ferrari et al., 2013)·(Katon et al., 2010).

Several studies have explored the underlying pathophysiology, as well as the characterization of the structural (Hellewell et al., 2019)·(Liao et al., 2013) and functional neurobiological correlates of MDD using neuroimaging techniques such as functional magnetic resonance imaging (fMRI) (Kerestes et al., 2014; Maglanoc et al., 2019; Mulders et al., 2015; Müller et al., 2017; Wang et al., 2012; Zou et al., 2016), namely in resting-state fMRI (RS-fMRI). The brain functional connectivity can also provide a meaningful way of understanding brain changes associated with an effective pharmacological regimen (Cheng et al., 2016)·(Gudayol-Ferré et al., 2015)·(Brakowski et al., 2017)·(An et al., 2017). An effective treatment was associated with a normalization of the connectivity in the DMN and also between cortico-limbic regions (Posner et al., 2013)·(Anand et al., 2007). Neuroimaging studies have focused on investigating alterations of the brain's resting state connectivity induced by antidepressants treatment to attempt to predict its efficacy at the early stages of pharmacological treatment (Cheng et al., 2016). More recently, other groups have studied the intrinsic connectomes of drug naïve MDD patients as a predictor of treatment response to escitalopram, sertraline or venlafaxine-XR. Their findings showed that responders presented an increased FC within the DMN and between the DMN and other RSNs (such as the visual,

limbic and frontoparietal and somatomotor networks) (Korgaonkar et al., 2019). Another study reported that sertraline treatment involved higher connectivity within the DMN and that higher connectivity between the DMN and the CEN predicted better clinical outcomes of sertraline treatment (Chin Fatt et al., 2020).

Clinical guidelines advise on switching to a different antidepressant after 4 to 12 weeks of sustained treatment with a therapeutic dose (Gelenberg et al., 2010). The possibility of identifying neurobiological predictors of clinical response earlier in the course of pharmacological therapy gives clinicians the advantage of evaluating and considering alternative treatments sooner, thus reducing the burden of disease. Herein we investigated the short-term (first day of treatment) and long-term (6-12 weeks after treatment initiation) effects of paroxetine in MDD patients aiming to find early indicators of treatment outcome in a sample of drug-naïve first episode MDD patients. To the best of our knowledge, this is the first attempt to describe FC alterations induced by paroxetine in drug-naïve patients, aiming to increase the knowledge about the neurobiological substrates underlying the response to this antidepressant, paving the way to uncover potential predictors of treatment response.

Subjects and Methods

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki (59th Amendment) and was approved by relevant local ethics review boards from University of Minho and Hospital de Braga (Braga, Portugal). The study goals, as well as the psychological and clinical assessments were explained to the participants, of whom all gave informed consent. The anonymity and confidentiality of the participants in the study were guaranteed during all research activities.

Study sample

A sample of patients diagnosed with the first depressive episode and without prior exposure to antidepressant treatment (treatment-naïve) were recruited by an experienced psychiatrist in the context of emergency psychiatric evaluations or the outpatient unit of the Psychiatry Department of the Hospital de Braga from January 2016 to January 2020. Diagnosis was confirmed with the SCID (structured clinical interview for DSM disorders) according to DSM-IV-TR (First et al., 2008).

The exclusion criteria were: age inferior to 18 years or superior to 65 years, prior medical history of neurological disorders or traumatic brain injury, prior medical history of addictive disorders, family history

of early onset dementia, cognitive impairments as defined by scores below or equal to 24 in the Mini-Mental Sate Examination (MMSE) test (Folstein et al., 1975). In accordance with the established criteria, 32 patients were first selected. From these, 2 were unable to perform the MRI due to claustrophobia, 2 were excluded due to brain abnormalities and 7 dropped out of the study before completion. Therefore, a total of 22 MDD subjects (8 males, 14 females) were included in the final analysis.

Participants performed neuropsychological evaluations in order to assess the dimensions of mood (Hamilton Depression scale – HAM-D) (Hamilton, 1960), anxiety (Hamilton Anxiety scale – HAM-A) (Hamilton, 1959), perceived stress (Perceived Stress Scale 10 – PSS-10) (Cohen, 1988), suicidal ideation (Beck Suicidal Ideation Scale - BSSI) (Beck et al., 1979) and cognition (Selective Reminding test – SRT (both long-term storage - LTS and consistent long-term retrieval - CLTR) (Buschke, 1973), Stroop test (Scarpina & Tagini, 2017), and Digit Symbol Substitution test - DSST) (Jaeger, 2018; Wechsler, 1997).

Study design

To evaluate the acute short-term effects of the SSRI paroxetine in the brain resting state functional connectivity of treatment naïve MDD patients, a single dose of paroxetine (20 mg) was orally administered, and each subject performed 3 different fMRI acquisitions in the first day of the pharmacological treatment (Figure 1). The first acquisition served as a baseline, before antidepressant administration, after which 2 more RS-fMRI were acquired in 2-hours intervals.

We chose to include patients prescribed with the SSRI paroxetine in our study in order to eliminate confounds associated with multiple drug targets. Also, paroxetine was preferred because it is one of the most potent and selective SSRI available, with given proof of its efficacy and effectiveness in treating MDD (K. et al., 2001; Nemeroff, 1994).

To observe disease progression and assess the chronic long-term effects of paroxetine treatment, patients were re-evaluated in the same fashion 6 to 12 weeks after treatment initiation (Figure 1). The percentage of depression symptoms reduction was calculated, using the HAM-D scores from both evaluation moments. Patients with a reduction inferior to 50% in the depressive symptoms were considered non-responders to the pharmacological treatment (Cusin et al., 2009).

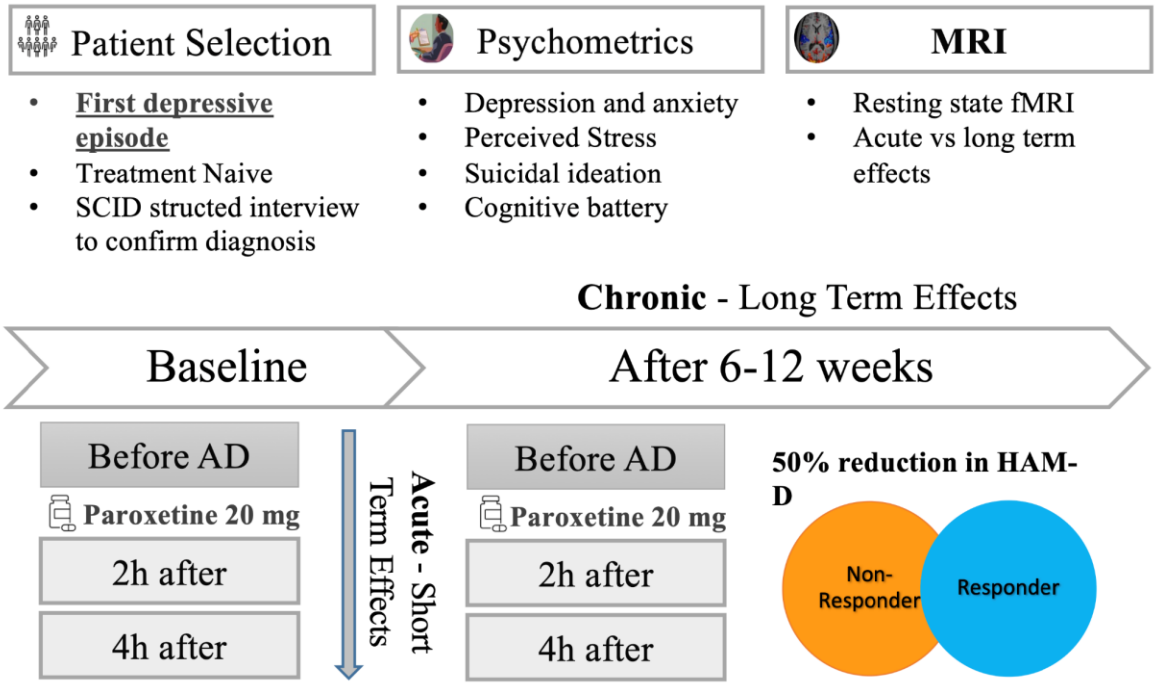


Figure 1: Graphical representation of the implemented **study design**. First depressive episode treatment naïve MDD patients were recruited and evaluated using a battery of neuropsychological tests. Acute (short term effects) and chronic (long term effects) of an oral dose of paroxetine (20 mg) were assessed using RS-fMRI. Patients were considered either responders or non-responders to the pharmacological treatment based on a 50% decrease in Hamilton Depression Rating Scale (HAM-D) scores after 6-12 weeks of pharmacological treatment.

MRI data acquisitions

MRI acquisitions were performed at Hospital de Braga with a clinical approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) that uses a 12-channel receive-only head coil. MRI acquisitions included morphological assessments and a resting state functional paradigm. Functional images were acquired using a BOLD sensitive echo-planar imaging (EPI) with the following parameters: 30 interleaved axial slices, repetition time (TR) = 2000 ms, echo time (TE) = 50 ms, flip angle (FA) = 90°, slice thickness = 3.5 mm, slice gap = 0.48 mm, in-plane resolution = 3.5 x 3.5 mm^2, field of view (FoV) = 224 mm and 190 volumes. Also, a T1-weighted structural image for anatomical reference was obtained using a magnetization-prepared rapid acquisition (MPRAGE) by gradient echo sequence with voxel resolution 1.0×1.0×1.0mm, FoV 234×234mm2, FA of 7°, 176 slices and TETR of 3.48/2730ms.

During functional resting state acquisitions, participants were instructed to remain in an awake and calm state, with their eyes closed while trying not to focus on any particular thought.

MRI data pre-processing

Preprocessing of fMRI data was done with custom scripts using FMRIB Software Library (FSL v6.01) tools (Smith et al., 2004).

In order to exclude possible magnetic field inhomogeneities at the beginning of the acquisition, the first step of preprocessing was the removal of the first 5 volumes (10 seconds). Then, because fMRI is measured using 2D imaging, there are temporal offsets between volumes that needed to be corrected. The slice timing correction was done with a temporal data interpolation that used the first acquired volume as reference. The next step was correcting the head motion, by using a function from FSL that searches motion parameters and uses them to correct movement in the time-series. Motion correction is an important step in fMRI preprocessing as the slightest movement can induce motion related artifacts. In order to further reduce contamination on functional connectivity due to motion, motion scrubbing was also performed to identify and exclude time-points where head motion could be critical. A non-linear spatial normalization to Montreal Neurological Institute (MNI) standard space was performed to register the set of brains to a common space/template. The procedure involved: i) skull stripping of the mean image of the functional acquisition; ii) rigid-body registration of the mean functional image to the skull stripped structural scan; iii) affine registration of the structural scan to the MNI T1 template; iv) non-linear registration of the structural scan to the MNI T1 template using the affine transformation estimated previously as the initial alignment; v) non-linear transformation of the functional acquisition to MNI standard space through the sequential application of the rigid-body transformation, followed by non-linear warp and resampling to 2x2x2 mm^3 voxel size. A linear regression using the mean of white matter and cerebrospinal fluid signals as well as motion outliers was performed, and the residuals of the regression were smoothed using a Gaussian kernel smoother with a full width half maximum of 6 mm (σ = 2.55 mm), band-pass temporal filtered (0.01–0.08Hz) and used for the subsequent analysis.

Resting-state Networks (RSNs)

Probabilistic independent component analysis (PICA) was performed with MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components), distributed with FSL v6.01. PICA is a data driven analysis that isolates components or non-overlapping spatial maps corresponding

to regions evidencing coherent time-courses(Beckmann & Smith, 2004; Soares et al., 2016). The software estimates group-wise spatial maps that correspond mainly to Resting State Networks (RSNs). A total number of 30 independent components were used. In order to study subject-specific components, a dual regression analysis was performed as well.

Thirteen RSNs (Figure 2) of interest were identified by visual inspection in combination with spatial correlations between the obtained components and RSN templates available from a public dataset from the Functional Imaging in Neuropsychiatric Disorders (FIND) Lab at Stanford University (Shirer et al., 2012).

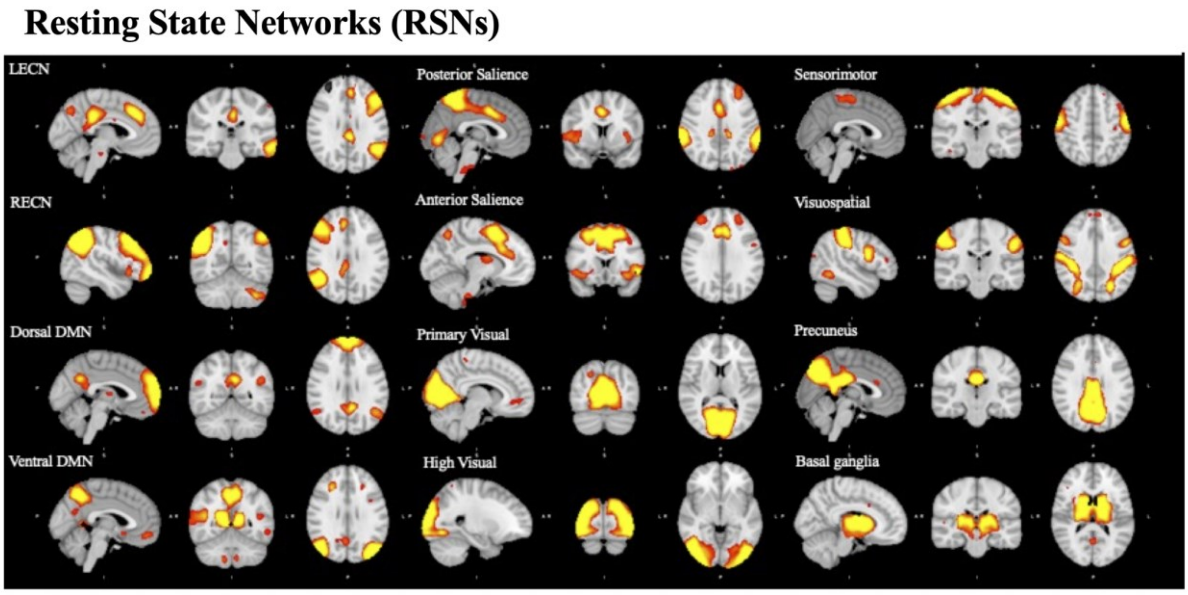


Figure 2: **Resting state networks** identified through MELODIC. Group MELODIC maps (LECN and RECN left and right executive networks, dorsal and ventral DMN, posterior and anterior salience networks, primary and high visual networks, sensorimotor network, visuospatial network, precuneus networks and basal ganglia network.

Statistics

Psychometric and demographic data

Statistical analysis of psychometric and demographic data was done using IBM SPSS Statistics V27. Normality was assessed using the Shapiro-Wilk Test. All demographic and psychometric variables followed a normal distribution except for the BSSI scale. Comparisons between responders and non-

responders were done using independent samples t-test. Differences were considered statistically significant for a p-value below 0.05.

Neuroimaging data

Intra-RSN FC was compared between groups (responders vs. non-responders), using independent samples t-tests with the non-parametric permutation procedure implemented in the *randomise* tool from FSL(Winkler et al., 2014). Threshold-free cluster enhancement (TFCE) was used to detect widespread significant differences and control the family-wise error rate (FWE-R) at α = 0.05. 5000 permutations were performed for each contrast.

Statistical group comparisons between groups (responders vs non-responders) across time points (acute and chronic) was done using a mixed model ANOVA. A non-parametric permutation procedure, PALM (permutation analysis of linear models) from FSL was used for these comparisons(Winkler et al., 2016). The same parameters were used (TFCE and FWE-R at α = 0.05, 5000 permutations for each contrast and the results were adjusted for the same confounding factors, sex and age).

For the analysis of inter-RSN FC, the time-series of each network were extracted and, using the Fisher's *Z*-transformed Pearson correlation coefficients, matrices of functional connectivity between pairs of RSNs were created. Individual correlations were statistically compared (α = 0.01) between groups and time-points. No results survived FDR or FWE corrections.

Results

Demographic data and Psychometric data

From the sample of 22 MDD patients included in this analysis, 14 (63.63%) were considered responders and 8 (36.36%) were considered non-responders after 6-12 weeks of sustained treatment with a daily dose of paroxetine (20 mg). Details about demographics and neuropsychological scales can be found in supplementary materials (Table S1). There was no significant difference in demographic variables (age and education) responders and non-responders (age: $t(20)=-0.859$, $p>0.05$; education: $t(20)=0.695$, $p>0.05$). Also, there was no significant difference in the re-evaluation period between responders and non-responders ($t(20)=-2.025$, $p>0.05$).

Participants were evaluated in the terms of their depression (HAM-D) and anxiety (HAM-A) as well as suicidal ideation (BSI) and perceived stress (PSS-10) scores. There was no significant differences, at baseline, between responders and non-responders in the scores of the HAM-D ($t(20)=0.286$, $p>0.05$), HAM-A ($t(20)=0.79$, $p>0.05$) and PSS-10 ($t(20)=-0.207$, $p>0.05$) scores (Figure 3a). Despite this, the non-responders scored higher than the responders in the Beck Suicidal Ideation scale (BSI) in the beginning of treatment (but not 6-12 weeks after).

Participants were also evaluated in the cognitive dimension using the SRT (long-term storage – LTS and consistent long-term retrieval – CLTR), Stroop, and DSST tests. No statistically significant differences were observed between responders and non-responders in any test (LTS: $t(20)=-0.912$, $p>0.05$, CLTR: $t(20)=-0.732$, $p>0.05$, Stroop: $t(20)=-0.630$, $p>0.05$ and DSST $t(20)=-1.571$, $p>0.05$) (Figure 3a) however non-responders tended to score less than responders in all tests.

RS-FC differences between responders and non-responders and correlates of clinical improvement

When comparing the patients that were responders and non-responders retrospectively, i.e., at the beginning of the treatment, the non-responders presented an increased connectivity to a small portion of the middle frontal gyrus within the ventral DMN. Responders however, presented an increased connectivity to the posterior middle temporal gyrus within the RECN when compared to non-responders (Figure 3b, Table S2).

