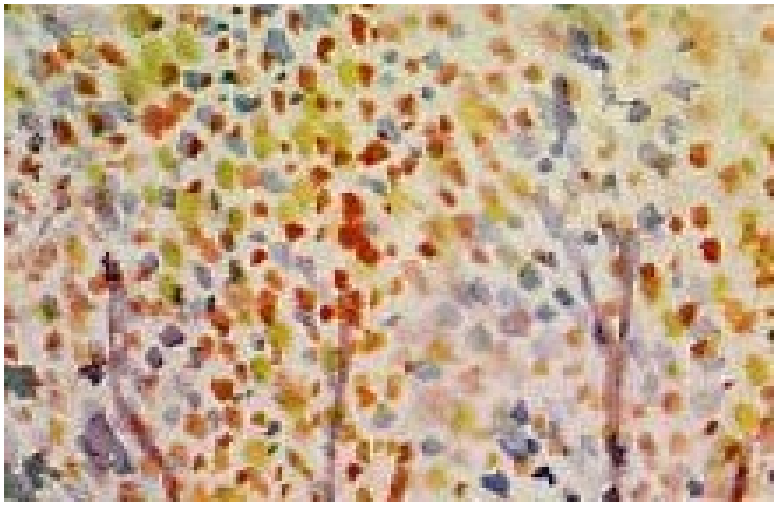
NEUROTOXICITY OF MAJOR DEPRESSIVE DISORDER: A NEUROIMAGING AND NEUROPSYCHOLOGICAL STUDY.



Universitat Autònoma de Barcelona

Thesis presented by

Maria Serra Blasco,

to obtain the degree of Doctor in Psychiatry.

Barcelona, 2015

Universitat Autònoma de Barcelona

Psychiatry Doctorate Program (2012-2015)
Departament de Psiquiatria i Medicina Legal

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Supervisors:
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Dr Yolanda Vives Gilabert (PhD in Telecommunication Engineering)

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Dr Maria J Portella Moll, PhD in neuroscience,
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Dr Yolanda Vives Gilabert, PhD in Telecommunication Engineering,

declare and confirm that they have supervised and guided the PhD thesis entitled NEUROTOXICITY OF MAJOR DEPRESSIVE DISORDER: A NEUROIMAGING AND NEUROPSYCHOLOGICAL STUDY, presented by **Maria Serra Blasco**. They hereby assert that this thesis fulfils the requirements to be defended for the Degree of Doctor.

Signature,

Maria Serra Blasco

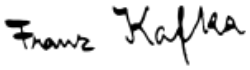
Dr Maria J Portella Moll Dr Enric Álvarez Martinez Dr Yolanda Vives-Gilabert

Barcelona, 2015

Als meus germans, Òscar i Elena
Als meus pares, Sara i Jordi.

«Du kannst Dich zurückhalten von den Leiden der Welt, das ist Dir freigestellt und entspricht Deiner Natur, aber vielleicht ist gerade dieses Zurückhalten
das einzige Leid, das Du vermeiden könntest.»

“You can hold yourself back from the sufferings of the world, that is something you are free to do and it accords with your nature, but perhaps this very holding back is the one suffering you could avoid.”



1883-1924

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Contents

Foreword

This dissertation, presented to obtain the degree of Doctor in Psychiatry of the “Universitat Autònoma de Barcelona”, is the result of different work carried out during a 4-year period (2011-2015) at the Port d'Informació Científica (PIC) and at the Department of Psychiatry, Hospital de la Santa Creu i Sant Pau.

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The following articles have been published in international journals, as a result of the work performed during the doctorate, with a global impact factor (IF) of 13.73 (ISI Web of Knowledge, Journal Citation Reports, 2013). The two articles used to present the thesis as a compendium of publications are highlighted in bold.

Serra-Blasco M, Portella MJ, Gómez-Ansón B, de Diego-Adeliño J, Vives-Gilabert Y, Puigdemont D, Granell E, Santos A, Álvarez E, Pérez, V. 2013. Effects of illness duration and treatment resistance on grey matter abnormalities in major depression. *British Journal of Psychiatry* 202:434-40. IF: 7.343.

Martín-Blanco A, Serra-Blasco M, Pérez-Egea R, de Diego-Adeliño J, Carceller-Sindreu M, Puigdemont D, Molet J, Álvarez E, Pérez V, Portella MJ. 2015. Immediate cerebral metabolic changes induced by discontinuation of deep brain stimulation of subcallosal cingulate gyrus in treatment-resistant depression. *Journal of Affective Disorders* 1;173:159-62. IF: 3.705.

Serra-Blasco M, de Vita S, Rodríguez M, de Diego-Adeliño J, Puigdemont D, Martín-Blanco A, Pérez-Egea R, Molet J, Álvarez E, Pérez V, Portella MJ. 2014. Cognitive functioning after deep brain stimulation in subcallosal cingulate gyrus for treatment-resistant depression: An exploratory study. *Psychiatry Research* 225(3):341-6. IF: 2.682.

The following article is currently under revision in an international journal:

Serra-Blasco M, de Diego-Adeliño J, Vives-Gilabert Y, Puigdemont D, Carceller-Sindreu M, Pérez V, Álvarez E, Portella MJ. 2015. Naturalistic course of major depressive disorder predicted by clinical and structural neuroimaging data: a 5-year follow-up. Submitted to *British Journal of Psychiatry* IF: 7.343.