Correlations with measures of clinical improvement (defined as the percentage of decline in depressive symptomatology after 6-12 weeks of sustained treatment, measured by the HAM-D scale) also revealed some interesting patterns of functional connectivity (Figure 3c, Table S2). A better clinical improvement was significantly correlated with higher connectivity within the left and right executive networks. Specifically, better clinical outcomes were observed in patients that presented higher connectivity to the angular gyrus within the LECN and to the posterior middle temporal gyrus within the RECN. Also, better clinical outcomes measured by the HAM-D scare were associated with higher connectivity within the visuospatial networks (both posterior frontal and parietal regions of the network).

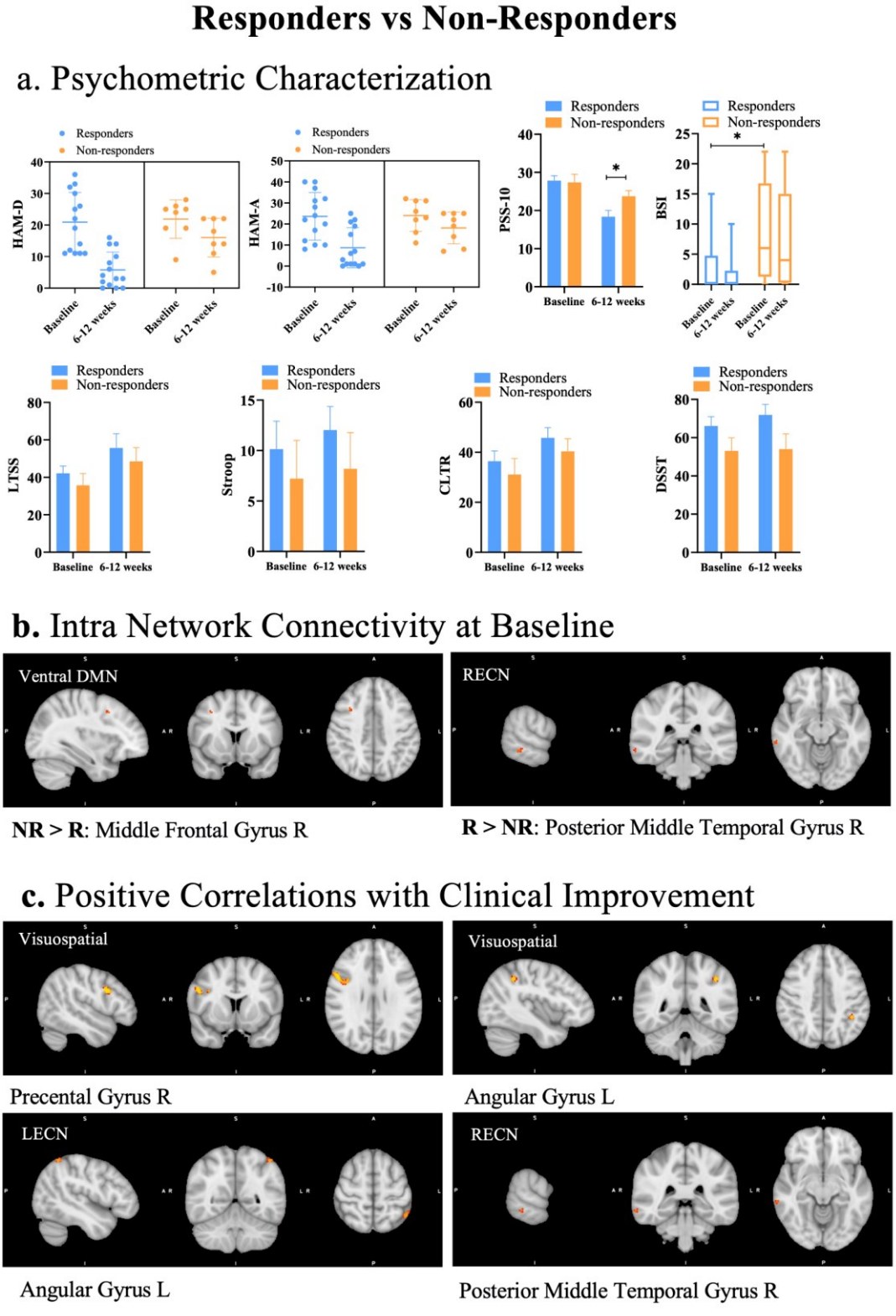


Figure 3: Differences between responders and non-responders in the a) psychometric tests at baseline and 6-12 weeks after treatment, b) intra network resting-sate functional connectivity at baseline and c) positive correlation with clinical improvement at baseline**.**

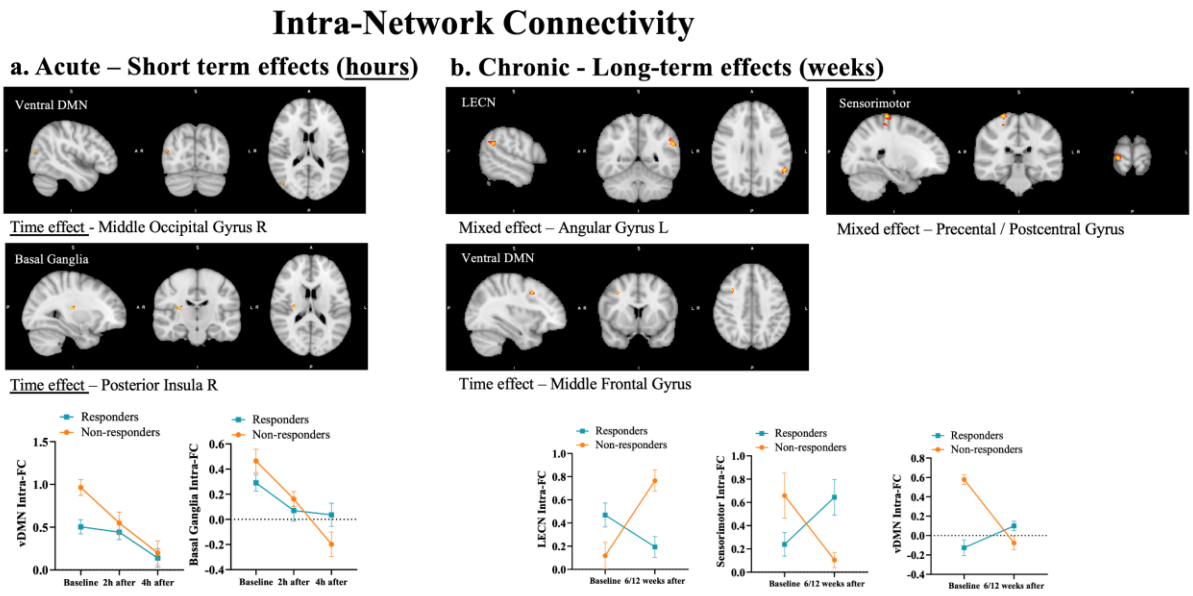


Figure 4: **Intra-Network connectivity** alterations between responders and non-responders, hours after the first antidepressant intake, (a) Acute short-term effects and after weeks of sustained treatment with a 20 mg dose of paroxetine (b) Chronic long-term effects (weeks).

RS-FC alterations following a single dose of paroxetine in drug-naïve patients

The acute effects of paroxetine in the intra-network connectivity revealed a time effect indicating that 20 mg dose of paroxetine had the short-term effect of reducing, for both groups, the connectivity to the middle occipital gyrus within the ventral DMN and the posterior insula within the basal ganglia network (Figure 4a, Table S2).

The acute effects of paroxetine in the inter-network connectivity (Figure 5a) showed a mixed effect (group*time after paroxetine) in the connectivity between the Sensorimotor and Anterior Salience networks. Also, the connectivity between the LECN and RECN was different between the groups (responders and non-responders) across all time points. The connectivity between the following networks: sensorimotor and primary visual, primary visual and RECN, LECN and Anterior Salience and ventral DMN and RECN, displayed a time effect. The connectivity between the sensorimotor and primary visual decreased after paroxetine intake for both groups (responders and non-responders). Differently, the connectivity between the primary visual and RECN, LECN and Anterior Salience and ventral DMN and RECN increased after the paroxetine intake for both groups of MDD. This result can indicate that a single dose of paroxetine could have and effect in altering the functional connectome of the brain.

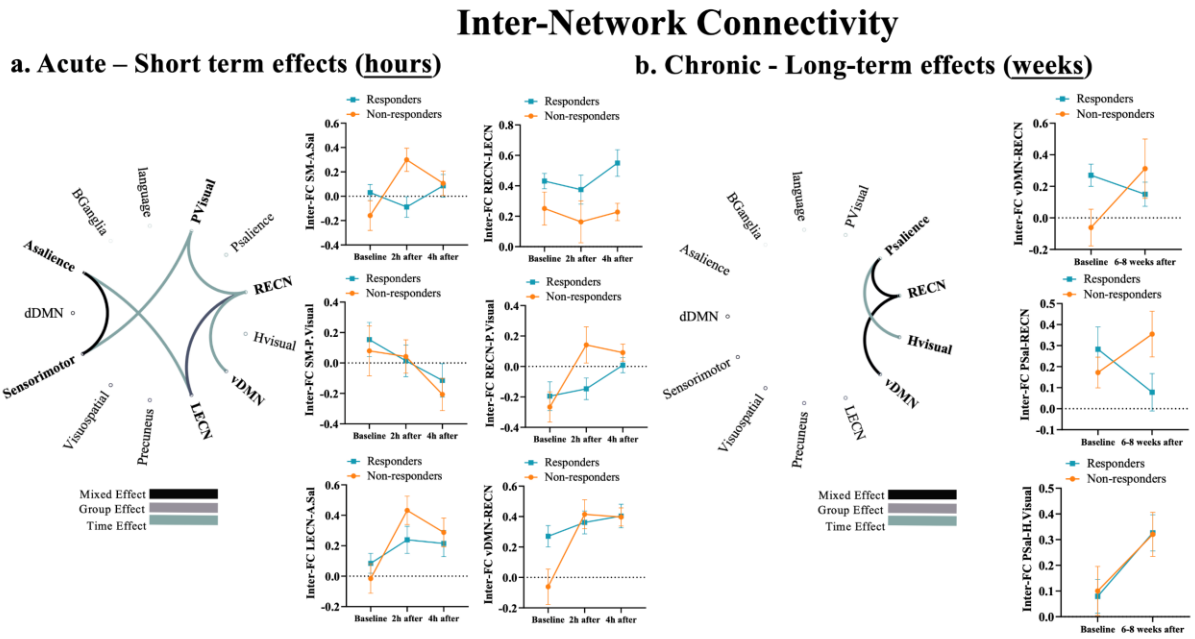


Figure 5: **Inter-Network connectivity** alterations between responders and non-responders, hours after the first antidepressant intake, (a) Acute short-term effects and after weeks of sustained treatment with a 20 mg dose of paroxetine (b) Chronic long-term effects (weeks).

RS-FC alterations following 6-12 weeks of paroxetine treatment

The long-term effects of paroxetine in the intra-network connectivity (Figure 4b) uncovered different patterns of FC between responders and non-responders across the LECN, Sensorimotor and ventral DMN. The intra-network FC within the LECN and sensorimotor networks showed a mixed effect (group*time after paroxetine intake for 6-12 weeks). The FC from the LECN to the angular gyrus was higher for the responders than the non-responders in the beginning of the pharmacological treatment and that difference inverted with the course of treatment. Differently, the connectivity from the sensorimotor network to the postcentral and precentral gyrus was higher for the non-responders in the beginning and lowered after 6-12 weeks of treatment. The intra-FC connectivity of the ventral DMN to the middle frontal gyrus was lowed after 6-12 weeks of treatment for both groups (Table S2).

The long-term effects of paroxetine in the inter-network connectivity (Figure 5b) showed a mixed effect (group*time after Paroxetine intake for 6-12 weeks) in the connectivity between the Posterior Salience and the RECN, and the ventral DMN and the RECN networks. The connectivity between the ventral DMN and the posterior salience and the RECN was increased for the responders in the beginning of the treatment, and the relationship inverted 6-12 weeks after treatment initiation. Also, the connectivity

between the high visual and the posterior salience networks increased after 6-12 weeks of treatment for both groups.

Discussion

The main goal of this study was to describe the alterations induced by the SSRI paroxetine in treatment naïve MDD patients. Such alterations can be of most importance in the pursue of neurobiological markers of AD response in depression.

From our sample of 22 drug naïve MDD patients, 8 were considered non-responders after 6-12 weeks of sustained treatment with a 20 mg dose of paroxetine. The definition for responsiveness to the treatment used in this study was the drop of 50% in depressive symptomatology measured by the HAM-D scale. Despite the evidence of demographic, genetic and clinical predictors of antidepressant treatment outcome (Gillett et al., 2020), in our sample, the patients that become non-responders after 6-12 weeks of treatment did not differ from the responders in any demographic variable (sex, age or education). Also, the groups did not differ in the anxiety and depression scores measured before treatment initiation. Such results can indicate that, in our sample of MDD patients, the responsiveness to the treatment cannot be attributed to a higher disorder severity present at baseline. Importantly, there were also no difference between groups in the time between baseline and re-evaluation. Similarly, no cognitive differences regarding verbal short-term memory or processing speed were found between responders and non-responders at baseline. To be noted, there was a trend in all cognitive tests (LTS, Stroop, DSST and CLTR) for worse performance in non-responders than responders. Such results indicate that in our sample, in line with the literature, MDD patients and in particular the non-responders presented poorer performance in the cognitive tests used (Bruder et al., 2014; Taylor et al., 2006).

Regarding neuroimaging results, the resting-state networks have proved to be a useful tool to obtain information about brain function independent of stimulus-based responses (Fox et al., 2006). In the beginning of the pharmacological treatment, the connectivity to the posterior middle temporal gyrus with the RECN was found to differentiate responders and non-responders. Also, a higher connectivity to the same region of the RECN was found to be correlated with a better clinical improvement after 6-12 weeks of sustained treatment with a 20 mg dose of paroxetine. Similarly, a higher connectivity to the angular gyrus with the LECN was correlated to a better clinical improvement. The angular gyrus is a hub where sensorial information is combined in order for the brain to orient attention, comprehend events and solve problems (Seghier, 2013). Moreover, the connectivity within the ventral DMN to a small portion of the

middle frontal gyrus was increased in non-responders compared to responders in the beginning of the treatment. After 6-12 weeks of treatment with paroxetine the difference was reduced and, even though continuing to exhibit symptoms of depression, non-responders were able to normalize the connectivity within the ventral DMN to the same region.

Overall, in our sample of MDD patients, a poorer clinical improvement seems to be associated with higher FC within the executive and visuospatial networks as well as a lower connectivity within the ventral DMN measured at baseline. These results can indicate that non-responders could present a dysfunction related with visuospatial and executive function as well as a bias towards introspective thoughts when compared with healthy controls and that these processes can impact the responsiveness to pharmacological treatment.

The connectivity within the ventral DMN was modulated hours after paroxetine intake. Both responders and non-responders were able to reduce connectivity to the middle occipital gyrus within the network. These results, together with previous evidence suggesting DMN connectivity as a potential biomarker for treatment response in MDD drug-naive patients, might indicate that, the decreased connectivity within the DMN (extensively associated with higher levels of rumination in depressed patients) might be potentially modified by paroxetine medication (Aizenstein et al., 2014; Nejad et al., 2013; Qin et al., 2015). A similar effect was found for the connectivity of the posterior insula within the Basal Ganglia network. Our results suggest that all patients decreased the connectivity to this region hours after paroxetine intake. Insula was described to have multiple functions in cognition and behavior ranging from sensorimotor to emotion, attention, and decision-making. Insula, specifically, its posterior part, was associated to sensorimotor processing functions, including somatic processing and pain.

The long-term assessment of MDD patients also revealed interesting patterns. Considering only the resting-state scans before the medication intake at baseline and 6-12 weeks after initiating the treatment, we showed a mixed effect (time*group) in the functional connectivity of the angular gyrus (within the LECN) and the precentral and postcentral regions (within the sensorimotor network). The connectivity to the angular gyrus was initially higher in the responders but, after a 6 to 12 weeks treatment with paroxetine, this difference was reversed. Overall, for the non-responders, the treatment seems to have increased the connectivity within the LECN (to the angular gyrus) and decreased within the sensorimotor network (to the pre and postcentral regions). Given the crucial role of the sensorimotor network in

processing sensorial and motor information (Göttlich et al., 2013), it could be relevant in the amelioration of the psychomotor symptoms of depression, such as psychomotor retardation.

Regarding inter-network FC analyses, the connectivity between the RECN and the ventral and dorsal portion of the DMN and the precuneus was increased in responders when compared to non-responders in the beginning of the treatment. The Connectivity between the RECN and the ventral DMN was also showed to be increased in responders compared to non-responders at baseline, suggesting a possible marker of acute treatment response in drug-naïve patients. In fact, this difference disappeared after 4 hours of paroxetine intake and continued to fade after 6-12 weeks of sustained treatment. Our results, along with the increased connectivity within the ventral DMN (to the middle frontal gyrus) might constitute a potential neurobiological markers of paroxetine treatment response visible in drug-naïve patients. Our Inter-network connectivity results showed that, a single dose of paroxetine in drug-naïve patients was able to have effects in the functional connectome of the brain, not only within specific networks but also in the way that networks are synchronized with each other.

After following the treatment for 6-12 weeks, inter-network connectivity alterations showed that both groups increased the FC between salience and visual areas. The mixed effects analysis revealed alterations in the connectivity between DMN and RECN regions. In fact, these differences observed at baseline appear to be reversed 6-12 weeks after treatment initiation (with the responders having higher FC levels than the non-responders). Interestingly, a single dose of paroxetine at baseline has had an effect of increasing the FC between DMN and RECN for both groups, and after 6-12 weeks treatment, the acute effect revealed that the non-responders presented higher values of FC that did not change after the administration of the same dose.

Altogether, the findings of this study reinforce the hypothesis that the FC between the ECNs and the DMN might be a potential marker of the responsiveness to the pharmacological treatment. Furthermore, the FC between these networks seems to be modulated by the paroxetine treatment, and the effects are visible just hours after the antidepressant intake.

The functional connectivity between the RECN and the ventral DMN was also showed to be increased in responders compared to non-responders at baseline, suggesting a possible marker of acute treatment response in drug-naïve patients. In fact, this difference disappeared after 4 hours of paroxetine intake and continued to fade after 6-12 weeks of sustained treatment. Our results, along with the increased

connectivity within the ventral DMN (to the middle frontal gyrus) might constitute a potential neurobiological markers of paroxetine treatment response visible in drug-naïve patients.

To the best of our knowledge, this study was the first to describe resting-state functional connectivity alterations in drug-naive MDD patients, measured on an hourly basis after the first dose intake of the SSRI paroxetine. Despite the innovative character of this study, there are some limitations that need to be considered when interpreting our findings. The sample of MDD patients included in this study, though relatively was small. was acquired between January 2016 and January 2020. Therefore, it would be of most importance to replicate the results described here in a larger sample, in order to validate them. The dose of 20 mg of paroxetine was administered orally and we have not considered the serum levels of the antidepressant for each patient. Such information could explain partially the heterogeneity of the results obtained in this study. Moreover, the MDD patients were recruited based on the prescribed medication being paroxetine, given its pharmacokinetics and potency. However, it would be interesting to follow the response to other SSRI, and even other classes of antidepressants, to compare the patterns of alterations produced in the RSN after the first exposure in drug naive MDD patients.

In conclusion, our study showed alterations produced by the SSRI paroxetine in the brain resting state functional connectivity, early in the course of pharmacological treatment. These alterations bring some light into the search for biological markers, and consequently lead to a better understanding of paroxetine treatment response in MDD patients. Such findings, although preliminary, can lead to other studies in the sense of replications and exploration of the predictive values, through the use of machine learning models.

Conflict of interest declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments

This work was funded by the Portuguese Foundation for Science and Technology (FCT) under scope of project PTDC/DTP-PIC/6936/2014.

This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020 and by the project NORTE-01-0145-FEDER-000039, supported by Norte Portugal Regional Operational Program (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

J.R. was supported by the FCT fellowship grant with the reference PDE/BDE/113602/2015 from the PhD-iHES program. R.V. was supported by the FCT fellowship grant with the reference PD/BDE/150619/2020 from the PhD-iHES program and UMINHO/BI/340/2018. C.P.N. was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Program in Ageing and Chronic Diseases. P.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113601/2015 from the PhD-iHES program. R.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113604/2015 from the PhD-iHES program. S.F. was supported by the FCT fellowship grant with the reference PD/BDE/127839/2016 from the PhD-iHES program.

References

Aizenstein, H. J., Khalaf, A., Walker, S. E., & Andreescu, C. (2014). Magnetic resonance imaging predictors of treatment response in late-life depression. *Journal of Geriatric Psychiatry and Neurology*. https://doi.org/10.1177/0891988713516541

American Psychiatric Association. (2013). American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.books.9780890425596.744053

Anand, A., Li, Y., Wang, Y., Gardner, K., & Lowe, M. J. (2007). Reciprocal effects of antidepressant treatment on activity and connectivity of the mood regulating circuit: An fMRI study. *Journal of Neuropsychiatry and Clinical Neurosciences*. https://doi.org/10.1176/jnp.2007.19.3.274

An, J., Wang, L., Li, K., Zeng, Y., Su, Y., Jin, Z., Yu, X., & Si, T. (2017). Differential effects of antidepressant treatment on long-range and short-range functional connectivity strength in patients with major depressive disorder. *Scientific Reports*. https://doi.org/10.1038/s41598-017-10575-9

Beck, A. T., Kovacs, M., & Weissman, A. (1979). Assessment of suicidal intention: The Scale for Suicide Ideation. *Journal of Consulting and Clinical Psychology*. https://doi.org/10.1037/0022-006X.47.2.343

Beckmann, C. F., & Smith, S. M. (2004). Probabilistic Independent Component Analysis for Functional Magnetic Resonance Imaging. *IEEE Transactions on Medical Imaging*, *23(2)*, 137-152. https://doi.org/10.1109/TMI.2003.822821

Brakowski, J., Spinelli, S., Dörig, N., Bosch, O. G., Manoliu, A., Holtforth, M. G., & Seifritz, E. (2017). Resting state brain network function in major depression – Depression symptomatology, antidepressant treatment effects, future research. In *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2017.04.007

Bruder, G. E., Alvarenga, J. E., Alschuler, D., Abraham, K., Keilp, J. G., Hellerstein, D. J., Stewart, J. W., & McGrath, P. J. (2014). Neurocognitive predictors of antidepressant clinical response. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2014.04.057

Buschke, H. (1973). Selective reminding for analysis of memory and learning. *Journal of Verbal Learning and Verbal Behavior*. https://doi.org/10.1016/S0022-5371(73)80034-9

Carvalho, A. F., Machado, J. R., & Cavalcante, J. L. (2009). Augmentation strategies for treatment-resistant depression. In *Current Opinion in Psychiatry*. https://doi.org/10.1097/YCO.0b013e32831be9ef

Cheng, Y., Xu, J., Arnone, D., Nie, B., Yu, H., Jiang, H., Bai, Y., Luo, C., Campbell, R. A. A., Shan, B., Xu, L., & Xu, X. (2016). Resting-state brain alteration after a single dose of SSRI administration predicts 8-week remission of patients with major depressive disorder. *Psychological Medicine*. https://doi.org/10.1017/S0033291716002440

Chin Fatt, C. R., Jha, M. K., Cooper, C. M., Fonzo, G., South, C., Grannemann, B., Carmody, T., Greer, T. L., Kurian, B., Fava, M., McGrath, P. J., Adams, P., McInnis, M., Parsey, R. V., Weissman, M., Phillips, M. L., Etkin, A., & Trivedi, M. H. (2020). Effect of Intrinsic Patterns of Functional Brain Connectivity in Moderating Antidepressant Treatment Response in Major Depression. *The American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2019.18070870

Cohen, S. (1988). Perceived stress in a probability sample of the United States. In *The social psychology of health.*

Cusin, C., Yang, H., Yeung, A., & Fava, M. (2009). Rating Scales for Depression. In *Handbook of Clinical Rating Scales and Assessment in Psychiatry and Mental Health*. https://doi.org/10.1007/978-1-59745-387-5_2

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., Vos, T., & Whiteford, H. A. (2013). Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. *PLoS Medicine*. https://doi.org/10.1371/journal.pmed.1001547

First, M. B., Williams, J. B. W., Karg, R. S., Spitzer, R. L., & Gibbon, M. (2008). Structured Clinical interview for DSM-IV Axis I Disorders (SCID-I). *Handbook of Psychiatric Measures*.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*. https://doi.org/10.1016/0022-3956(75)90026-6

Fox, M. D., Corbetta, M., Snyder, A. Z., Vincent, J. L., & Raichle, M. E. (2006). Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0604187103

Fried, E. I., & Nesse, R. M. (2015). Depression is not a consistent syndrome: An investigation of unique symptom patterns in the STAR*D study. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2014.10.010

Gelenberg, A.J., Freeman, M.P., Markowitz, J.C., Rosenbaum, J.F., Thase, M.E.,... Trivedi, M. H. (2010). Practice guideline for the treatment of patients with major depressive disorder. In *American Psychiatric Association*. https://doi.org/10.1176/appi.books.9780890423387.654001

Gillett, G., Tomlinson, A., Efthimiou, O., & Cipriani, A. (2020). Predicting treatment effects in unipolar depression: A meta-review. In *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2020.107557

Göttlich, M., Münte, T. F., Heldmann, M., Kasten, M., Hagenah, J., & Krämer, U. M. (2013). Altered resting state brain networks in Parkinson's disease. *PloS One*, *8*(10), e77336. https://doi.org/10.1371/journal.pone.0077336

Gudayol-Ferré, E., Peró-Cebollero, M., González-Garrido, A. A., & Guàrdia-Olmos, J. (2015). Changes in brain connectivity related to the treatment of depression measured through fMRI: A systematic review. In *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2015.00582

Hamilton, M. (1959). The assessment of anxiety states by rating. *British Journal of Medical Psychology*. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry*, *23*, 56–62.

Hellewell, S. C., Welton, T., Maller, J. J., Lyon, M., Korgaonkar, M. S., Koslow, S. H., Williams, L. M., Rush, A. J., Gordon, E., Grieve, S. M., & Gordon, E. (2019). Profound and reproducible patterns of reduced regional gray matter characterize major depressive disorder. *Translational Psychiatry*. https://doi.org/10.1038/s41398-019-0512-8

Jaeger, J. (2018). Digit symbol substitution test. *Journal of Clinical Psychopharmacology*. https://doi.org/10.1097/JCP.0000000000000941

Katon, W., Unützer, J., & Russo, J. (2010). Major depression: The importance of clinical characteristics and treatment response to prognosis. *Depression and Anxiety*. https://doi.org/10.1002/da.20613

Kerestes, R., Davey, C. G., Stephanou, K., Whittle, S., & Harrison, B. J. (2014). Functional brain imaging studies of youth depression: A systematic review. In *NeuroImage: Clinical*. https://doi.org/10.1016/j.nicl.2013.11.009

Kessler, R. C. (2012). The costs of depression. In *Psychiatric Clinics of North America*. https://doi.org/10.1016/j.psc.2011.11.005

K., K., S.L., W., R., S., A., G., G.J., E., R., D., P., S., X.-H., Z., R., H., Kroenke, K., West, S. L., Swindle, R., Gilsenan, A., Eckert, G. J., Dolor, R., Stang, P., Zhou, X. H., Hays, R., & Weinberger, M. (2001). Similar effectiveness of paroxetine, fluoxetine, and sertraline in primary care: A randomized trial. *Journal of the American Medical Association*.

Koenig, A. M., & Thase, M. E. (2009). First-line pharmacotherapies for depression - What is the best choice? In *Polskie Archiwum Medycyny Wewnetrznej*. https://doi.org/10.20452/pamw.743

Korgaonkar, M. S., Goldstein-Piekarski, A. N., Fornito, A., & Williams, L. M. (2019). Intrinsic connectomes are a predictive biomarker of remission in major depressive disorder. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0574-2

Liao, Y., Huang, X., Wu, Q., Yang, C., Kuang, W., Du, M., Lui, S., Yue, Q., Chan, R. C. K., Kemp, G. J., & Gong, Q. (2013). Is depression a disconnection syndrome? Meta- analysis of diffusion tensor imaging studies in patients with MDD. *Journal of Psychiatry and Neuroscience*. https://doi.org/10.1503/jpn.110180

Maglanoc, L. A., Landrø, N. I., Jonassen, R., Kaufmann, T., Córdova-Palomera, A., Hilland, E., & Westlye, L. T. (2019). Data-Driven Clustering Reveals a Link Between Symptoms and Functional Brain Connectivity in Depression. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. https://doi.org/10.1016/j.bpsc.2018.05.005

Mulders, P. C., van Eijndhoven, P. F., Schene, A. H., Beckmann, C. F., & Tendolkar, I. (2015). Resting-state functional connectivity in major depressive disorder: A review. In *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2015.07.014

Müller, V. I., Cieslik, E. C., Serbanescu, I., Laird, A. R., Fox, P. T., & Eickhoff, S. B. (2017). Altered Brain Activity in Unipolar Depression Revisited. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2016.2783

Nejad, A. B., Fossati, P., & Lemogne, C. (2013). Self-referential processing, rumination, and cortical midline structures in major depression. In *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2013.00666

Nemeroff, C. B. (1994). The Clinical Pharmacology and Use of Paroxetine, a New Selective Serotonin Reuptake Inhibitor. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. https://doi.org/10.1002/j.1875-9114.1994.tb02799.x

Posner, J., Hellerstein, D. J., Gat, I., Mechling, A., Klahr, K., Wang, Z., McGrath, P. J., Stewart, J. W., & Peterson, B. S. (2013). Antidepressants normalize the default mode network in patients with dysthymia. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2013.455

Qin, J., Shen, H., Zeng, L. L., Jiang, W., Liu, L., & Hu, D. (2015). Predicting clinical responses in major depression using intrinsic functional connectivity. *NeuroReport*. https://doi.org/10.1097/WNR.0000000000000407

Scarpina, F., & Tagini, S. (2017). The stroop color and word test. In *Frontiers in Psychology*. https://doi.org/10.3389/fpsyg.2017.00557

Seghier, M. L. (2013). The angular gyrus: Multiple functions and multiple subdivisions. In *Neuroscientist*. https://doi.org/10.1177/1073858412440596

Shirer, W. R., Ryali, S., Rykhlevskaia, E., Menon, V., & Greicius, M. D. (2012). Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cerebral Cortex*, *22 (1)*, 158–165. https://doi.org/10.1093/cercor/bhr099

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*(SUPPL. 1), S208–S219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Soares, J. M., Magalhães, R., Moreira, P. S., Sousa, A., Ganz, E., Sampaio, A., Alves, V., Marques, P., & Sousa, N. (2016). A Hitchhiker's guide to functional magnetic resonance imaging. *Frontiers in Neuroscience*, *10*, 515. https://doi.org/10.3389/fnins.2016.00515

Taylor, B. P., Bruder, G. E., Stewart, J. W., McGrath, P. J., Halperin, J., Ehrlichman, H., & Quitkin, F. M. (2006). Psychomotor slowing as a predictor of fluoxetine nonresponse in depressed outpatients. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.163.1.73

Wang, L., Hermens, D. F., Hickie, I. B., & Lagopoulos, J. (2012). A systematic review of resting-state functional-MRI studies in major depression. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2012.04.013

Wechsler, D. (1997). Wechsler adult intelligence scale - Third Edition (WAIS-III). *San Antonio*.

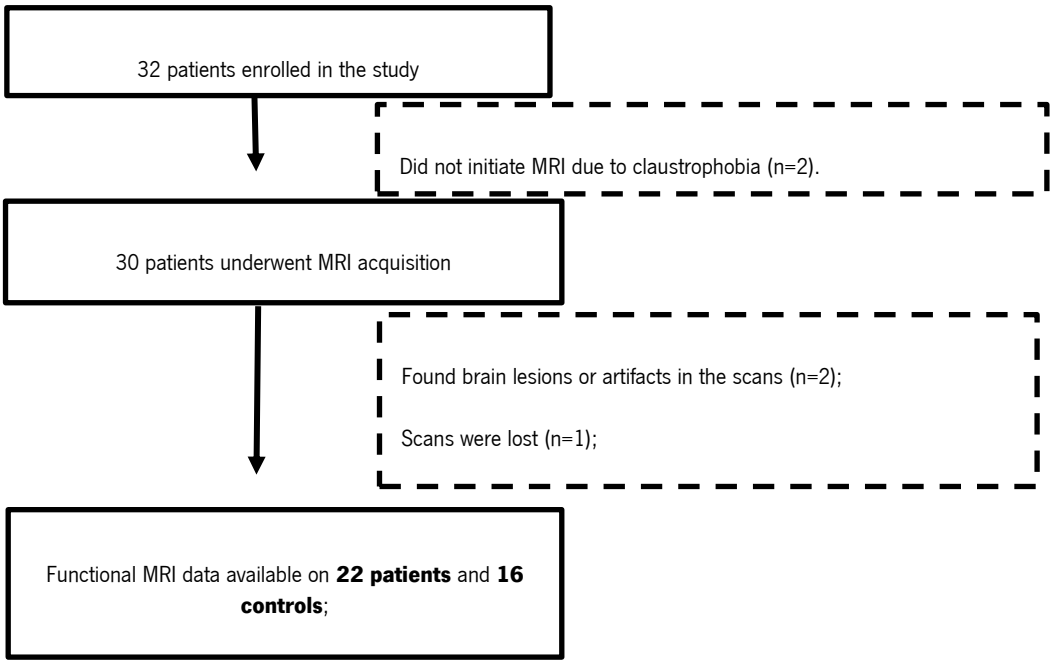
Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, *92*, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060

Winkler, A. M., Webster, M. A., Brooks, J. C., Tracey, I., Smith, S. M., & Nichols, T. E. (2016). Non-parametric combination and related permutation tests for neuroimaging. *Human Brain Mapping*. https://doi.org/10.1002/hbm.23115

World Health Organization. (2017). Depression and other common mental disorders: global health estimates. *World Health Organization*. https://doi.org/CC BY-NC-SA 3.0 IGO

Zou, K., Gao, Q., Long, Z., Xu, F., Sun, X., Chen, H., & Sun, X. (2016). Abnormal functional connectivity density in first-episode, drug-naive adult patients with major depressive disorder. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2015.12.081

Supplementary material



Supplementary Figure 1. Detailed information on subject recruitment and exclusion.

Supplementary Table 4: Descriptive statistics between non-responders and responders after 6-12 weeks of sustained treatment with 20 mg of paroxetine. Table describes demographic variables (years of education and age), depression (HAM-D), anxiety (HAM-A), suicidal ideation (BSSI), perceived stress (PSS-10) as well as scores in cognitive tests (LTSS, Stroop, DSST and CLTR) at baseline and 6-12 weeks after treatment initiation.

			Baseline		6-12 weeks	
	Group	**N**	**Mean**	**Std. Error Mean**	**Mean**	**Std. Error Mean**
Education (years)	Non-responders	8	10.25	1.87		
	Responders	14	12.21	1.36		
Age	Non-responders	8	39.75	5.67		
	Responders	14	35.43	2.55		
HAM-D	Non-responders	8	21.88	2.16	16	2.18
	Responders	14	20.93	2.51	5.79	1.51
HAM-A	Non-responders	8	24	2.69	18.13	2.68
	Responders	14	23.64	3.03	8.71	2.54
BSI	Non-responders	8	8	2.99	7.38	2.93
	Responders	13	2.46	1.30	2.29	1.23
PSS-10	Non-responders	8	27.38	2.15	23.75	1.46
	Responders	14	27.86	1.27	18.38	1.65
LTSS	Non-responders	8	35.75	6.30	48.5	7.41
	Responders	14	42.14	3.90	55.67	7.61
Stroop	Non-responders	8	7.21	3.80	8.19	3.59
	Responders	14	10.14	2.76	12.03	2.34
DSST	Non-responders	8	53.13	6.78	54	7.94
	Responders	14	66.07	4.89	71.92	5.46
CLTR	Non-responders	8	31.13	6.38	40.38	5.06
	Responders	14	36.43	4.11	45.75	4.07

Supplementary Table 5: Significant neuroimaging results obtained for the acute and long-term comparison including t-values, corrected p-values, cluster-sizes as well as MNI coordinates and labels of the peak value of each cluster.