Finally, the results obtained during these years have been presented to international conferences:

Serra-Blasco M. Breaking news communication: Cingulate cortex volumetric changes at different stages of major depressive disorder. 23rd European College of Neuropsychopharmacology, Amsterdam, The Netherlands 2010.

Serra-Blasco M, Portella MJ, de Diego-Adeliño J, Vives-Gilabert Y, Gómez-Ansón B, Santos A, Puigdemont D, Álvarez E, Pérez V. Larger grey matter volume reductions are related to longer duration of Major Depressive Disorder. ECNP Workshop on Neuropsychopharmacology for Young Scientists in Europe, Nice, France 2012.

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Serra-Blasco M, de Diego-Adeliño J, Puigdemont D, Vives-Gilabert Y, Santos A, Crespo I, Martín-Blanco A, Álvarez E, Pérez V, Portella MJ. Amygdala shape differences in patients with major depressive disorder. 27 th European College of Neuropsychopharmacology. Berlin, Germany 2014.

Serra-Blasco M, de Diego-Adeliño J, Vives-Gilabert Y, Puigdemont D, Carceller-Sindreu M, Pérez V, Álvarez E, Portella MJ. Predictive Value of Structural MRI in Major Depressive Disorder Outcomes: a 5-year Follow-up. Society of Biological Psychiatry 70th Annual Scientific Meeting, Toronto, Ontario – Canada 2015.

Glossary of Abbreviations

MDD Major depressive disorder
TRD Treatment-resistant depression
ECT Electroconvulsive therapy
DBS Deep brain stimulation
SCG Subgenual cingulate gyrus
GMV Grey matter volume
COs Clinical outcomes
DSM IV-TR Diagnostic and Statistical Manual of Mental Disorders IV Edition Text Revision
MDE Major depressive episode
FE First episode
NT Neurotransmitters
MAOI Monoamine oxidase inhibitor
TCAs Tricyclic antidepressants
5-HT Serotonin
NA Noradrenaline
cAMP Cyclic adenosine monophosphate
CREB cAMP response element-binding
BDNF Brain-derived neurotrophic factor
CRH Corticotropine-relasing hormone
ACTH Adrenocorticotrophic hormone
HPA Hypothalamic–pituitary–adrenal
CNS Central nervous system
AD Antidepressant
CSF Cerebrospinal fluid
SSRI Selective serotonin reuptake inhibitor
PET Positron emission tomography
rCMRglu Regional cerebral glucose metabolism rates
ACG Anterior cingulate gyrus
DLPFC Dorsolateral prefrontal cortex
fMRI Functional magnetic resonance imaging
DMN Default mode network
Glu Glutamate
vmPFC Ventromedial prefrontal cortex
Glx Glutamate/glutamine
Cho Choline
NMDA N-methyl-D-aspartate
ReHo Regional homogeneity

CBF Cerebral blood flow
BOLD Blood-oxygen-level dependent
MRI Magnetic resonance imaging
sMRI structural magnetic resonance imaging
DTI Diffusion tensor imaging
FSL FMRIB software library
SPM Statistical parametric mapping
PFC Prefrontal cortex
IFG Inferior frontal gyrus
ROI Region of interest
SVM Support vector machine
OFC Orbitofrontal cortex
CG Cingulate gyrus
MFG Middle frontal gyrus
BA Brodmann area
RAVLT Rey Auditory Verbal Learning Test
TMT-B Trail making test part B
WAIS-III Wechsler Adult Intelligence Scale third version
TOL Tower of London
TMT-A Trail making test part A
ANOVA Analysis of variance
PLUM Polytomous Universal Model
DARTEL Diffeomorphic image registration algorithm
VBM Voxel-based morphometry
TIV Total intracranial volume
HDRS Hamilton depression rating scale
RSC *Retrosplenial cingulate*

1. Introduction

1. Introduction

1.1. Approach

Major Depressive Disorder (MDD) is a psychiatric illness characterized by feelings of sadness or apathy, accompanied by physical and cognitive disturbances. The nature of its symptomatology makes this disorder very crippling, and the huge incidence in the worldwide population, a global concern. Besides the suffering of patients and their relatives, MDD has enormous costs for community, as most of the patients can not work for long periods of time or even do not come back to work never again.

Already in ancient Greece, fears and despondencies that lasted a long time were referred to as melancholia, a disease with mental and physical symptoms. Since then, efforts of science community to find out causes and effective treatments for depression are countless. Nowadays, there is an agreement of what depression is, there are quite well established models of the underlying causes, and several effective drugs, psychological and somatic therapies are available to treat this disorder. However, proposed hypotheses of the pathophysiology of MDD are still far from being complete and there are a 30% of patients who do not achieve a complete remission.

Given the elevate recurrence rates of MDD, many research groups are focused on how life stress and biological susceptibility trigger further episodes. The kindling model, postulated by Post (1992), hypothesized that the brain changes occurring during an episode of depression could facilitate the appearance of a new one. Volume losses observed in specific brain structures seen in depressed patients give support to this model, but specific brain structures vary from study to study and their characterization is hard to define.