	Network	*P* value (corrected)	Cluster size	MNI Coordinates	Label
R vs NR Correlations with CI	**Ventral DMN** - NR>R	0.031	4	34 12 42	Middle Frontal Gyrus R
	RECN- R>NR	0.030	19	64 -36 -12	Posterior Middle Temporal Gyrus R
	LECN - Correlation CI (positive)	0.018	41	-46 -54 58	Angular Gyrus L
	RECN - Correlation CI (positive)	0.027	13	66 -36 -10	Posterior Middle Temporal Gyrus R
	Visuospatial - Correlation CI (positive)	0.005	184	50 10 28	Precentral Gyrus R
		0.007	36	-40 -46 42	Angular Gyrus L
Acute Short term effects (hours)	**Ventral DMN**– Time effect	0.037	8	46 -76 16	Middle Occipital Gyrus R
	Basal Ganglia – Time effect	0.017	19	30 -18 16	Posterior Insula R
Chronic Long-term effects (weeks)	**LECN** – Mixed effect	0.013	77	-56 -52 28	Angular Gyrus L
	Sensorimotor – Mixed effect	0.021	76	24 -30 74	Precentral Gyrus R
		0.038	15	24 -32 60	Postcentral Gyrus R
	Ventral DMN – Time effect	0.008	13	34 10 42	Middle Frontal Gyrus

CHAPTER III

Suicidal Ideation is associated with reduced functional connectivity and white matter integrity in drug-naïve patients with major depression

Joana Reis, Rita Vieira, Carlos Portugal-Nunes, Ana Coelho, Ricardo Magalhães, Pedro Silva Moreira, Sónia Ferreira, Maria Pico-Pérez, Nuno Sousa, Nuno Dias, João Miguel Bessa

Article published in Frontiers in Psychiatry

March, 2022

DOI: 10.3389/fpsyt.2022.838111

Link: https://doi.org/10.3389/fpsyt.2022.838111

Suicidal Ideation is associated with reduced functional connectivity and white matter integrity in drug-naïve patients with major depression

Joana Reis[1,2,3], Rita Vieira[1,2,3], Carlos Portugal-Nunes[1,2,3], Ana Coelho[1,2,3], Ricardo Magalhães[1,2,3], Pedro Silva Moreira[1,2,3], Sónia Ferreira[1,2,3], Maria Pico-Pérez[1,2,3], Nuno Sousa[1,2,3], Nuno Dias[5], João Miguel Bessa[1,2,]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

[2]ICVS-3Bs PT Government Associate Laboratory, Braga/Guimarães, Portugal.

[3]Clinical Academic Center – Braga, Braga, Portugal

[4]Psychology Research Centre (CIPsi), School of Psychology, University of Minho, Braga, Portugal

[5]2Ai—School of Technology, Instituto Politécnico do Cávado e do Ave (IPCA), Barcelos, Portugal

***Corresponding author:** João Miguel Bessa, MD/PhD; Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal e-mail: joaobessa@med.uminho.pt

Abstract

Depression is a highly prevalent psychiatric disorder affecting millions of people worldwide. Depression is characterized by decreased mood or loss of interest in daily activities, changes in feeding and circadian rhythms and significant impairments in cognitive and executive function. In addition, the occurrence of recurrent thoughts of death and suicidal ideation confers depressed patients a higher risk of suicide than the general population. With this study, we aimed to explore the neural correlates of suicidal ideation in drug-naïve patients diagnosed with depression.

Twenty-five patients were scanned using two-different magnetic resonance imaging (MRI) modalities, resting state functional MRI (fMRI) and diffusion tensor imaging (DTI). Resting state allowed the exploration of connectivity patterns in the absence of a specific stimulus and DTI allowed a detailed analysis of structural white matter integrity with measures like fractional anisotropy (FA). Probabilistic independent component analysis (PICA), network-based statistics and tract-based spatial statistics (TBSS) were applied to analyze resting-state fMRI and DTI data, respectively. Our results showed that, in our sample of drug-naïve patients, suicidal ideation was negatively associated with resting-state functional connectivity in the visual networks and with FA in the genu of corpus callosum and in the right anterior corona radiata. In addition, a significant association was identified between suicidal ideation and a functional connectivity network that included connections between regions in the superior and orbitofrontal cortex, the cerebellum, the cingulate gyrus as well as temporal and occipital regions.

In conclusion, this work has expanded our knowledge about the possible functional and structural neuronal correlates of suicidal ideation in drug-naïve patients with depression, paving the way for future personalized therapeutic approaches.

Keywords: Major depression, Suicide, Suicidal ideation, fMRI, DTI

Introduction

Depression affected nearly 300 million people around the world in 2019 (Abbafati et al., 2020) and it is considered by the world health organization (WHO) as the single largest contributor for disability worldwide(World Health Organization, 2017). Nowadays, its prevalence might be even higher due to the challenges imposed by the COVID-19 pandemic (Bueno-Notivol et al., 2021; Luo et al., 2020). The symptoms of depression include depressed mood or loss of interest in daily activities but also, changes in body weight, sleep disturbances, psychomotor retardation, fatigue, feelings of worthlessness, diminished ability to concentrate, recurrent thoughts of death and suicidal ideation(American Psychiatric Association, 2013). In fact, depression is one of the most common diagnosis at the time of death by suicide (Ferrari et al., 2013; Nock et al., 2009) being depressed patients 20 times more likely to commit suicide than the general population (Lépine & Briley, 2011; Ösby et al., 2001). It has been reported that more than 700 000 deaths are a result of suicide (Kessler, 2012; World Health Organization (WHO), 2021) and it is estimated that for each death by suicide, there is a much higher number of suicidal attempts, increasing the personal, social, and economic burden of the disorder (Crosby et al., 2011; Lépine & Briley, 2011).

Suicide is a complex phenomenon in which many factors could be implicated, especially in MDD patients. Even though the specific pathophysiological pathways of suicide are not yet fully understood, recent research suggest that suicide is a result of not a single factor but rather a complex interaction between genetic vulnerability as well as exogenous and endogenous stressors (D. Wasserman, 2016; Orsolini et al., 2020).

The first step on this complex pathway is suicidal ideation, reported as one of the major risk factors for suicide (Franklin et al., 2017). Its presence as a symptom of depression was previously associated with poorer treatment response(Lopez-Castroman et al., 2016) and deficits in cognitive functions, such as executive functions(Marzuk et al., 2005; Pu et al., 2017) and verbal learning(Lan et al., 2020). One third of the patients experiencing suicidal ideation will act on their thoughts(Nock et al., 2008). Hence, suicidal ideation is a red flag for clinical concern, and it is an important part of the suicide risk assessment guidelines(Kleber et al., 2007).

In the past decade, there has been an exponential growth of studies employing neuroimaging techniques to investigate structural, functional, and metabolic brain processes related to suicidal thoughts and behaviors(Schmaal et al., 2020). A commonly used neuroimaging technique is magnetic resonance

imaging (MRI), given that its different modalities, such as structural MRI, diffusion tensor imaging (DTI) and functional MRI (fMRI), allow a wide characterization of the brain. Specifically, they provide information on gray and white matter anatomy, structure, as well as brain activity and connectivity. In the literature, these MRI modalities have been used to explore the neural correlates of suicidal ideation due to its importance on the suicide pathway.

At the functional level, previous studies have described the functional networks associated with suicidal ideation in depressed patients, showing a relation between decreased functional connectivity with suicidal ideation(Chen, Chou, et al., 2021; Kim et al., 2017). The functional networks included similar frontal brain regions, such as the orbitofrontal gyrus, but also distinct regions like the middle occipital gyrus, superior parietal gyrus, thalamus, and caudate(Chen, Chou, et al., 2021; Kim et al., 2017). Other studies, using seed-based approaches reported increased resting-state functional connectivity (rsFC) between both sides of hippocampus(Chen, Chou, et al., 2021), amygdala-precuneus/cuneus (Wei et al., 2018) and between the left habenula and the left cerebellum (Qiao et al., 2020) when comparing depressed patients with and without suicidal ideation. In contrast, decreased rsFC between the right anterior cingulate cortex and the right middle temporal pole and the orbitomedial prefrontal cortex(Du et al., 2017), as well as between the right habenula and the right precuneus and the left inferior frontal gyrus (Qiao et al., 2020) were also described when comparing the same groups of patients with depression.

Alterations in gray and white matter have been reported in depressed patients with suicidal ideation(Chen, Chou, et al., 2021; Myung et al., 2016; Taylor et al., 2015). In particular, patients with suicidal ideation showed decreased cortical thickness in the left hemisphere, mainly in the insula when compared to patients without suicidal ideation(Taylor et al., 2015); however, there were no statistically significant differences in their volumes(Taylor et al., 2015). The authors also found that depressed patients with suicidal ideation presented increased radial diffusivity (RD) and decreased fractional anisotropy (FA) in the hippocampal part of the cingulum buddle, superior and posterior corona radiata (CR) as well as anterior thalamic radiation (ATR), when compared to depressed controls(Taylor et al., 2015). These findings suggest a disruption in the white matter integrity of bundles described above, responsible to connect frontal brain regions and basal ganglia(Taylor et al., 2015). Another study also reported decreased white matter integrity in depressed patients with suicidal ideation in tracts connecting frontal regions, namely corpus callosum(Chen, Kao, et al., 2021). Indeed, Myung and

collaborators(Myung et al., 2016) also identified a brain network with decreased structural connectivity in frontal, parietal, occipital, and subcortical regions (i.e., thalamus, caudate and pallidum).

These findings suggest that alterations in structure and function of frontal-subcortical networks are implied in suicidal ideation in MDD. Nevertheless, more studies are required in order to consolidate these results and establish the brain patterns related to suicidal ideation. Thus, combining multimodal MRI techniques seems crucial to level up the characterization of the pathophysiology and etiology of suicidal ideation in MDD, which may lead to the development of useful approaches to identify high-risk patients and targeted treatments for suicidal ideation.

In the present study, our main objective was to explore the neural correlates of suicidal ideation in drug-naïve depressed patients at the whole-brain level, using two different MRI modalities, namely resting-state fMRI, and DTI. For that purpose, probabilistic independent component analysis (PICA), network-based statistics (NBS) and tract-based spatial statistics (TBSS) were applied to analyze resting-state fMRI and DTI data, respectively. Our main hypothesis was that suicidal ideation would be associated with decreased functional connectivity as well as white matter integrity.

Subjects and Methods

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki (59th Amendment) and was approved by relevant local ethics review boards from University of Minho and Hospital de Braga (Braga, Portugal). The study goals, as well as the psychological and clinical assessments were explained to the participants, of whom all gave informed consent. The anonymity and confidentiality of the participants in the study were guaranteed during all research activities.

Characterization of participants

A sample of treatment-naïve (first depressive episode and without prior exposure to antidepressant treatment) MDD patients were recruited in the context of emergency psychiatric evaluations or the outpatient unit of the Psychiatry Department of the Hospital de Braga. The recruitment took place

between January of 2016 and January of 2020. The diagnosis was confirmed with the SCID (structured clinical interview for DSM disorders) according to DSM-IV-TR(First et al., 2008).

The inclusion criteria were; being drug-naive, first-episode MDD patient, aged 18-65 years-old, without psychiatric or neurological comorbidities, The exclusion criteria were: age inferior to 18 years or superior to 65 years, presenting any MRI contradictions, comorbid psychiatric disorders (e.g. bipolar disorder, addictive disorders and schizophrenia), prior medical history of neurological disorders or traumatic brain injury and sign of cognitive impairment defined by scores below or equal to 24 in the Mini-Mental Sate Examination (MMSE) test (Folstein et al., 1975).

In accordance with the established criteria, 32 patients were first selected. From these, 7 were not included in this analysis because either they were unable to perform the MRI, or they had to be excluded due to brain abnormalities. Therefore, a total of 25 MDD subjects (8 males, 17 females) were included in the final analysis of resting-state MRI data and 23 MDD subjects (7 males, 16 females) were included in the final analysis of diffusion data (2 subjects did not perform diffusion acquisitions).

All participants performed a psychological assessment in the same day of the MRI acquisition. 17-item Hamilton Depression Rating Scale HAM-D)(Hamilton, 1960) and 14-item Hamilton Anxiety Rating Scale HAM-A)(Hamilton, 1959) were used for the assessment of depression and anxiety severity in the past week, respectively. Both scales are clinician-administered. 10-item Perceived Stress Scale PSS-10 (Cohen, 1988) was used to evaluate the global perceived stress in the past month. Beck Scale for Suicidal Ideation BSSI (Beck, Aaron T and Steer, 1991) is a self-reported scale used to detect and measure the severity of current suicidal ideation. It comprises 21 items: the first 19 items focus on several characteristics of suicidal ideation; the last two items assess the number of previous suicide attempts and the intention to die associated with it. Each item is rated on a 3-point scale (0-2). The total score is computed by adding the first 19 items of the scale, ranging from 0 to 38. There are no specific cut-off scores; higher total scores reflect higher severity of suicidal ideation.

MRI acquisitions

MRI acquisitions were performed at Hospital de Braga with a clinical approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) using a 12-channel receive-only head coil. All patients underwent the same MRI acquisition protocol. Even though the protocol included other acquisitions, for the present study only the resting-state functional images and the diffusion-weighted imaging (DWI) sequences were considered.

Functional images were acquired using a BOLD sensitive echo-planar imaging (EPI) with the following parameters: 30 interleaved axial slices, repetition time (TR) = 2000 ms, echo time (TE) = 50 ms, flip angle (FA) = 90°, slice thickness = 3.5 mm, slice gap = 0.48 mm, in-plane resolution = 3.5 x 3.5 mm^2, field of view (FoV) = 224 mm and 190 volumes. Also, a T1-weighted structural image for anatomical reference was obtained using a magnetization-prepared rapid acquisition (MPRAGE) by gradient echo sequence with voxel resolution 1.0×1.0×1.0mm, FoV 234×234mm2, FA of 7°, 176 slices and TE/TR of 3.48/2730ms. During functional resting state acquisitions, participants were instructed to remain in an awake and calm state, with their eyes closed while trying not to focus on any particular thought.

DWI scans were performed using a spin echo–echo planar imaging (SE-EPI) sequence: TR = 8,800 ms, TE = 99 ms, FoV = 240 mm × 240 mm, acquisition matrix = 120 × 120, 61 two-millimeter axial slices with no gap, 30 non-collinear gradient directions with b = 1,000 s mm^{-2}, one b = 0 s mm^{-2} acquisition, and one repetition.

Before data pre-processing, the raw acquisitions from all participants were visually inspected to discard any brain lesions, critical head motion, or artifacts that could compromise the data.

MRI data pre-processing

Resting-state fMRI

Preprocessing of fMRI data was performed using custom scripts using FMRIB Software Library (FSL v6.01) tools(Smith et al., 2004). In order to exclude possible magnetic field inhomogeneities at the beginning of the acquisition, the first step of preprocessing was the removal of the first 5 volumes (10 seconds). Then, because fMRI is measured using 2D imaging, there are temporal offsets between volumes that needed to be corrected. The slice timing correction was done with a temporal data interpolation that used the first acquired volume as reference. The next step was correcting the head motion, by using a function from FSL that searches motion parameters and uses them to correct movement in the time-series. Motion correction is an important step in fMRI preprocessing as the slightest movement can induce motion related artifacts. To further reduce contamination on functional connectivity due to motion, motion scrubbing was also performed to identify and exclude time-points where head motion could be critical. A non-linear spatial normalization to Montreal Neurological Institute (MNI) standard space was performed to register the set of brains to a common space/template. The procedure involved: i) skull stripping of the mean image of the functional acquisition; ii) rigid-body registration of the mean functional image to the skull stripped structural scan; iii) affine registration of

the structural scan to the MNI T1 template; iv) non-linear registration of the structural scan to the MNI T1 template using the affine transformation estimated previously as the initial alignment; v) non-linear transformation of the functional acquisition to MNI standard space through the sequential application of the rigid-body transformation, followed by non-linear warp and resampling to 2x2x2 mm^3 voxel size. A linear regression using the mean of white matter and cerebrospinal fluid (CSF) signals as well as motion outliers was performed, and the residuals of the regression were smoothed using a Gaussian kernel smoother with a full width half maximum of 6 mm (σ = 2.55 mm), band-pass temporal filtered (0.01–0.08Hz) and used for the subsequent analysis.

Resting-state Networks (RSNs)

Probabilistic independent component analysis (PICA) was performed with MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components), distributed with FSL v6.01. PICA is a data driven analysis that isolates components or non-overlapping spatial maps corresponding to regions evidencing coherent time-courses(Beckmann & Smith, 2004; Soares et al., 2016). The software estimates group-wise spatial maps that correspond mainly to Resting State Networks (RSNs). A total number of 30 independent components were used. In order to study subject-specific components, a dual regression analysis was performed as well.

Twelve RSNs (Supplementary Figure 2) of interest were identified by visual inspection in combination with spatial correlations between the obtained components and RSN templates available from a public dataset from the Functional Imaging in Neuropsychiatric Disorders (FIND) Lab at Stanford University(Shirer et al., 2012).

Matrices of functional connectivity

The matrices of functional connectivity were constructed using the 116 cortical, subcortical, and cerebellar areas of the Anatomical Automatic Labeling (AAL) atlas[28]. The mean signal for each brain region was extracted and the Pearson's correlations coefficients between each possible pair of regions were calculated, originating a symmetric adjacency matrix, R. Coefficients in matrix R were then transformed to Z-scores using Fisher's r-to-z transform.