Patients with treatment-resistant depression (TRD), who have bad response to numerous treatments (drugs, electroconvulsive therapy -ECT- and even psychotherapy), are now facing a new strategy to achieve good response. Deep brain stimulation (DBS) of subgenual cingulate gyrus (SCG) is now tested as a promising therapy for TRD. DBS is a somatic therapy and is potentially reversible, as electrodes can be removed. Stimulation of SCG produces large changes in the activity of brain regions similar as those produced by antidepressant (AD) medication, cognitive behavioural therapy

or ECT, suggesting a common neural substrate.

Taking into consideration that every new depressive episode entails more vulnerability to the brain, it would be desirable to avoid a chronic course of the illness by achieving remission with the minimum number of antidepressant trials. Ideally, by knowing a number of demographic, clinical and biological characteristics of the patient, therapists may optimize antidepressant treatment individually. To do so, researchers have started to compile relevant data to predict clinical outcome of patients. But what if clinicians were able to know what are the probabilities of a particular patient to relapse or even to become chronic?

1.2. General objectives of the thesis

The general aim of this dissertation is to find out the brain structures involved in the pathophysiology of MDD across different courses of the illness. To investigate brain and cognitive characteristics of depression, structural and functional neuroimaging techniques as well as neuropsychological tests were used. The current thesis is conformed by different studies, designed to draw a clearer picture of MDD clinical courses and the subsequent brain effects, with a focus on TRD.

First, a cross-sectional study was carried out to investigate the effect of MDD on grey matter volumes (GMV) of depressed patients and to determine the weight of clinical characteristics on such effect (Chapter 6.1). Previous studies were mainly focused on comparing depressed patients as a whole with a group of healthy controls, without considering any of the clinical characteristics in the depressed sample. We aimed to analyse brain volume characteristics of each depressive stage. To do so, depressed patients were divided into those who were suffering their first depressive episode, those who had had three or more previous episodes and those who were considered TRD with a chronic course, comparing GMV between them and with a group of healthy controls. At the same time, GMV were correlated with clinical variables such as age at onset, duration of illness or depressive symptomatology to study their weight on the hypothesized brain volume damage.

TRD patients showed considerably less GMV compared to healthy

controls, and duration of illness was the most related variable more related with GMV reduction. DBS of SCG is hypothesized to restore the brain network deregulated in MDD patients and it might prevent GMV shrinkage. However, little is known about the effects of DBS on the neural substrate. Therefore, the second study aimed to determine the immediate effects of DBS on the brain areas, presumably involved in TRD (Chapter 6.2).

The third study aimed to assess cognitive safety of DBS technique on SCG (Chapter 6.3), as a plausible treatment alternative for TRD patients. The purpose was to evaluate the impact on cognition of this experimental somatic treatment (that induced remission in about 50% of TRD patients). This assessment is particularly important given that iatrogenic effects of DBS such as cognitive impairment would compromise even more the long-term patients' functioning. In addition, by comparing neuropsychological function of TRD patients with a less severe depressive group, we aimed to help to characterize TRD as a distinct subgroup of MDD.

Although DBS seems an effective and safety approach for TRD patients, it is also an invasive and highly cost technique, apart from still being under investigation. It would be desirable to know the approximate probabilities of a given patient to become treatment resistant or chronic. With this purpose, the fourth article (Chapter 6.4, under revision) aimed to find out predictive models for clinical outcomes (COs). The depressed sample of the first study was followed-up after 5 years and then categorized into four different clinical trajectories. Models including demographic, clinical and neuroimaging data were run to predict the long-term illness categories.

2. Theoretical framework of Major Depressive Disorder

2. Theoretical framework of Major Depressive Disorder

2.1. Introduction

Depression is the most frequently diagnosed psychiatric disorder among adults. It constitutes a 10% of the chronic diseases to disability-adjusted life-years, being the medical condition with most years lost to disability. Despite all the efforts of the health community to reduce MDD morbidity, by 2030 will be the leading contributor to the worldwide burden of disease (World Health Organization -WHO-, 2008).

The pathophysiology of MDD is still far from being well characterized. The definition of the brain areas playing a primary role on the onset and maintenance of the disease should lead to more optimal treatments. There is consistent evidence of the GMV losses in depressed patients, but there is also a disparity of results regarding which brain areas are involved. Yet, the biological pathways underneath these findings (i.e. glucocorticoid and glutamatergic toxicity, reductions of neurotrophic factors or neurogenesis) have no solid evidence either, probably due to the inappropriateness of tools to examine them (Hasler, 2010).

Neuroimaging have provided a solid approach to study the effects of depression on the brain and is adding new evidence to build a unified neurobiological model underlying MDD pathophysiology. Brain imagery is recently used to study the relationship between brain structure/function and MDD clinical outcomes. The prediction of depressive courses will help to categorize patients at an early stage of the illness, identifying those who will need exhaustive or combined therapies. Although there are clues of the brain areas implied in different illness courses (Frodl et al., 2008; MacQueen, 2009; Schmaal et al., 2014; Soriano-Mas et al., 2011) or the cerebral mechanisms underlying AD treatments (Chen et al., 2007; Fu et al., 2013), there is still long way to go to achieve a model with optimal predictive capacity for clinical use.

2.2. Major Depressive Disorder

2.2.1. Clinical features

Diagnostic and Statistical Manual of Mental Disorders IV Edition Text Revision (DSM IV-TRD), describes a major depressive episode (MDE) as a period of at least 2 weeks during which there is either depressed mood or loss of interest or pleasure. These symptoms have to be present in almost all activities for most of the day and nearly every day and accompanied by

at least four out of the seven following effects: sleep disturbances, changes of appetite or weight, psychomotor agitation or physical retardation, fatigue, guiltiness, diminished ability to concentrate or thoughts of death. MDD is characterized by the affectation of the basic human behaviours (i.e. sleeping or eating) or emotions (fear) to more complex feelings such as sense of hopelessness, uselessness or guiltiness.

In addition to the core symptoms of disturbed mood and affect, there is strong evidence that cognitive function is also impaired. Depressed people commonly suffer from deficits and biases in cognition (reviewed in Gotlib & Joormann, 2010), which interact with other clinical symptoms worsening the course of the illness. Neuropsychological deficits in attention (Paelecke-Habermann et al., 2005; Preiss et al., 2009), memory (Brand et al., 1992; Burt et al., 1995) or executive functioning (Elliott, 1998), specially in cognitive flexibility (Veiel, 1997; Austin et al., 2001) seem to be part of MDD per se rather than an epiphenomenon (Beblo et al., 2011; Gotlib & Joormann, 2010). An understanding of these neuropsychological deficits has practical and theoretical implications for the study of MDD. As reviewed in (Martinez-Aran & Vieta, 2015), cognitive impairment does not fully improve after antidepressant treatment and can deteriorate over time. Thus, cognitive symptoms should be considered a critical goal and an essential therapeutic objective. At a theoretical level, understanding neuropsychological functioning, its interaction with depression and its relation to neuropathology is crucial to a complete comprehension of the disorder.

2.2.2. Neuropsychobiological hypotheses

Given that depression is a heterogeneous disorder, many different models have been proposed to understand the biological mechanisms, based on reliable evidence of the relationship between specific body systems and MDD pathophysiology. However, it is now broadly understood that such a complex disorder is unlikely to be the result of a unique brain region or a neurotransmitter system.

2.2.2.1. The role of monoamines

The monoamine theory suggests that the monoaminergic system is down-

regulated in MDD. The serotonergic, noradrenergic and dopaminergic neurons originate in midbrain and brainstem nuclei and project to extensive cerebral areas. Thus, a deficit of such communications would presumably lead to depressive symptomatology.

This hypothesis arose in the late fifties, when the first antidepressant was accidentally discovered. It turned that patients treated for tuberculosis with a non-selective, irreversible monoamine oxidase inhibitor (MAOI) iproniazid (Loomer et al., 1957) and for psychosis with imipramine (Kuhn, 1957) showed improvements in mood. These two drugs potentiate serotonin (5-HT) and noradrenaline (NA), increasing the availability in the brain connections. Thenceforth, MAOI and imipramine were used as first-line MDD pharmacological treatments (Davidson, 2010). A strong point of the monoamine theory is its predictability, as most of compounds inhibiting NA or 5HT reuptake are effective antidepressants. However, one of every three patients does not respond to these agents and, neurobiologically speaking, direct measures of reduced monoamine neurotransmission have not yielded definitive findings. Research focused on finding reduced levels of NA and 5-HT metabolites in plasma, urine, and cerebrospinal fluid of depressed patients (Owens & Nemeroff, 1994; Roy, 1988; Garcia-Sevilla et al., 2004, Garcia-Sevilla et al., 2011) supports only partially this hypothesis. When 5-HT and NA are depleted experimentally in humans by oral treatments, there is no depression induction in healthy individuals. By contrast, 5-HT and NA depletion does cause a relapse in MDD patients who responded to a selective serotonin-reuptake inhibitor (SSRI) in the past (Ruhé, et al., 2007).

Another way to study monoamine hypothesis has been trough downstream effects of NT involved. Via these indirect measures, monoamine under functioning such as decreased levels of p11 (protein which enhances the efficiency of 5HT-1B receptor signaling), reduced sensitivity of 5HT-1A receptor, heightened α2-NA receptor (inhibits NA release) sensitivity as well as reduced inositol (second-messenger system for 5HT) levels in frontal cortex have been found in depressed patients. The cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) is a transcription factor affected by AMP in the cell. It interferes in the genetic expression of proteins with a key role in neuronal plasticity and neurotrophic processes (i.e. brain derived neurotrophic factor, BDNF). Most of studies report that long-term treatment with antidepressants stimulates CREB functioning. CREB and phospho-CREB levels, in turn, have been found

to be reduced in post-mortem studies of depressive patients who did not took antidepressants compared to controls. (Reviewed in Reid & Stewart, 2001).

These findings point to the vulnerability trait of a lack of monoamines to suffer MDD at least in two thirds of patients, who respond to first-line AD treatments. However, current results fail to demonstrate a causal relation, highlighting the necessity of additional neurobiological factors to induce depression.