DWI Image Pre-processing and Tensor Fitting

Diffusion data were pre-processed using the FMRIB Diffusion Toolbox (FDT) provided with FSL v6.0.3. DWI images were corrected for motion artifacts and eddy current distortions. Then, the affine transformations were used to register each volume and were applied to rotate gradient vectors. Afterwards, the first b_0 volume of each subject was extracted and skull stripped, creating a brain mask applied to the remaining volumes to remove non-brain structures.

Tensor fitting and the scalar maps computation steps were performed with DTIFIT, also included in the FDT toolbox. In this step, a diffusion tensor model is fitted at each voxel and scalar maps of FA and Mean Diffusivity (MD), as well as eigenvector and eigenvalue maps, were generated. Axial Diffusivity (AD) was defined as the principal diffusion eigenvalue, and RD was computed using the mean of the second and third eigenvalues.

Tract-Based Spatial Statistics

Voxel-wise analyses of the scalar maps between subjects were performed using TBSS procedures(Smith et al., 2006), also part of FSL. To remove potential outliers from the tensor fitting, all FA templates were slightly eroded, and the end slices were zeroed. Afterward, all the FA templates were non-linearly registered into a 1-mm × 1-mm × 1-mm standard space. This step was performed by non-linearly registering each subject's FA template to each other to find the "most representative one" (i.e., the one that requires the least warping to align all images), subsequently used as the study-specific target image. Next, the selected target image was affine transformed into the MNI 152 standard space, and each subject's FA template was transformed into this standard space by combining the non-linear transformation to the study-specific target with the affine transformation into the MNI space. Then, the FA templates of all subjects were averaged, and the resulting image skeletonized. After visual inspection of the skeletonized image, we threshold it at 0.35 to remove from the skeleton regions encompassing other tissues, such as gray matter or CSF. Finally, all scalar maps (FA, AD, MD, and RD) were projected into this FA skeleton using the same transformation applied to the FA templates.

Statistics

Psychometric and demographic data

Descriptive statistics of psychometric and demographic data were performed using IBM® SPSS® Statistics (version 27; IBM Corp., Armonk, NY, United States). Normality was assessed using the Shapiro-Wilk Test. All demographic and psychometric variables followed a normal distribution except for the BSSI scale where the distribution was tailed towards left-hand. Correlations between non-parametric variables were performed using the Kendall's tau (τ). Results were considered significant for a *p-value* below 0.05.

RSN Analysis

RSN FC were correlated with the standardized score (z-score) obtained in the BSSI scale using a regression model with the non-parametric permutation procedure implemented in the *randomise* tool from FSL(Winkler et al., 2014). Threshold-free cluster enhancement (TFCE) was used to detect widespread significant differences and control the family-wise error rate (FWE-R) at α = 0.05. 5000 permutations were performed for each contrast. Results were corrected for sex, age, and years of education.

Network based statistics (NBS)

To assess whether suicidal ideation (BSSI score) was associated with the functional connectome, a regression model using the Network Based Statistics (NBS) was applied[31]. The NBS method provides a correction for multiple comparisons equivalent to FWE-R by estimating the probability of identifying in a random permutation of the data, networks larger in extent than the ones identified by the hypothesis tested. The method consists in two parts. In the first part, the statistical hypothesis is tested for each connection, filtered by a user-chosen statistical edge threshold. and significant network components are identified. In a significant network component, a node can be reached from any other through significant connections. The size of the component is calculated by the number of significant connections in it and although the user-defined threshold is not a determinant of the significance of the network, larger thresholds reveal more widespread networks and smaller thresholds produce smaller and more focused results. A set of different primary thresholds (0.005, 0.001, 0.0005 and 0.0001) was used, as suggested by the toolbox authors, to access different levels of sensibility. In the second part, random permutations of the data were created, and the same methodology described in the first part is applied to each permutation and the size of the network components found is calculated. The calculated distribution of components size is used to estimate the probability of finding random components with a size greater

than the one found in our hypothesis. A total of 5000 permutations were used, together with an FWE corrected network significance of 0.05. Results were corrected for sex, age, and years of education.

Diffusion Data

In a similar way to the analyses performed with RSN FC, non-parametric permutation methods were employed using the *randomise* tool from FSL (Winkler et al., 2014), in order to analyze the skeletonized maps of FA, AD, MD, and RD. Particularly, linear regression models were performed to investigate whether WM microstructure measures were associate with suicidal ideation (z-scores of BSSI score). These models were adjusted for age, gender/sex and years of education.

Widespread significant differences were detected with TFCE, and multiple comparisons were corrected using FWE-R at $\alpha = 0.05$ and cluster extent threshold of $K > 50$. Clusters showing significant results were identified using the John Hopkins University ICBM-DTI-81 WM Labels Atlas and dilated with the *tbss_fill* tool (included in FSL) for visualization purposes.

Results

Psychometric and demographic data

Descriptive statistics of psychometric and demographic data are detailed in Table 1. Our cohort was composed of 25 drug-naïve MDD patients (9 males and 16 females) with an age mean of 37 years (ranging from 19 to 59 years of age). In our sample 10 patients presented no current suicidal ideation (BSSI score=0) and 22 patients presented no previous history of suicide attempt.

In our cohort, suicidal ideation (BSSI) was not found to be significantly correlated with depression (HAM-D, τ=0.155, p-value=0.375), anxiety (HAM-A, τ=0.069, p-value =0.659) or perceived stress (PSS-10, τ=0.083, p-value =0.602) as well as demographic variables such as, years of education (τ=0.165, p-value =0.292) or age (τ=0.101, p-value =0.511).

Table 1. Demographic and psychological characterization of the sample.

	N	Mean	Std. Error Mean	95% CI lower	95% CI upper	Min	Max

Age (years)	25	37.44	2.47	32.35	42.53	19	59
Sex (Male/Female)	9/16						
Education (years)	25	11.64	0.99	9.60	13.68	4	20
HAM-D	25	20.72	1.45	17.72	23.72	9	33
HAM-A	25	22.96	1.77	19.30	26.62	8	40
PSS-10	25	27.36	1.07	25.15	29.57	17	36
BSSI	25	5.12	1.46	2.10	8.14	0	23
Previous SA	3						

Notes. HAM-D, Hamilton Depression Rating Scale; HAM-A, Hamilton Anxiety Rating Scale; PSS-10, Perceived Stress Scale 10 items; BSSI, Beck Suicidal Ideation Scale; SA, suicide attempts; CI, confidence interval; Min, minimum; Max, maximum.

RS-FC correlates of suicidal ideation in drug-naïve MDD

Resting State Networks (RSNs)

Thirty independent components were obtained using the MELODIC approach. From these components, 12 were consistent with the typical and identifiable RSN (Supplementary Figure 2) (Damoiseaux et al., 2006).

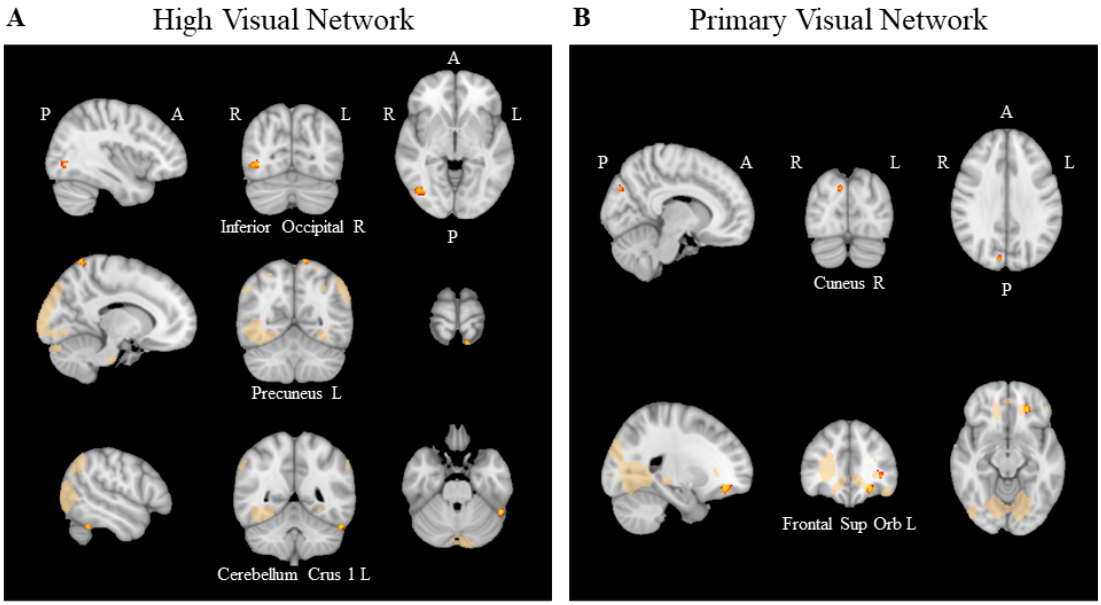
Our results revealed positive and negative correlations between suicidal ideation and functional connectivity to the primary and high visual networks (Figure 1). Statistical analysis revealed that in our cohort of drug naïve MDD patients, the FC between the high visual network and the right inferior occipital gyrus was positively correlated with suicidal ideation (FWE-corrected p-value=0.013, Figure 1A and 1C). In the other hand, the FC between the same network and the left precuneus and cerebellum were negatively correlated with suicidal ideation (FWE-corrected p-value=0.011, FWE-corrected p-value=0.007, respectively, Figure 1A and 1C). As for the primary visual network, the connectivity to the right cuneus had a positive association with suicidal ideation, inversely the connectivity to the frontal superior orbital gyrus was negatively correlated (FWE-corrected p-value=0.015, FWE-corrected p-value=0.007, respectively, Figure 1B and 1C). Figure 1C presents details on statistical analysis obtained for each cluster (including MNI coordinates, FWE-corrected peak p-values and cluster sizes).

Network-based Statistics

From the connectivity analysis performed using the NBS method, a single network was found to be significantly correlated with suicidal ideation (BSSI). In our cohort of MDD patients, a higher FC in this network was related to lower scores in the BSSI scale. The NBS analysis was performed using thresholds between 0.005 and 0.0001 (Supplementary Tables 1 and 2). In figure 2, we present the results found

for the significant threshold p-value=0.001 t(threshold)=3.78, df=23, p-value (network)=0.025, 15 nodes and 15 edges. From the set of tested thresholds this was the network that showed a higher mean FC for the lowest p-value.

Of this network we emphasize the connections between the cerebellum (node #106) and the nodes in the left superior frontal gyrus (node #3), the frontal middle orbital cortex (nodes #25 and #26), the rectus (nodes #27 and #28) and the right inferior and superior occipital gyrus (nodes #54 and #50). This network also presented relevant connections between the left superior frontal gyrus (node #3) and the left middle temporal gyrus (node #85) and the cuneus (nodes #45 and #46) and the posterior cingulate gyrus (nodes #35 and #36).



C

RSNs	MNI coordinates at signal peak			Cluster size	FWE-corrected p	Correlation	AAL label
	x	y	z				
High Visual	38	-72	-4	97	0.013	Positive	Inferior Occipital R
	-12	-58	74	34	0.011	Negative	Precuneus L
	-52	-50	-28	30	0.007	Negative	Cerebellum Crus 1 L
Primary Visual	10	-82	32	21	0.015	Positive	Cuneus R
	-22	38	-12	155	0.008	Negative	Frontal Sup Orb L

Figure 3: Resting State Networks (RSNs) using ICA A) Significant correlations between suicidal ideation and functional connectivity with the high visual network and the B) primary visual network. Significance threshold was set to $p < 0.05$ (FWE-R correction for multiple comparisons). C) Table illustrating the clusters positively and negatively associated with suicidal ideation score in each network.

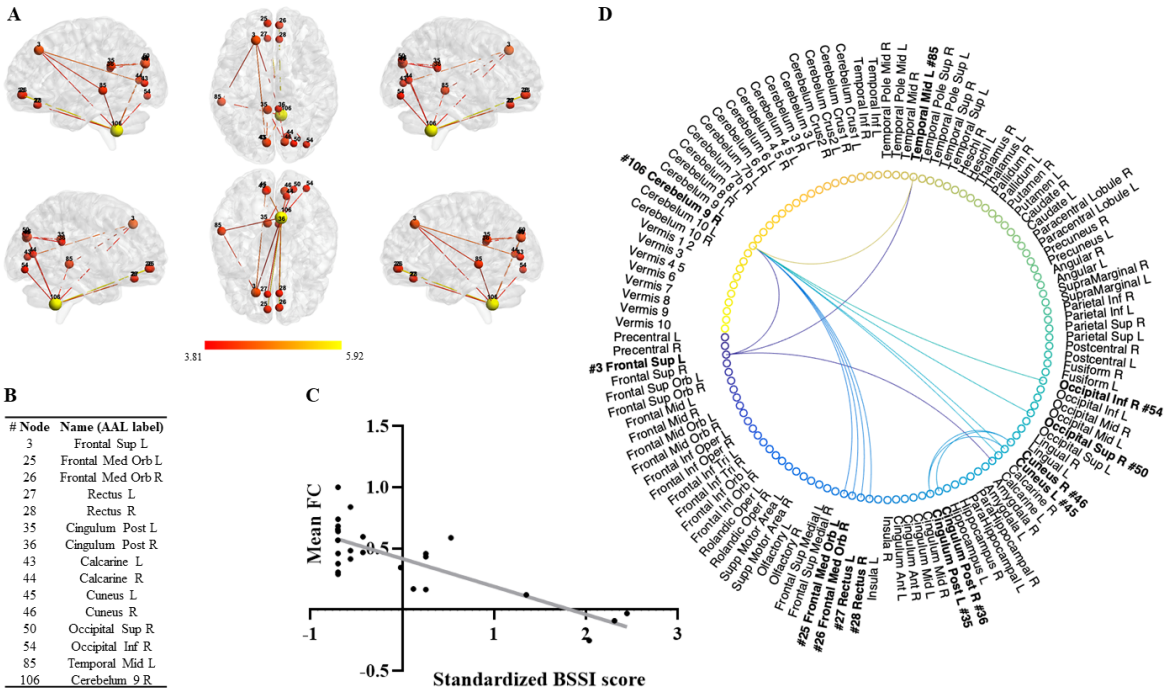
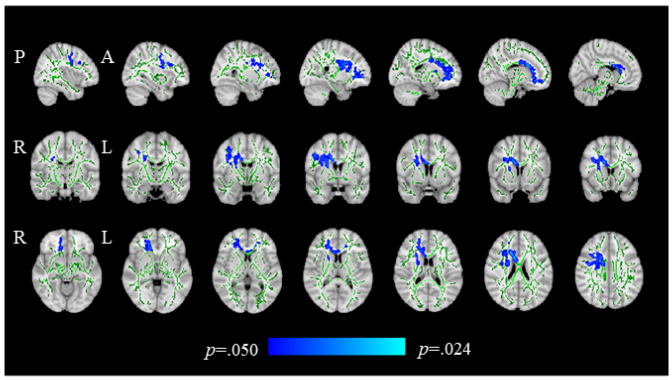


Figure 4: Network Based Statistics (NBS) A. Significant negative correlation between suicidal ideation and a functional connectivity network (adjusted for age, sex, and years of education) using the NBS method. Network presented for the significant threshold p-value=0.001 t(threshold)=3.78, df=23, p-value (network)=0.025, 15 nodes and 15 edges. **B)** Nodes comprising the network and the correspondent AAL label. **C)** Scatterplot illustrating the negative correlation between FC and suicidal ideation for each patient. The x-axis represents average values of FC and y-axis the standardized suicide ideation severity score on BSSI. The grey line represents the overall regression line. **D)** Circular graphical representation of the nodes and edges of the network.

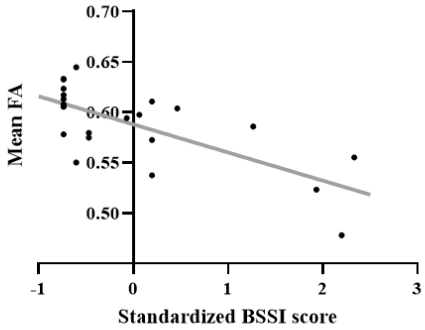
WM microstructure correlates of suicidal ideation in drug-naïve MDD

The statistical analysis of the skeletonized maps revealed a statistically significant negative correlation between suicidal ideation scores and FA maps (Figure 3A). Patients with higher suicidal ideation scores showed a significant decrease in FA maps in clusters including the genu of the Corpus Callosum and the right anterior Corona Radiata (Figure 3B). Figure 3C presents details on statistical analysis obtained for each cluster (including MNI coordinates, FWE-corrected peak p-values and cluster sizes). No significant correlations were found between suicidal ideation score and AD, MD, and RD maps.

A



C



B

Tract at signal peak	MNI coordinates at signal peak			Cluster size	FWE-corrected p
	x	y	z		
Genu of Corpus Callosum	15	21	21	1624	0.024
	17	27	-6	659	0.042
	1	25	7	104	0.047
	16	26	16	61	0.050
R Anterior Corona Radiata	24	15	16	51	0.049

Figure 5: Tract-Based Spatial Statistics (TBSS) A**.** Significant negative correlation between suicidal ideation and FA maps in drug-naïve patients with Major Depression (adjusted for age, sex, and years of education). Blue/light-blue voxels indicate a significant decrease in FA. Significance threshold was set to $p < 0.05$ (FWE-R correction for multiple comparisons). The WM skeleton (represented in green) is superimposed on a T1-weighted MNI template. **B.** Table illustrating the clusters negatively associated with suicidal ideation score. **C.** Scatterplot illustrating the negative correlation between FA of the significant clusters and suicidal ideation score. The x-axis represents average values of FA and y-axis the standardized suicide ideation severity score on BSSI. The grey line represents the overall regression line.

Discussion

The present study aimed to investigate the neuronal correlates of suicidal ideation in drug-naïve depressed patients using a multimodal MRI approach, specifically resting-state fMRI, and DTI. In our sample of MDD patients, three (12%) reported previous history of suicide attempt, whereas in previous studies including drug-naïve depressed patients 16%-20% reported suicide attempts in the past (Yang et al., 2019; Zhao et al., 2020; Zhou et al., 2020).

Our results showed that, in our cohort of MDD patients, suicidal ideation was negatively correlated with rsFC in the visual networks and FA in the genu of corpus callosum and the right anterior CR. Moreover,

we identified a specific rsFC network associated with suicidal ideation that included connections between regions in the superior and orbitofrontal cortex, the cerebellum, as well as temporal and occipital regions. Moreover, patients' scores on HAM-D were not correlated with BSSI scores, suggesting that in our cohort of patients with depression, higher suicidal ideation is not associated with higher depression severity.

Regarding the functional correlates of suicidal ideation in MDD, the results in our cohort showed that suicidal ideation was correlated with functional connectivity with the visual RSN, both high and primary visual networks. These results seem to indicate a dysregulation in rsFC involving brain regions such as the right inferior occipital gyrus (positive correlation), the left precuneus and cerebellum (negative correlation) and their interaction with the regions that compose the high visual network. In accordance, the connectivity between primary visual network and the right cuneus was positively correlated with suicidal ideation. Finally, the connectivity between left frontal superior orbital gyrus and the primary visual network was negatively correlated with the suicidal ideation in MDD. These results in the RSN seem to be in line with the ones obtained using a different approach to FC. In fact, the NBS method revealed a network of FC that was also shown to be negatively correlated with suicidal ideation in our cohort of MDD patients before the initiation of an antidepressant. The regions of this network overlap, to some extent, with the ones previously found using the typical RSNs, reinforcing the idea of their involvement in the suicidal ideation processes. The areas that compose this network range from regions involved in higher order cognitive functions (such as the medial frontal orbital gyrus, left frontal superior gyrus, posterior cingulate and rectus) to visual processing functions (such as the calcarine, superior and inferior left occipital gyrus and cuneus) and semantic and conceptual processing (left middle temporal gyrus) and motor control (cerebellum).

In a previous study using NBS, Chen et al. reported a specific network of FC that distinguished MDD patients with and without suicidal ideation(Chen, Chou, et al., 2021). The reported network was composed by areas that overlap partially with the network found in our cohort, specifically the orbital frontal gyrus, the posterior cingulate cortex, the rectus, and the occipital gyrus. Moreover, another study by Kim et al, also using NBS(Kim et al., 2017), reported a network of FC that distinguished MDD patients with and without suicidal ideation. That network also presents some regions that overlap with our observations, specifically the left middle temporal gyrus, the middle frontal orbitofrontal gyrus and the superior frontal gyrus. Thus, together with previous evidence, our results seem to indicate a possible involvement of a specific network of rsFC in the suicidal ideation processes.

Differently, our results showed a possible involvement of the cerebellum in the network of functional connectivity associated with suicidal ideation. The association between the cerebellum and suicidal ideation has been previously hypothesized, however its involvement in the process is still unclear. Our findings seem to be in line with previous studies that revealed that the cerebellum plays a role in higher order cognitive processes but also mood regulation in disorders such as major depression and bipolar disorder (BD) (Schmahmann, 2010; Stoodley & Schmahmann, 2010) with reported reduced cerebellar volumes in BD patients with previous suicide attempts(Johnson et al., 2017). Also in line with our results, Shaffer et al. suggested a role of the cerebellum in the neuronal circuits involved in suicidal behavior(Shaffer et al., 2021).

Regarding the white matter microstructure, our findings revealed that increased suicidal ideation was associated with decreased FA in the genu of corpus callosum and the anterior CR, mainly in the right hemisphere. These findings suggest that alterations in the white matter microstructure are associated with higher suicidal ideation in MDD. Taylor and colleagues(Taylor et al., 2015) also suggested alterations in the white matter microstructure in depressed patients with suicidal ideation, although they reported widespread alterations in CgH, superior and posterior CR, and ATR. The incongruent findings between the studies might be explained by the characteristics of the sample (first depressive episode vs. recurrent depressive episodes), previous exposure to antidepressants (drug-naïve vs. drug washout for at least 1 month), or even the characteristics of the diffusion acquisition (30 directions vs. 20 directions). Moreover, in line with our results, Chen and colleagues(Chen, Kao, et al., 2021) reported decreased generalized fractional anisotropy and normalized quantitative anisotropy values in corpus callosum and anterior cingulate in depressed patients with suicidal ideation, suggesting focal alterations in the white matter integrity associated with suicidal ideation.

The genu of the corpus callosum and the anterior CR were the 2 major white matter structures displaying a significant association with suicidal ideation. The genu of the corpus callosum comprises fibers from orbital, medial, and dorsolateral frontal cortex, whereas the anterior CR is formed by thalamic and motor projections from the internal capsule to the cortex(Catani et al., 2002). Previous studies reported a decreased FA in the genu of the corpus callosum associated with an increased number of suicide attempts in depressed and bipolar patients(Cyprien et al., 2016), as well as reduced number of projecting fibers from the internal capsule to orbitofrontal cortex and thalamus in depressed suicide attempters when compared with depressed controls(Jia et al., 2014). Taken together with our findings, these

observations suggest that alterations in the white matter microstructure and connections of these bundles might contribute to an increased suicide risk, given its association with both suicidal ideation and behaviors. Also supporting this hypothesis, Myung and collaborators(Myung et al., 2016) described that depressed patients with suicidal ideation present a reduced structural connectivity in a specific network, including the orbitofrontal cortex and thalamus.

The present work has expanded our knowledge into the possible neuronal correlates of suicidal ideation in MDD patients before antidepressant medication is initiated. Besides the alterations in the white-matter integrity (in the genu of corpus callosum and the right anterior CR), our results also revealed abnormal functional connectivity in the visual RSN but also in a specific network composed by frontal, cerebellar, occipital, and parietal regions. Importantly, in our cohort of MDD patients, suicidal ideation was not found to be associated with worse depression severity and thus suggesting that these results were specific to suicidal ideation. It is often difficult to separate the suicidal ideation process from factors such as disease severity, duration of illness and medication status as these variables are often clinically linked and not independent.

The present study presents some important limitations, first being the limited sample size and the strength of the MRI magnetic field being 1.5 Tesla that limited the quality of the signal and the number of directions considered in the DTI acquisitions, as well as the absence of a control group. Due to these limitations, caution should be applied when interpreting these results as more studies should be done in the sense of replication of the patterns presented in this paper. The present study shared some light into the brain alterations associated with suicidal ideation in MDD patients. Such knowledge could be at use in the guidance of better treatments that could target deregulations linked to suicidal ideation in MDD patients.

Conflict of interest declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments

This work was funded by the Portuguese Foundation for Science and Technology (FCT) under scope of project PTDC/DTP-PIC/6936/2014.
This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020 and by the project NORTE-01-0145-FEDER-000039, supported by Norte Portugal Regional Operational Program (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).
J.R. was supported by the FCT fellowship grant with the reference PDE/BDE/113602/2015 from the PhD-iHES program. R.V. was supported by the FCT fellowship grant with the reference PD/BDE/150619/2020 from the PhD-iHES program and UMINHO/BI/340/2018. C.P.N. was supported by the Portuguese Science Foundation (FCT) doctoral scholarship PD/BD/106050/2015 via Inter-University PhD Program in Ageing and Chronic Diseases. P.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113601/2015 from the PhD-iHES program. R.M. was supported by the FCT fellowship grant with the reference PDE/BDE/113604/2015 from the PhD-iHES program. S.F. was supported by the FCT fellowship grant with the reference PD/BDE/127839/2016 from the PhD-iHES program.

References

Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., Adekanmbi, V., Adeoye, A. M., Adetokunboh, O. O., ... Wiangkham, T. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396(10258)*, 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

American Psychiatric Association. (2013). American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.books.9780890425596.744053

Beck, Aaron T and Steer, R. A. (1991). *Manual for the Beck scale for suicide ideation* (63rd ed.). Psychological Corporation.

Beckmann, C. F., & Smith, S. M. (2004). Probabilistic Independent Component Analysis for Functional Magnetic Resonance Imaging. *IEEE Transactions on Medical Imaging*, *23(2)*, 137-152. https://doi.org/10.1109/TMI.2003.822821

Bueno-Notivol, J., Gracia-García, P., Olaya, B., Lasheras, I., López-Antón, R., & Santabárbara, J. (2021). Prevalence of depression during the COVID-19 outbreak: A meta-analysis of community-based studies. *International Journal of Clinical and Health Psychology*, *21 (1)*. https://doi.org/10.1016/j.ijchp.2020.07.007

Catani, M., Howard, R. J., Pajevic, S., & Jones, D. K. (2002). Virtual in Vivo interactive dissection of white matter fasciculi in the human brain. *NeuroImage*. https://doi.org/10.1006/nimg.2002.1136

Chen, V. C. H., Chou, Y. S., Tsai, Y. H., Huang, Y. C., McIntyre, R. S., & Weng, J. C. (2021). Resting-State Functional Connectivity and Brain Network Abnormalities in Depressive Patients with Suicidal Ideation. *Brain Topography*. https://doi.org/10.1007/s10548-020-00817-x

Chen, V. C. H., Kao, C. J., Tsai, Y. H., Cheok, M. T., McIntyre, R. S., & Weng, J. C. (2021). Assessment of Disrupted Brain Structural Connectome in Depressive Patients With Suicidal Ideation Using

Generalized Q-Sampling MRI. *Frontiers in Human Neuroscience*, *15*(August), 1–11. https://doi.org/10.3389/fnhum.2021.711731

Cohen, S. (1988). Perceived stress in a probability sample of the United States. In *The social psychology of health.*

Crosby, A. E., Han, B., Ortega, L. A. G., Parks, S. E., Gfroerer, J., & Centers for Disease Control and Prevention (CDC). (2011). Suicidal thoughts and behaviors among adults aged ≥18 years–United States, 2008-2009. *Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington, D.C. : 2002)*.

Cyprien, F., de Champfleur, N. M., Deverdun, J., Olié, E., Le Bars, E., Bonafé, A., Mura, T., Jollant, F., Courtet, P., & Artero, S. (2016). Corpus callosum integrity is affected by mood disorders and also by the suicide attempt history: A diffusion tensor imaging study. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2016.07.026

Damoiseaux, J. S., Rombouts, S. A. R. B., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., & Beckmann, C. F. (2006). Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences*, *103*(37), 13848–13853. https://doi.org/10.1073/pnas.0601417103

Du, L., Zeng, J., Liu, H., Tang, D., Meng, H., Li, Y., & Fu, Y. (2017). Fronto-limbic disconnection in depressed patients with suicidal ideation: A resting-state functional connectivity study. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2017.02.027

D. Wasserman. (2016). The suicidal process. In *Suicide: An unnecessary death* (2nd editio).

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., Vos, T., & Whiteford, H. A. (2013). Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. *PLoS Medicine*. https://doi.org/10.1371/journal.pmed.1001547

First, M. B., Williams, J. B. W., Karg, R. S., Spitzer, R. L., & Gibbon, M. (2008). Structured Clinical interview for DSM-IV Axis I Disorders (SCID-I). *Handbook of Psychiatric Measures*.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*. https://doi.org/10.1016/0022-3956(75)90026-6

Franklin, J. C., Ribeiro, J. D., Fox, K. R., Bentley, K. H., Kleiman, E. M., Huang, X., Musacchio, K. M., Jaroszewski, A. C., Chang, B. P., & Nock, M. K. (2017). Risk factors for suicidal thoughts and behaviors: A meta-analysis of 50 years of research. *Psychological Bulletin*. https://doi.org/10.1037/bul0000084

Hamilton, M. (1959). The assessment of anxiety states by rating. *British Journal of Medical Psychology*. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry*, *23*, 56–62.

Jia, Z., Wang, Y., Huang, X., Kuang, W., Wu, Q., Lui, S., Sweeney, J. A., & Gong, Q. (2014). Impaired frontothalamic circuitry in suicidal patients with depression revealed by diffusion tensor imaging at 3.0 T. *Journal of Psychiatry and Neuroscience*. https://doi.org/10.1503/jpn.130023

Johnson, S. L., Carver, C. S., & Tharp, J. A. (2017). Suicidality in Bipolar Disorder: The Role of Emotion-Triggered Impulsivity. *Suicide and Life-Threatening Behavior*. https://doi.org/10.1111/sltb.12274

Kessler, R. C. (2012). The costs of depression. In *Psychiatric Clinics of North America*. https://doi.org/10.1016/j.psc.2011.11.005

Kim, K., Kim, S. W., Myung, W., Han, C. E., Fava, M., Mischoulon, D., Papakostas, G. I., Seo, S. W., Cho, H., Seong, J. K., & Jeon, H. J. (2017). Reduced orbitofrontal-thalamic functional connectivity related to suicidal ideation in patients with major depressive disorder. *Scientific Reports*. https://doi.org/10.1038/s41598-017-15926-0

Kleber, H. D., Weiss, R. D., Anton, R. F., George, T. P., Greenfield, S. F., Kosten, T. R., O'Brien, C. P., Rounsaville, B. J., Strain, E. C., Ziedonis, D. M., Hennessy, G., Connery, H. S., McIntyre, J. S., Charles, S. C., Anzia, D. J., Cook, I. A., Finnerty, M. T., Johnson, B. R., Nininger, J. E., ... Regier, D. (2007). Treatment of patients with substance use disorders, second edition. American Psychiatric Association. *The American Journal of Psychiatry*, *164*(4 Suppl), 5–123.

Lan, X., Zhou, Y., Zheng, W., Zhan, Y., Liu, W., Wang, C., Jiang, M., Yu, M., Zhang, B., & Ning, Y. (2020). Association between cognition and suicidal ideation in patients with major depressive disorder: A longitudinal study. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2020.03.141

Lépine, J. P., & Briley, M. (2011). The increasing burden of depression. *Neuropsychiatric Disease and Treatment*, *7 (SUPPL.)*, 3–7. https://doi.org/10.2147/NDT.S19617

Lopez-Castroman, J., Jaussent, I., Gorwood, P., & Courtet, P. (2016). SUICIDAL DEPRESSED PATIENTS RESPOND LESS WELL TO ANTIDEPRESSANTS IN THE SHORT TERM. *Depression and Anxiety*. https://doi.org/10.1002/da.22473

Luo, M., Guo, L., Yu, M., & Wang, H. (2020). The psychological and mental impact of coronavirus disease 2019 (COVID-19) on medical staff and general public – A systematic review and meta-analysis. *Psychiatry Research*, *291*(June), 113190. https://doi.org/10.1016/j.psychres.2020.113190

Marzuk, P. M., Hartwell, N., Leon, A. C., & Portera, L. (2005). Executive functioning in depressed patients with suicidal ideation. *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/j.1600-0447.2005.00585.x

Myung, W., Han, C. E., Fava, M., Mischoulon, D., Papakostas, G. I., Heo, J. Y., Kim, K. W., Kim, S. T., Kim, D. J. H., Kim, D. K., Seo, S. W., Seong, J. K., & Jeon, H. J. (2016). Reduced frontal-subcortical white matter connectivity in association with suicidal ideation in major depressive disorder. *Translational Psychiatry*. https://doi.org/10.1038/tp.2016.110

Nock, M. K., Borges, G., Bromet, E. J., Alonso, J., Angermeyer, M., Beautrais, A., Bruffaerts, R., Wai, T. C., De Girolamo, G., Gluzman, S., De Graaf, R., Gureje, O., Haro, J. M., Huang, Y., Karam, E., Kessler, R. C., Lepine, J. P., Levinson, D., Medina-Mora, M. E., ... Williams, D. (2008). Cross-national prevalence and risk factors for suicidal ideation, plans and attempts. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.bp.107.040113

Nock, M. K., Hwang, I., Sampson, N., Kessler, R. C., Angermeyer, M., Beautrais, A., Borges, G., Bromet, E., Bruffaerts, R., De Girolamo, G., De Graaf, R., Florescu, S., Gureje, O., Haro, J. M., Hu, C., Huang, Y., Karam, E. G., Kawakami, N., Kovess, V., ... Williams, D. R. (2009). Cross-national analysis of the associations among mental disorders and suicidal behavior: Findings from the

WHO World Mental Health Surveys. *PLoS Medicine*, *6 (8)*. https://doi.org/10.1371/journal.pmed.1000123

Orsolini, L., Latini, R., Pompili, M., Serafini, G., Volpe, U., Vellante, F., Fornaro, M., Valchera, A., Tomasetti, C., Fraticelli, S., Alessandrini, M., La Rovere, R., Trotta, S., Martinotti, G., Di Giannantonio, M., & De Berardis, D. (2020). Understanding the complex of suicide in depression: From research to clinics. In *Psychiatry Investigation*. https://doi.org/10.30773/pi.2019.0171

Ösby, U., Brandt, L., Correia, N., Ekbom, A., & Sparén, P. (2001). Excess mortality in bipolar and unipolar disorder in Sweden. *Archives of General Psychiatry*, *58 (9)*, 844–850. https://doi.org/10.1001/archpsyc.58.9.844

Pu, S., Setoyama, S., & Noda, T. (2017). Association between cognitive deficits and suicidal ideation in patients with major depressive disorder. *Scientific Reports*. https://doi.org/10.1038/s41598-017-12142-8

Qiao, D., Zhang, A., Sun, N., Yang, C., Li, J., Zhao, T., Wang, Y., Xu, Y., Wen, Y., Zhang, K., & Liu, Z. (2020). Altered Static and Dynamic Functional Connectivity of Habenula Associated With Suicidal Ideation in First-Episode, Drug-Naïve Patients With Major Depressive Disorder. *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2020.608197

Schmaal, L., Pozzi, E., C. Ho, T., van Velzen, L. S., Veer, I. M., Opel, N., Van Someren, E. J. W., Han, L. K. M., Aftanas, L., Aleman, A., Baune, B. T., Berger, K., Blanken, T. F., Capitão, L., Couvy-Duchesne, B., R. Cullen, K., Dannlowski, U., Davey, C., Erwin-Grabner, T., … Veltman, D. J. (2020). ENIGMA MDD: seven years of global neuroimaging studies of major depression through worldwide data sharing. In *Translational Psychiatry*. https://doi.org/10.1038/s41398-020-0842-6

Schmahmann, J. D. (2010). The role of the cerebellum in cognition and emotion: Personal reflections since 1982 on the dysmetria of thought hypothesis, and its historical evolution from theory to therapy. In *Neuropsychology Review*. https://doi.org/10.1007/s11065-010-9142-x

Shaffer, J. J., Willour, V., Fiedorowicz, J. G., Christensen, G. E., Long, J. D., Johnson, C. P., Schmitz, S. L., Williams, A. J., Wemmie, J., & Magnotta, V. A. (2021). Distinct patterns of altered quantitative

T1ρ and functional BOLD response associated with history of suicide attempts in bipolar disorder. *Brain Imaging and Behavior*. https://doi.org/10.1007/s11682-021-00552-2

Shirer, W. R., Ryali, S., Rykhlevskaia, E., Menon, V., & Greicius, M. D. (2012). Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cerebral Cortex*, *22 (1)*, 158–165. https://doi.org/10.1093/cercor/bhr099

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., Watkins, K. E., Ciccarelli, O., Cader, M. Z., Matthews, P. M., & Behrens, T. E. J. (2006). Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2006.02.024

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*(SUPPL. 1), S208–S219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Soares, J. M., Magalhães, R., Moreira, P. S., Sousa, A., Ganz, E., Sampaio, A., Alves, V., Marques, P., & Sousa, N. (2016). A Hitchhiker's guide to functional magnetic resonance imaging. *Frontiers in Neuroscience*, *10*, 515. https://doi.org/10.3389/fnins.2016.00515

Stoodley, C. J., & Schmahmann, J. D. (2010). Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex*. https://doi.org/10.1016/j.cortex.2009.11.008

Taylor, W. D., Boyd, B., McQuoid, D. R., Kudra, K., Saleh, A., & MacFall, J. R. (2015). Widespread white matter but focal gray matter alterations in depressed individuals with thoughts of death. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. https://doi.org/10.1016/j.pnpbp.2015.05.001

Wei, S., Chang, M., Zhang, R., Jiang, X., Wang, F., & Tang, Y. (2018). Amygdala functional connectivity in female patients with major depressive disorder with and without suicidal ideation. *Annals of General Psychiatry*. https://doi.org/10.1186/s12991-018-0208-0

Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, *92*, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060

World Health Organization. (2017). Depression and other common mental disorders: global health estimates. *World Health Organization*. https://doi.org/CC BY-NC-SA 3.0 IGO

World Health Organization (WHO). (2021). *Suicide worldwide in 2019: global health estimates*.