2.2.2.2. The neuroendocrine model

The hypothalamic–pituitary–adrenal (HPA) axis is the major neuroendocrine stress response system to adapt the organism to maintain stability and health (McEwen, 2005). When human cortical areas (consciously or unconsciously) perceive a threatening situation, the hypothalamus releases corticotropine-relasing hormone (CRH) and vasopressin. These hormones induce the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland, stimulating the adrenal gland to release cortisol into plasma. HPA main functions consist in the regulation of metabolism and immunity, but it also shows effects on central nervous system (CNS). A hyperactivity of the HPA axis has been described in MDD patients (Arborelius et al., 1999; Heinrichs & Koob, 2004; Mitchell, 1998; Nemeroff, 1996; Wong & Licinio, 2001). Cortisol has been found to regulate neuronal survival, neurogenesis, hippocampus structure, anterograde amnesia and emotional appraisal of events (Herbert et al., 2006). A significant percentage of depressed patients show increased levels of cortisol in saliva, plasma and urine, and increased size and activity of the pituitary and adrenal glands (Nemeroff & Vale, 2005). It has also been reported a reduced glucocorticoid receptor function in peripheral tissues (i.e. peripheral blood mononuclear cells and skin cells), which resolved with successful AD treatments (reviewed in Pariante, 2006). HPA dysfunction it is thought to be an instigator of MDD. People with history of physical or sexual abuse as children have shown increased CRH levels in cerebrospinal fluid (CSF) (Lee et al., 2006), probably caused by such early neuroendocrine changes (Heim et al., 2008). Thus, HPA hyperactivity might act as a risk factor trough two ways: 1) via epigenetic modification from genes encoding glucocorticoid receptor and ACTH releasing factor of early life experiences which would program molecular changes and genetic

liability; and/or 2) as a result of a malfunctioning of the neural circuitry implicated in emotional, neuroendocrine and autonomic control in response to challenge.

HPA axis is also activated by pro-inflammatory cytokines (i.e., interleukin-1α, tumour necrosis factor-α, and interleukin-6). Interestingly, "Sickness behaviour" resulting from such inflammatory response share many MDD symptoms (i.e. fatigue, anhedonia, psychomotor retardation and cognitive impairment) (Dantzer et al., 2008). Therefore, it has been suggested that cytokines may play a role in the pathophysiology of the subgroup of depressed patients showing comorbid physical symptoms. In this regard, Mendlewicz and colleagues (2006) reported an accelerating effect of acetylsalicylic acid in combination with SSRIs in the treatment of MDD, supporting the possible clinical relevance of psychoneuroimmunology in clinical depression. HPA axis hyperactivity and inflammation in adult depressed individuals might be part of the same pathophysiological process with a mutual feedback. The HPA hyperactivity would act as a marker of ineffective action of glucocorticoid hormones on target tissues (glucocorticoid resistance), leading to immune activation. Inflamation, in turn, could stimulate HPA axis activity via both a direct action of cytokines on the brain and by inducing glucocorticoid resistance (Raison et al., 2006).

2.2.2.3. The diathesis/stress model

The diathesis/stress hypothesis answers the classical question about why the same stressful event can affect people so differently. Perhaps, differential vulnerability to depression explains this phenomenon. The diathesis/stress model confers different weights to vulnerability (diathesis) and precipitation (stress) for a given psychiatric illness. In the case of MDD, the model assumes that as diathesis increases, the level of stress needed to trigger a new MDE decreases.

Individual vulnerability to become depressed is known to be multifactorial, and the strongest factors contributing to that susceptibility are, in descending order: stressful life events, genetic factors, previous history of major depression, and neuroticism personality trait (Kendler et al., 1993). Stressful life events are known to raise the risk for depression, but the processes mediating this effect are not clear enough. One explanation is that individuals with early adverse experiences became stress sensitized,

that is to say, that less stress in adulthood could end up in a depressive episode (reviewed in Heim et al., 2008). The experiences more frequently reported to increase the probability of suffering MDD in adulthood are early parental loss and early stress (reviewed in Heim & Nemeroff, 2001).

Genetic predisposition could act as a vulnerability factor to illness and it could influence the individual's response to stress and the probability of stressful events exposure (Agid et al., 2000). MDD has solid evidence to be considered a familial disorder given some genetic penetrance (Sullivan et al., 2000). Depression heritability (30-42%) is polygenetic, and has its incidence via epigenetic factors like stressful life events. To date, there is statistically significant evidence for six MDD susceptibility genes (APOE, DRD4, GNB3, MTHFR, SLC6A3 and SLC6A4, López-León et al., 2008), but specific loci associated with MDD have yet to be defined (Hasler, 2010).

Prior experience to depression is another diathesis factor reported as significantly predictive of recurrences in MDD (Colman et al., 2011). One of the most integrative theories explaining this relationship (kindling hypothesis) is extensively described below (section **2.2.2.4**). Finally, neuroticism has also been strongly associated with MDD risk (revised in Ormel et al., 2013). This personality trait first proposed by Eysenck (1950), refers to the frequency and facility of an individual to be bothered. Healthy subjects with high neuroticism display similar characteristics than those observed in MDD patients, like HPA hyperactivity (Portella et al., 2005) or cognitive biases (Chan et al., 2007).

Actually, neuroticism has been found to mediate MDD treatment effect (Quilty et al., 2008), as individuals with relatively low neuroticism showed better outcomes in depressive symptoms (reviewed in Ormel et al., 2013). However, like any of the above-mentioned vulnerability factors, it does not determine the occurrence of a depressive episode, but add susceptibility to suffer it. Taken together, the diathesis/stress findings indicate that the more risk factors an individual accumulate (stressful life events, neurobiological predisposition, genetic risk or/and neuroticism) the more chances to succumb to depression.

2.2.2.4. The kindling hypothesis

The contribution of life stress to MDD is beyond doubt. However, one of the

most potent diathesis is prior experience of depression, meaning that the number of stressful events needed to elicit a new episode change over time. This fact was already observed by Kraepelin (1921) who wrote that a patient became depressed "... after the death first of her husband, next of her dog, and then of her dove" (p. 179). This observation it is a clear example of the shift from episodes that are triggered to those that occur almost autonomously (Post, 1992). Thus, the concept of kindling (which originally refers to the sensitization of brain tissue to seizure-inducing electrical current, Goddard et al., 1969) has been suggested as an analogy for the stress sensitization seen in human mood disorders. In the 1980s, Post and Ballenger used the kindling model to explain how depressive episodes – initially triggered by stressful life events– begin to appear automatically (Post et al., 1982). An extensive research literature consistently supports the kindling premise that there is a greater role for major life stress "...in association with the first episode of major affective disorder than with subsequent episodes" (Post, 1992, pp. 999–1000) reviewed in Monroe & Harkness (2005).

In addition to clinical observations, there is also evidence that a brain does not fully recover after an MDE. Imaging studies have different lines of evidence regarding neurobiological basis of stress sensitization in humans. On the one hand, structural magnetic resonance imaging (sMRI) studies of MDD patients report decreases in hippocampal volume (presumed as a result from the toxic effects of corticosteroids). Small hippocampal volumes have shown negative correlations with illness duration (Bell-McGinty et al., 2002; MacQueen et al., 2003; Sheline et al., 1999; Sheline et al., 1996) and with the total number of previous depressive episodes (Videbech & Ravnkilde, 2004). There are also studies that find no relation of hippocampal volume losses with clinical characteristics (Frodl et al., 2002). Other structures such as the cingulate (Caetano et al., 2006), prefrontal (Bora et al., 2012), insula (Soriano-Mas et al., 2011) or the whole cerebral GMV (Lampe et al., 2003) have also shown volume reductions even in remitted patients. At a functional level, positron emission tomography (PET) studies have found that regional cerebral glucose metabolism (rCMRglu) correlates negatively with number of previous episodes/illness duration in fronto-cingulate areas and positively in middle temporal gyrus/lingual-parahippocampal gyrus, respectively (Kimbrell et al., 2002). In the same line, MDD euthymic patients showed cingulate cortex hyperactivation for

working memory tasks compared to controls, pointing to systematic changes in neuronal networks (Schöning et al., 2009). Another study that examined neural activation of recovered participants when presented with pleasant (chocolate) and unpleasant (mouldy strawberries) stimuli showed decreased ventral striatum activation in the first condition and hyperactivity of caudate nuclei in the second (McCabe et al., 2009). The observed structural and functional abnormalities present in recovered patients may be mirroring the impact that previous episodes of depression have exerted on brain.

This brain impairment is also reflected in neuropsychological functioning. MDD non-symptomatic patients have shown more cognitive impairment than healthy participants in attention, memory and executive functioning (Paradiso et al., 1997; Preiss et al., 2009). Austin and colleagues (2001) carried out a revision and reported persistent neuropsychological impairments in mnemonic and executive functioning. However, they also spotted that the specific factors mediating these alterations needed to be determined. Interestingly, cognitive functions impaired once depression has remitted (namely memory, attention and executive functioning), strongly rely on cingulate, frontal and temporal brain regions, which have demonstrated to suffer morphometric and functional alterations in depressed patients. Thus, vulnerability (diathesis) to depression might be linked with cortico-limbic abnormalities (independent of mood state) that remain after recovery, facilitating new depressive episodes.

2.2.3. The course of depressive illness

There are several clinical trajectories in MDD. The most benign consists on achieving complete recovery after a first MDE without any more relapses. However, about a 50% of patients with a first MDE suffer relapses (Faravelli et al., 1986; Richards, 2011) and among them, about a 15% will experience a chronic course. Patients with a chronic course have an illness duration of 17 years, on average, (Gilmer et al., 2005) and are generally linked to a more severe and debilitating subphenotype (Angst et al., 2009; Eaton et al., 2008). Residual symptomatology is a robust clinical marker associate with very rapid relapses (Judd et al., 1998), facilitating chronic trajectories and resistance to AD treatments (see section **2.2.4**). MDE per se also constitutes a risk factor for future episodes, incrementing the chances to relapse with

each new episode (Solomon et al., 2000). Therefore, MDD could not be only described as an episodic neither as a chronic illness, but as a disease initially episodic with some probability of ending up being chronic. Although the literature often subdivides chronic depression (dysthymia, chronic major depression, recurrent major depression with incomplete remission during episodes and double depression), the current general approach is to see the disease as a broad category including variants with more similarities than differences and possibly sharing an underlying aetiology (Dunner et al., 2005; Klein & Santiago, 2003). Some characteristics shared by chronic patients are the resistance to AD treatments (see section 2.2.4), impaired functioning, (Jaeger et al., 2006) and lifelong duration of symptomatology (Richards, 2011).