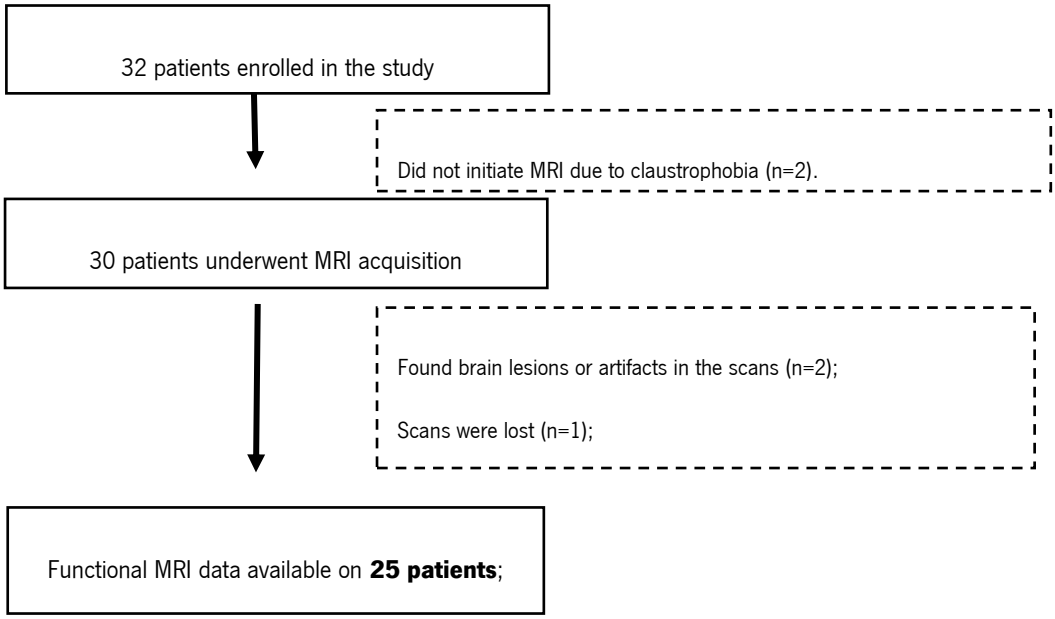
Yang, W., Zhang, G., Jia, Q., Qian, Z. K., Yin, G., Zhu, X., Alnatour, O. I., Trinh, T. H., Wu, H. E., Lang, X. E., Du, X., & Zhang, X. (2019). Prevalence and clinical profiles of comorbid anxiety in first episode and drug naïve patients with major depressive disorder. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2019.06.052

Zhao, K., Zhou, S., Shi, X., Chen, J., Zhang, Y., Fan, K., Zhang, X., Wang, W., & Tang, W. (2020). Potential metabolic monitoring indicators of suicide attempts in first episode and drug naive young patients with major depressive disorder: A cross-sectional study. *BMC Psychiatry*. https://doi.org/10.1186/s12888-020-02791-x

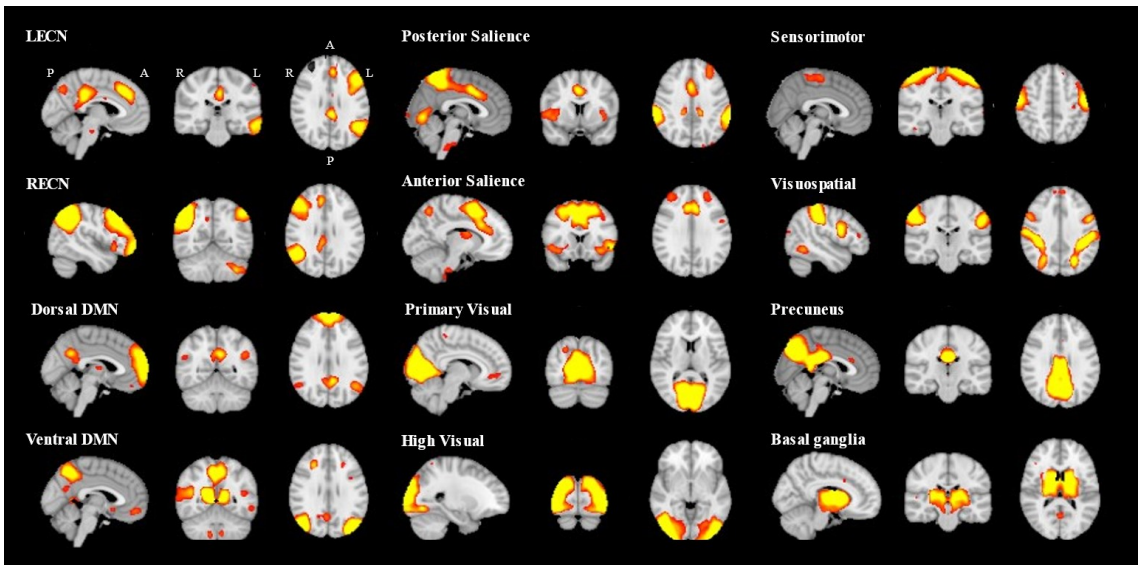
Zhou, Y., Li, Z., Wang, Y., Huang, H., Chen, W., Dong, L., Wu, J., Chen, J., Miao, Y., Qi, L., Zhang, S., Lang, X., & Zhang, X. (2020). Prevalence and clinical correlates of psychotic depression in first-episode and drug-naïve outpatients with major depressive disorder in a Chinese Han population. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2019.10.051

Supplementary material

Supplementary Figure 1. Detailed information on subject recruitment and exclusion.



Supplementary Figure 2. Resting State Networks. Resting state networks identified through MELODIC. Group MELODIC maps (LECN and RECN left and right executive networks, dorsal and ventral DMN, posterior and anterior salience networks, primary and high visual networks, sensorimotor network, visuospatial network, precuneus networks and basal ganglia network.



Supplementary Table 1: Results of the functional connectivity analysis using NBS. For each threshold used in the NBS analysis, we report the p-value of the significant network, the number of nodes and edges in the network as well as the mean connectivity for all patients.

Threshold (t, p)	p (network)	N. Nodes	N. Edges	Mean FC
3.10, 0.005	0.046	44	71	0.31
3.78, 0.001	0.025	15	15	0.42
4.05, 0.0005	0.029	7	6	0.39
4.69, 0.0001	0.037	3	2	0.41

Supplementary table 2: Nodes of the networks with connecting edges that have a significant correlation with BSI. Results are represented for the 4 tested thresholds using the AAL atlas and are, name and number of each node as well as the sum t-statistic value (t) over its significant connections.

		p-value			
# Node	**Name (AAL label)**	**0.005**	**0.001**	**0.0005**	**0.0001**
1	Precentral L	0	0		0
2	Precentral R	0	0	0	0
3	Frontal Sup L	16.09	12.501	0	0
4	Frontal Sup R	0	0	0	0
5	Frontal Sup Orb L	0	0	0	0
6	Frontal Sup Orb R	3.799	0	0	0
7	Frontal Mid L	0	0	0	0
8	Frontal Mid R	0	0	0	0
9	Frontal Mid Orb L	0	0	0	0
10	Frontal Mid Orb R	0	0	0	0
11	Frontal Inf Oper L	0	0	0	0
12	Frontal Inf Oper R	0	0	0	0
13	Frontal Inf Tri L	0	0	0	0
14	Frontal Inf Tri R	0	0	0	0
15	Frontal Inf Orb L	0	0	0	0
16	Frontal Inf Orb R	3.5361	0	0	0
17	Rolandic Oper L	0	0	0	0
18	Rolandic Oper R	0	0	0	0
19	Supp Motor Area L	0	0	0	0
20	Supp Motor Area R	0	0	0	0
21	Olfactory L	0	0	0	0
22	Olfactory R	10.282	0	0	0
23	Frontal Sup Medial L	3.1574	0	0	0
24	Frontal Sup Medial R	3.4138	0	0	0
25	Frontal Med Orb L	19.11	5.5733	5.5733	5.5733
26	Frontal Med Orb R	16.168	5.9173	5.9173	5.9173
27	Rectus L	10.485	3.9627	0	0
28	Rectus R	10.959	4.4431	4.4431	0
29	Insula L	0	0	0	0
30	Insula R	0	0	0	0
31	Cingulum Ant L	3.2607	0	0	0
32	Cingulum Ant R	3.3402	0	0	0
33	Cingulum Mid L	0	0	0	0

34	Cingulum Mid R	0	0	0	0
35	Cingulum Post L	18.461	8.3048	4.4125	0
36	Cingulum Post R	20.179	3.8201	0	0
37	Hippocampus L	3.3725	0	0	0
38	Hippocampus R	0	0	0	0
39	ParaHippocampal L	0	0	0	0
40	ParaHippocampal R	0	0	0	0
41	Amygdala L	0	0	0	0
42	Amygdala R	0	0	0	0
43	Calcarine L	14.947	4.5207	0	0
44	Calcarine R	7.7281	4.1677	4.1677	0
45	Cuneus L	16.101	9.0764	9.0764	0
46	Cuneus R	17.777	7.7124	0	0
47	Lingual L	9.857	0	0	0
48	Lingual R	6.5521	0	0	0
49	Occipital Sup L	10.064	0	0	0
50	Occipital Sup R	7.1502	3.8056	0	0
51	Occipital Mid L	0	0	0	0
52	Occipital Mid R	0	0	0	0
53	Occipital Inf L	0	0	0	0
54	Occipital Inf R	10.809	3.946	0	0
55	Fusiform L	0	0	0	0
56	Fusiform R	6.777	0	0	0
57	Postcentral L	0	0	0	0
58	Postcentral R	0	0	0	0
59	Parietal Sup L	0	0	0	0
60	Parietal Sup R	0	0	0	0
61	Parietal Inf L	0	0	0	0
62	Parietal Inf R	0	0	0	0
63	SupraMarginal L	0	0	0	0
64	SupraMarginal R	0	0	0	0
65	Angular L	3.1785	0	0	0
66	Angular R	0	0	0	0
67	Precuneus L	0	0	0	0
68	Precuneus R	0	0	0	0
69	Paracentral Lobule L	0	0	0	0
70	Paracentral Lobule R	0	0	0	0
71	Caudate L	3.2659	0	0	0
72	Caudate R	3.3572	0	0	0
73	Putamen L	3.1799	0	0	0
74	Putamen R	0	0	0	0
75	Pallidum L	0	0	0	0
76	Pallidum R	0	0	0	0
77	Thalamus L	0	0	0	0
78	Thalamus R	0	0	0	0
79	Heschl L	7.6825	0	0	0
80	Heschl R	4.4322	0	0	0
81	Temporal Sup L	0	0	0	0
82	Temporal Sup R	3.3558	0	0	0
83	Temporal Pole Sup L	0	0	0	0
84	Temporal Pole Sup R	0	0	0	0
85	Temporal Mid L	21.58	8.103	0	0
86	Temporal Mid R	7.571	0	0	0
87	Temporal Pole Mid L	0	0	0	0
88	Temporal Pole Mid R	20.737	0	0	0
89	Temporal Inf L	0	0	0	0
90	Temporal Inf R	0	0	0	0
91	Cerebelum Crus1 L	0	0	0	0

92	Cerebelum Crus1 R	0	0	0	0
93	Cerebelum Crus2 L	0	0	0	0
94	Cerebelum Crus2 R	3.1324	0	0	0
95	Cerebelum 3 L	6.5205	0	0	0
96	Cerebelum 3 R	0	0	0	0
97	Cerebelum 4 5 L	0	0	0	0
98	Cerebelum 4 5 R	0	0	0	0
99	Cerebelum 6 L	0	0	0	0
100	Cerebelum 6 R	0	0	0	0
101	Cerebelum 7b L	0	0	0	0
102	Cerebelum 7b R	0	0	0	0
103	Cerebelum 8 L	0	0	0	0
104	Cerebelum 8 R	0	0	0	0
105	Cerebelum 9 L	6.5426	0	0	0
106	Cerebelum 9 R	79.203	44.434	24.765	11.491
107	Cerebelum 10 L	30.634	0	0	0
108	Cerebelum 10 R	20.137	0	0	0
109	Vermis 1 2	4.561	0	0	0
110	Vermis 3	0	0	0	0
111	Vermis 4 5	0	0	0	0
112	Vermis 6	0	0	0	0
113	Vermis 7	0	0	0	0
114	Vermis 8	0	0	0	0
115	Vermis 9	20.865	0	0	0
116	Vermis 10	10.39	0	0	0

DISCUSSION

DISCUSSION

Major depressive disorder (MDD) is an increasingly prevalent mood disorder for which the diagnostic and prognostic tools remain a challenge to this day. There is still a lack of biomarkers (based on neuroimaging or other physiological parameters) implemented in the regular clinical practice that could guide psychiatrists when prescribing medication to patients initializing therapy (Kennis et al., 2020; Strawbridge et al., 2017).

The current criteria used for the diagnosis of depression, based on the DSM-V requires the presence of at least 5 symptoms during a 2-week period. At least one of those symptoms should be depressed mood or loss of interest or pleasure in daily activities but other symptoms can range from disturbed sleep or appetite patterns, tiredness (loss of energy), psycho-motor changes, feelings of guilt, worthlessness or low self-worth, diminished ability to concentrate and recurrent thoughts of death, to suicidal ideation and/or attempt (American Psychiatric Association, 2013). These criteria allow for a vast range of combination of different symptom profiles that reflect the extraordinary heterogeneity of depression. Such heterogeneity is often associated with the difficulty of understanding the pathophysiology of depression as well as the difficulty to determine the best treatment course as the diagnostic tools (such as the DSM-V) often assign one label to a disorder that could be caused by distinct pathological processes, which would require different treatments options (Buch & Liston, 2021).

Although psychiatric disorders create a burden of disease superior than any other medical diagnosis, the disparity in terms of funding when comparing to other medical conditions remains problematic. Apart from such difficulties, the field of psychiatry suffers from problems regarding the consensus in disease classification, diagnosis and treatment. Problems that are highly apparent in mood disorder, especially in the most prevalent one, MDD (World Health Organization, 2017). Indeed, data shows that 60% of patients diagnosed with MDD will suffer some degree of treatment resistance due to lack of ability to choose the best treatment course to take (Rush et al., 2006).

Several other studies have tried to find neuroimaging-based biomarkers for either the diagnosis of depression (and depression subtypes) but also to predict treatment outcomes). Similar to the work presented in this Thesis, fMRI is a neuroimaging modality widely used in depression research as it can be applied either during the execution of a task and during awake rest. A vast range of strategies can be applied in the analysis of fMRI data and while some studies focus on the detection of abnormal brain

activation in different regions, others focus on connectivity and how different brain regions are synchronized and linked constituting networks of functional connectivity (Pilmeyer et al., 2022; Soares et al., 2016).

In this Thesis we focused mainly on the functional connectivity measured in functional MRI, either to describe alterations in MDD patients when compared with healthy controls but also to find possible alterations related with pharmacological treatment, specifically the SSRI paroxetine. The comparison between a healthy sample of subjects with a sample of MDD patients before treatment initiation produced interesting results (detailed in Chapter 1). In alignment with the available literature, MDD patients showed alterations in FC networks related with executive functioning (such as the ECN) and mind wondering/rumination processes (such as the DMN) (Kaiser et al., 2015). Patients also presented worse performance in tasks designed to test cognitive functioning and that are sensitive to alterations in processing speed, executive functioning and working memory (Jaeger, 2018). Such results point to the existence of difficulties in cognitive functioning that could be related with common depression symptomatology such as anhedonia, psychomotor retardation or inability to concentrate.