2.2.4. Treatment-resistant depression

TRD was first described in late sixties, when after a series of controlled trials of imipramine and derived tricyclic antidepressants (TCA), several authors noticed cases of non-respondent patients. However, patients occasionally responded to intravenous clomipramine or combination of antidepressants (Ayuso & Aliño, 1971; Freyhan, 1974; Kielholz, 1986; López-Ibor, 1971). Quitkin and colleagues (1986) considered AD resistance when patients did not respond to imipramine (300 mg/day) or to an equivalent TCA, neither to phenelzine nor to ECT. In subsequent revisions (Álvarez et al., 1985; Fawcett & Kravitz, 1985), criteria for treatment-resistant depression were established: patients diagnosed from primary unipolar depression who do not respond to 300 mg of imipramine per day or an equivalent TCA nor to a MAOI, after a minimum period of 6 months, and an optimal compliance. Since then, many definitions have been proposed (Ananth, 1998; Souery et al., 1999), but the most recent and accepted may be the one defined by (Sackeim, 2001). It states that an adequate antidepressant therapy consists of one or more trials with AD medications with established efficacy in MDD, with effective doses (superior to placebo in controlled clinical trials), sufficient duration and compliance. Sackeim's TRD definition also includes relapsing when patients are following the same treatment that was effective in the past. A meta-analysis (Fava & Davidson, 1996) reported a 19-34% of patients as non-responsive to AD treatments. Both single (Golden et al., 2002) and sequential antidepressant treatments (Petersen et al., 2005)

have reported that only 50% of patients achieved full remission. Thase & Rush (1997) proposed a model of staging the various levels of resistance in TRD patients. Although the model make some assumptions that have not been contrasted enough (i.e. the switch of antidepressant within the same class is less effective than the switch of antidepressants within a different class or the implicit hierarchy of AD treatments –MAOIs>TCAs>SSRIs– (Mace & Taylor, 2000)) and do not consider augmentation or combination strategies (Fava, 2003), it is a useful tool which help clinicians and researchers to achieve a general agreement in TRD stages. Historically, a 50% of symptoms reduction in most of depression scales was considered response and subsequently an optimal outcome. Nowadays full recovery or complete remission of symptoms is the optimal therapeutic goal, and it is only achieved when patients come back to their previous psychosocial functioning with a minimal burden of residual symptoms (Bakish, 2001).

In order to find more adequate and effective long-term treatments, or even better, an early identification of potential TRD patients, this sub-group of MDD patients have to be defined and its risk factors detected. Female gender is sometimes considered to increase the risk for TRD, but when studies have examined predictors of outcome, gender has not been consistently reported (Kornstein & Schneider, 2001). Likewise, a positive family history of depression has been also proposed, but there is no well-designed studies corroborating this association (Nelsen & Dunner, 1995). There exist a controversy with the onset of depression, as an earlier or a later could yield to different illness trajectories. In this regard, Akiskal et al. (1981) found that early onset together with family MDD history were associated to illness chronicity, entailing lower response rates and residual symptomatology. On the other hand, a late illness onset (>60 years) was associated with several important features that may lead to treatment resistance. However, these features may be more related to other associated clinical conditions (named psychotic depression or a higher rate of comorbid medical conditions) than with late onset subgroup per se (Brodaty et al., 1991; Brown et al., 1983). The severity of MDD symptomatology is one of the clinical variables most related to TRD, as it tends to be associated to functional impairment, longer illness duration, lower likelihood of spontaneous remission, and a greater risk of recurrence (Thase, 2000). Finally, chronicity, which refers to the MDD condition in which patients have either longer illness episodes (≥2 years) or a partial remission

between episodes (Keller & Hanks, 1994), is closely associated to TRD, as chronicity tends to worsen the overall prognosis of depression (Keller et al., 1984, reviewed in Korstein et al., 2001).

The neurobiology of TRD has also been investigated. Neuroimaging studies (see section **2.3.2**) have defined grey and white matter structural alterations of TRD patients, as well as their possible functional abnormalities (fMRI, Default Mode Network –DMN–, MR spectroscopy). There are only few studies of TRD patients including a treatment-responsive group and ideally controlling for medication effects. Shah et al. (1998) reported smaller right medial frontal and striatal volumes in TRD patients than in those treatment-responsive and control subjects. Regarding white matter alterations, de Diego-Adeliño et al. (2013) reported significant reductions of fractional anisotropy in cingulum, corpus callosum, superior and inferior longitudinal fascicule in TRD/chronic patients compared with first-episode MDD and controls. Some brain metabolites (measured by magnetic resonance spectroscopy) can provide useful information regarding neuron cells functioning. Price and Drevets (2010), reported lower concentrations of GABA in occipital cortex in TRD patients compared to those who responded to AD treatments and to healthy volunteers. Portella et al. (2011) reported significant decreases of Glutamate (Glu) levels in ventromedial prefrontal cortex (vmPFC) in remitted-recurrent and chronic (and TRD) patients compared with both first-episode and controls. However, Glu negatively correlate to illness duration, pointing to a possible relation of this metabolite with the time being depressed more than with refractoriness to AD treatments. TRD and remitted-recurrent patients also showed lower levels of Glutamate/glutamine (Glx) and N-acetylaspartate (NAA) in the right hippocampus (de Diego-Adeliño et al. 2013) than healthy controls. Glx correlated with longer illness duration, and levels of Choline (Cho) were specifically higher in TRD/chronic patients compared to FE patients and controls, pointing to specific metabolite abnormalities in this chronic condition. Regarding fMRI studies, the vast majority lack a non-depressed group (Duhameau et al., 2010; Guo et al., 2011; Kumari et al., 2003). Therefore, results may be mirroring differences between depressed patients and healthy controls, not being attributable to TRD subgroup. Wen-bin Guo and his colleagues have addressed this issue by adding patients with treatment-sensitive depression to their works. After a series of fMRI studies (Guo et al., 2012; Guo et al., 2012b; Guo et al., 2013), they reported

affected brain circuits of TRD patients to be partly different from those of treatment-sensitive depression patients. Likewise, Wu and colleagues (2011) found that TRD patients had more cerebral regions with altered regional homogeneity (ReHo) than did treatment-refractory. Regarding resting-state studies, Lui et al. (2011) used a proper TRD group (named refractory), and found a more distributed decrease in connectivity than in the refractory patients in prefrontal areas and in bilateral thalamus.