With the aim of identifying possible Imaging predictors of clinical response early in the course of pharmacological treatment with paroxetine. To do so, we investigated the short-term (first day of treatment) and long-term (6-12 weeks after treatment initiation) effects of paroxetine in the same cohort of drug-naïve patients using rs-fMRI. The work is fully detailed in Chapter 2 and our main goal was to use rs-FC to identify indicators of treatment outcomes present before treatment initiation or very early in the course of pharmacological treatment. The results obtained revealed alteration in the brain of non-responders (when comparing to responders) before treatment Initiation (at baseline). These results revealed abnormal connectivity within networks of functional connectivity previously (chapter 1) reported to be altered in MDD, the ECN and the DMN but also the visuospatial network (Kaiser et al., 2015). Indeed, a better response to treatment was associated with higher connectivity within networks associated with executive function (such as the RECN) and also higher FC within networks associated with mind-wandering introspective thoughts (such as the DMN). Poorer clinical improvement was also associated with lower rs-FC within the visuospatial network. We hypothesized that such results could be related with visuospatial attention and executive function but also a bias towards ruminative thoughts more predominant maybe in patients that will respond worse to the pharmacological treatment.

The FC in the DMN has been associated with treatment response in the literature medication (Aizenstein et al., 2014; Nejad et al., 2013; Qin et al., 2015). In our sample of patients, the connectivity within the ventral DMN was modulated hours after a single dose of paroxetine and also after 6-12 of sustained treatment with the same dose. In both cases the treatment was able to reduce FC however without differentiating responders from non-responders. Our results can then Indicate that paroxetine has an effect in reducing abnormally high connectivity within the DMN in a short period of time, although this effect could not be associated with a better prognosis at an early stage of treatment. Although also not differentiating between responders and non-responder, functional connectivity within the basal ganglia and the sensorimotor networks was also modulated (reduced) hours and weeks after treatment initiation, respectively. Importantly, in our sample all patients presented clinical improvement measured by a reduction in the score of the HAM-D scale, however the one that we categorized as responders were able to achieve at least an Improvement by 50%.

In our study we reported long-term effects that were able to distinguish responders and non-responders, namely the connectivity within regions of the LECN and sensorimotor networks. Other patterns of inter-network connectivity seem to indicate significant changes in network dynamics related to paroxetine treatment such as the connectivity between right ECN and the ventral DMN. We hypothesized that due to the Important role of these networks in processing sensorial and motor could be related to the amelioration of psychomotor symptoms of depression (Göttlich et al., 2013).

Our study showed alterations produced by paroxetine in the rs-FC, early in the course of the pharmacological treatment, that can lead to a better understanding of paroxetine treatment response in MDD. To the best of our knowledge, this study is Innovative as It describes specific resting-state functional connectivity alterations in drug-naive MDD patients, measured on an hourly basis after the first dose intake of the SSRI paroxetine.

Depression is also associated with higher levels of suicidal ideation and suicidal attempts (Franklin et al., 2017). In fact, depression is one of the most common diagnosis at the time of dead by suicide and depressed patients are 20 times more likely than the general population to commit suicide (Lépine & Briley, 2011; Ösby et al., 2001). Even though in our sample of MDD patients, responders and non-responders to the pharmacological treatment were not different in the severity of their depression and anxiety at the beginning of the treatment, non-responders scored higher in a scale that measured suicidal ideation (BSSI scale). Suicide is a complex phenomenon that recent research pointed to be a complex

interaction between genetic vulnerability as well as exogenous and endogenous factors (D. Wasserman, 2016; Orsolini et al., 2020). In the final work of this Thesis, described in Chapter 3, we explore the neural correlates of suicidal ideation in drug-naïve MDD patients at the whole-brain level, using two different MRI modalities, namely resting-state fMRI, and DTI. Our results showed that besides the alterations in the white-matter integrity (in the genu of corpus callosum and the right anterior CR), suicidal ideation was also associated with abnormal functional connectivity the visual RSN but also in a specific network composed by frontal, cerebellar, occipital, and parietal regions. These findings suggest that alterations in structure and function of frontal-subcortical networks are implied in suicidal ideation in MDD.

Neuroimaging limitations

While there are a few fMRI biomarkers for depression and treatment response that are more consistent across literature, a lot remain to be replicated due to the lack of robust data acquisition and analysis protocols. Indeed, there are limitations on the acquisition, processing and analysis of fMRI data that can underlie inconsistency in neuroimaging results (Pilmeyer et al., 2022; Zhuo et al., 2019). Some of these limitations also affected the present Thesis and their implications is further discussed in the next paragraphs.

Functional MRI is a powerful tool that is based on the detection of changes in the blood deoxyhemoglobin concentrations that reflect oxygen consumptions in the brain. The signal captured during fMRI is an indirect measurement of concentration of brain activity via the hemodynamic responses that occur as a consequence of the oxygen consumption (Huettel et al., 2014). Conventional fMRI acquisitions can be limited by several factors including susceptibility to noise and artifacts, temporal or spatial resolution, and signal-to-noise ratio (SNR) (Pilmeyer et al., 2022). In fact, there are tissue-air boundaries in the head (nasal cavity, mastoid air cells, and sphenoid sinus) that can affect the fMRI signal as they create distortions in the magnetic fields (Kirilina et al., 2016). Regions often reported in depression studies such as frontal and limbic regions could be affected by this phenomenon. Another common limitation factor is the temporal resolution of EPI acquisitions. Often neuroimaging studies report using a 2-second TR. This trade-off between spatial and temporal resolution creates relatively low temporal resolution time-series that are greatly affected by noise and thus decreasing statistical power (Demetriou et al., 2018).

Another important limitation of the fMRI acquisitions is the susceptibility to noise such as participant motion, other physiological signals (cardiac and respiratory) as well as scanner related artifacts (Pais-Roldán et al., 2018). Indeed, current methods used to denoise fMRI signals (such as CompCor or ICA-

AROMA) could benefit largely from strategies like neural network-based approaches that help creating higher quality fMRI signals (Kam et al., 2019). Current fMRI acquisitions can be largely improved by strategies such as novel acquisition strategies such as multiband and multiecho Imaging but also using MRI scanner with higher magnetic field strengths. While scanners with a higher magnetic field strength (such as 3T or higher) produce better SNR (SNR Increases linearly with the strength of the magnetic field) the optimization of fast Imaging protocols (like multiband Imaging) could prevent physiological noise from aliasing the BOLD signal (Collins, 2016). Actually, using multiband Imaging techniques one can acquire more than one slice simultaneously thus reducing total scan time with improved temporal resolution (using TRs around or lower than 1 second). Overall these advances In MRI acquisition can Improve Image quality and BOLD sensitivity and thus help in the search for Imaging biomarkers of depression as well as possible predictors of clinical improvement (Demetriou et al., 2018; Hamilton et al., 2017).

Study Limitations

Even though the findings presented in this Thesis are promising, the sample size is relatively modest which could be problematic when trying to generalize the results to the population. Due to our very restrict inclusion/exclusion criteria the recruitment process was very slow which constituted an important constraint of this study. The cohort was recruited between January 2016 and January 2020 illustrating how the difficulties along the way.

However, and also because of the very restrictive criteria, our sample is constituted exclusively by drug-naïve patients and the baseline evaluations were performed before treatment initiation, allowing us to eliminate potential medication confounding effects when interpreting results. Such confounders are often present in depression literature and are hard to dissociate from disease status or treatment response.

Another import limitation of the work presented in this Thesis is the strength of the MRI scanner used for imaging acquisitions. As discussed before, the usage of the 1.5 Tesla scanner imposes limitations on the signal quality and sensitivity and well as on the number of directions considered in the DTI acquisitions. However, at the time of this study, the 1.5 T scanner was the only one available for research. Due to these limitations, caution should be applied when interpreting the results reported and discussed in this Thesis as more studies should be done in the sense of replication of the patterns presented.

References

Aizenstein, H. J., Khalaf, A., Walker, S. E., & Andreescu, C. (2014). Magnetic resonance imaging predictors of treatment response in late-life depression. *Journal of Geriatric Psychiatry and Neurology*. https://doi.org/10.1177/0891988713516541

American Psychiatric Association. (2013). Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. In *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition*. https://doi.org/10.1176/appi.books.9780890425596.893619

Buch, A. M., & Liston, C. (2021). Dissecting diagnostic heterogeneity in depression by integrating neuroimaging and genetics. *Neuropsychopharmacology*, *46*(1), 156–175. https://doi.org/10.1038/s41386-020-00789-3

Collins, C. M. (2016). Electromagnetics in magnetic resonance imaging: Physical principles, related applications, and ongoing developments. In *Electromagnetics in Magnetic Resonance Imaging: Physical Principles, Related Applications, and Ongoing Developments*. https://doi.org/10.1088/978-1-6817-4083-6

Demetriou, L., Kowalczyk, O. S., Tyson, G., Bello, T., Newbould, R. D., & Wall, M. B. (2018). A comprehensive evaluation of increasing temporal resolution with multiband-accelerated protocols and effects on statistical outcome measures in fMRI. *NeuroImage*, *176*. https://doi.org/10.1016/j.neuroimage.2018.05.011

D. Wasserman. (2016). The suicidal process. In *Suicide: An unnecessary death* (2nd editio).

Franklin, J. C., Ribeiro, J. D., Fox, K. R., Bentley, K. H., Kleiman, E. M., Huang, X., Musacchio, K. M., Jaroszewski, A. C., Chang, B. P., & Nock, M. K. (2017). Risk factors for suicidal thoughts and behaviors: A meta-analysis of 50 years of research. *Psychological Bulletin*. https://doi.org/10.1037/bul0000084

Göttlich, M., Münte, T. F., Heldmann, M., Kasten, M., Hagenah, J., & Krämer, U. M. (2013). Altered resting state brain networks in Parkinson's disease. *PloS One*, *8*(10), e77336. https://doi.org/10.1371/journal.pone.0077336

Hamilton, J., Franson, D., & Seiberlich, N. (2017). Recent advances in parallel imaging for MRI. In *Progress in Nuclear Magnetic Resonance Spectroscopy* (Vol. 101). https://doi.org/10.1016/j.pnmrs.2017.04.002

Huettel, S. A., Song, A. W., & McCarthy, G. (2014). chapter 4 Functional Magnetic Resonance Imaging, Third Edition. *Functional Magnetic Resonance Imaging, Third Edition*.

Jaeger, J. (2018). Digit Symbol Substitution Test: The Case for Sensitivity Over Specificity in Neuropsychological Testing. *Journal of Clinical Psychopharmacology*, *38*(5), 513–519. https://doi.org/10.1097/JCP.0000000000000941

Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D., & Pizzagalli, D. A. (2015). Large-scale network dysfunction in major depressive disorder: A meta-analysis of resting-state functional connectivity. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2015.0071

Kam, T. E., Wen, X., Jin, B., Jiao, Z., Hsu, L. M., Zhou, Z., Liu, Y., Yamashita, K., Hung, S. C., Lin, W., Zhang, H., & Shen, D. (2019). A Deep Learning Framework for Noise Component Detection from Resting-State Functional MRI. *Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, *11766 LNCS*. https://doi.org/10.1007/978-3-030-32248-9_84

Kennis, M., Gerritsen, L., van Dalen, M., Williams, A., Cuijpers, P., & Bockting, C. (2020). Prospective biomarkers of major depressive disorder: a systematic review and meta-analysis. In *Molecular Psychiatry* (Vol. 25, Issue 2, pp. 321–338). Springer Nature. https://doi.org/10.1038/s41380-019-0585-z

Kirilina, E., Lutti, A., Poser, B. A., Blankenburg, F., & Weiskopf, N. (2016). The quest for the best: The impact of different EPI sequences on the sensitivity of random effect fMRI group analyses. *NeuroImage*, *126*. https://doi.org/10.1016/j.neuroimage.2015.10.071

Lépine, J. P., & Briley, M. (2011). The increasing burden of depression. *Neuropsychiatric Disease and Treatment*, *7 (SUPPL.)*, 3–7. https://doi.org/10.2147/NDT.S19617

Nejad, A. B., Fossati, P., & Lemogne, C. (2013). Self-referential processing, rumination, and cortical midline structures in major depression. In *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2013.00666

Orsolini, L., Latini, R., Pompili, M., Serafini, G., Volpe, U., Vellante, F., Fornaro, M., Valchera, A., Tomasetti, C., Fraticelli, S., Alessandrini, M., La Rovere, R., Trotta, S., Martinotti, G., Di Giannantonio, M., & De Berardis, D. (2020). Understanding the complex of suicide in depression: From research to clinics. In *Psychiatry Investigation*. https://doi.org/10.30773/pi.2019.0171

Ösby, U., Brandt, L., Correia, N., Ekbom, A., & Sparén, P. (2001). Excess mortality in bipolar and unipolar disorder in Sweden. *Archives of General Psychiatry*, *58 (9)*, 844–850. https://doi.org/10.1001/archpsyc.58.9.844

Pais-Roldán, P., Biswal, B., Scheffler, K., & Yu, X. (2018). Identifying respiration-related aliasing artifacts in the rodent resting-state fMRI. *Frontiers in Neuroscience*, *12*(NOV). https://doi.org/10.3389/fnins.2018.00788

Pilmeyer, J., Huijbers, W., Lamerichs, R., Jansen, J. F. A., Breeuwer, M., & Zinger, S. (2022). Functional MRI in major depressive disorder: A review of findings, limitations, and future prospects. In *Journal of Neuroimaging* (Vol. 32, Issue 4, pp. 582–595). John Wiley and Sons Inc. https://doi.org/10.1111/jon.13011

Qin, J., Shen, H., Zeng, L. L., Jiang, W., Liu, L., & Hu, D. (2015). Predicting clinical responses in major depression using intrinsic functional connectivity. *NeuroReport*. https://doi.org/10.1097/WNR.0000000000000407

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., Niederehe, G., Thase, M. E., Lavori, P. W., Lebowitz, B. D., McGrath, P. J., Rosenbaum, J. F., Sackeim, H. A., Kupfer, D. J., Luther, J., & Fava, M. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR*D report. *American Journal of Psychiatry*, *163*(11). https://doi.org/10.1176/ajp.2006.163.11.1905

Soares, J. M., Magalhães, R., Moreira, P. S., Sousa, A., Ganz, E., Sampaio, A., Alves, V., Marques, P., & Sousa, N. (2016). A Hitchhiker's guide to functional magnetic resonance imaging. In *Frontiers in Neuroscience* (Vol. 10). https://doi.org/10.3389/fnins.2016.00515

Strawbridge, R., Young, A. H., & Cleare, A. J. (2017). Biomarkers for depression: Recent insights, current challenges and future prospects. In *Neuropsychiatric Disease and Treatment* (Vol. 13, pp. 1245–1262). Dove Medical Press Ltd. https://doi.org/10.2147/NDT.S114542

World Health Organization. (2017). Depression and other common mental disorders: global health estimates. *World Health Organization*, 1–24. https://doi.org/CC BY-NC-SA 3.0 IGO

Zhuo, C., Li, G., Lin, X., Jiang, D., Xu, Y., Tian, H., Wang, W., & Song, X. (2019). The rise and fall of MRI studies in major depressive disorder. In *Translational Psychiatry* (Vol. 9, Issue 1). https://doi.org/10.1038/s41398-019-0680-6

CONCLUSION AND FUTURE PERSPECTIVES

CONCLUSION AND FUTURE PERSPECTIVES

Major depressive disorder (MDD) is very complex and heterogeneous. For people diagnosed with this mood disorder the prognosis remains disadvantageous since data shows that only around 30 % of patients are able to achieve remission after the first treatment. In that sense, the diagnosis and prognosis of depression would largely benefit from the discovery of robust personalized, evidence-based tool to help psychiatrists diagnose and choose the most appropriate treatment course for their patients.

In this Thesis we explored neuroimaging biomarkers of depression and depression treatment in a cohort of MDD patients first diagnosed with MDD.

In **chapter 1** we showed that patients diagnosed with depression presented overall poorer cognitive functioning when compared with healthy controls. Our results showed patients presented abnormal rs-FC connectivity within and between networks of functional connectivity often related with executive functioning and goal directed behavior (ECN) or internal mind wondering processes (DMN). As our sample being composed exclusively drug-naïve patients allows as to exclude medication confounding factors, thus, we hypothesize that these alterations could be related with depression symptomology such as anhedonia, rumination, psychomotor retardation and inability to concentrate.

In **chapter 2** we described potential neuroimaging biomarkers associated with treatment outcomes in the same cohort of MDD patients. We described that patients with better clinical outcomes also presented higher rs-FC In the visuospatial network and as well as in the right and left executive networks. In this work we also described that a single dose of paroxetine was able to produce effects, reducing the connectivity within the ventral DMN and the Basal ganglia network, however these alterations did not differ responders from non-responders. Nevertheless, the alterations produced over a longer period of time (6-12 weeks) were able to differentiate between responders and non-responders. Non-responders increased FC with the left executive networks and decreased FC within the sensorimotor network. This study showed alterations produced by the SSRI paroxetine in the brain resting state functional connectivity, early in the course of pharmacological treatment. These alterations bring some light into the search for biological markers, and consequently lead to a better understanding of paroxetine treatment response in MDD patients

In **chapter 3** we studied suicidal ideation, and its neuronal correlates in terms of brain functional connectome and white matter integrity. Suicide is a very complex phenomenon and patients diagnosed

with depression often suffer from recurrent thoughts of death and suicidal ideation. In this work we identified specific networks as well as white-matter track associated with higher suicidal ideation increasing our knowledge about it's possible neuroimaging correlates in treatment-naïve patients.

Overall the work developed in this Thesis allowed to expand our knowledge about the physiopathology of depression as well as depression treatment. We hope that this new data can be used in the sense of replication in order to guide better treatments for depression.

The search for predictors of treatment response or differential treatment response has the benefit of not only helping clinicians choose the best treatment course for their patients, but also targeting research efforts at the gaps in the existing treatment options. The predictors that are more likely to be adopted into clinical practice, apart from their accuracy must also be practical and affordable. In summary, the ideal predictive model of treatment response in depression must be a combination of symptom profiling and patient medical history with a selection of accurate and practical biomarkers. Machine learning and deep learning algorithms can be very useful in the search for such biomarkers.