Summing up, the findings clearly point to specific structural and functional brain characteristics in TRD patients. However, particular brain areas and their precise contribution to such refractoriness remain to be defined. These brain abnormalities are reflected at a cognitive level as well. Neuropsychological profile of TRD patients has been addressed in studies assessing DBS cognitive safety. These studies have found that TRD patients scored below average compared to normative data in attention and visual perception (Grubert et al., 2011), in executive functioning –Iowa gambling test– (McNeely et al., 2008) and that they have lower functioning than control participants (Moreines et al., 2014) in processing speed. However, as noticed, studies are scarce and none of them control for repeated measures or for acute symptoms, which may blur the pure TRD cognitive impairment.

There is a wide range of treatment strategies for TRD patients (Vieta & Colom, 2011). Pharmacological strategies consist on drug optimization, switching to different AD class or combining different medications and adding a second agent to enhance the antidepressant effect. Among the somatic therapies, we find ECT, repetitive transcranial magnetic stimulation and vagus nerve stimulation (approved by the US Food and Drug Administration for the treatment of intractable seizure disorders and treatment-resistant depression), or DBS (see section **2.2.4.1**), magnetic seizure therapy or transcranial direct current stimulation, which are still in experimental stages. The usefulness of integrated approaches which combine antidepressants or/and somatic therapies with psychotherapy have shown promising results (Riva-Posse et al., 2013).

2.2.4.1. Deep brain stimulation

DBS is a new somatic treatment currently under investigation for TRD patients. This method consists on the stereotactic implantation of electrodes

into precise neuroanatomical structures where constant stimulation is applied via a stimulator device. DBS has benefits over other somatic treatments: is reversible, targets small specific structures, has both acute and maintained effects, can be adjusted to achieve an optimal therapeutic effect (reviewed in Anderson et al., 2012), and has some evidence reporting safeness of cognitive functioning (Grubert et al., 2011; McNeely et al., 2008; Moreines et al., 2014). The goal of this new technique is, assuming that depression is a brain system-wide disorder; modulate the activity of this circuit to adequately response under cognitive, emotional and somatic stress circumstances (Mayberg, 2003). Thus, the models of depression driving DBS target selection consider brain structures as a "nodes" within dysfunctional neural circuits that modulate different aspects of the syndrome via connections to limbic, cortical and subcortical areas (reviewed in Anderson et al., 2012). These networks include the limbic–cortical–striatal–pallidal–thalamic network, formed by connections between the orbital and medial PFC, amygdala, hippocampal subiculum, ventromedial striatum, mediodorsal and midline thalamic nuclei and ventral pallidum (Ongür et al., 2003). The orbital and medial PFC are associated with two extended networks, the orbital prefrontal network (which process the affective characteristics of the stimuli –reward, aversion, and relative value; Drevets et al., 2008) and the medial prefrontal network (involved in more introspective functions like mood, emotion and visceral reactions). DBS, as well as psychotherapy, has been shown through neuroimaging studies to normalize the dysfunction (elevated metabolism) in these neural circuits following successful treatment (reviewed in Anderson et al., 2012). Five brain targets are being investigated with encouraging results (i.e. lateral habenula, inferior thalamic peduncle, ventral capsule/ventral striatum, nucleus accumbens and SCG). SCG, perhaps the most studied until now, is located ventral to the genu of the corpus callosum and has shown hyperactivity (Glucose metabolism and cerebral blood flow –CBF–) in depressed patients in comparison with healthy controls (Mayberg, 2003). SCG has also shown hyperactivity in healthy volunteers during sadness induced tasks (Mayberg et al., 1999). The hyperactivity observed, in addition, decreases after distinct but successful antidepressant treatments (Drevets, 2002; Mayberg et al., 2000; Mottaghy et al., 2002; Nobler et al., 2001) and even in placebo responders (Benedetti et al., 2005).

The efficacy of DBS of the SCG has been demonstrated in three

independent studies. The very first one was carried out by the group of Mayberg and colleagues (2005), which lead to a sustained remission of depression in four of six patients. When the sample was extended with 14 patients (Lozano et al., 2008), 60% of them responded and 35% remitted at both follow-up assessments at 6 and 12 months. In addition, remitted patients increased up to 42.9% in the 3–6 years follow-up (Kennedy et al., 2011). Second trial of DBS of the SCG (Puigdemont et al., 2012) showed, at a year follow-up, rates of response (62.5%) and remission (50%) comparable to those above reported by Mayberg and colleagues. Third independent study (Holtzheimer et al., 2012) showed moderate rates of response and remission (36% and 36%, respectively) after 1 year of chronic stimulation, which significantly increased (58% and 92%) after 2 years. Given that several patients had medication and psychotherapy changes after chronic stimulation, the reasons for the long-term clinical improvement cannot be attribute to SCG DBS alone. In any case, these findings are encouraging as demonstrate antidepressant efficacy of DBS of SCG for TRD patients.

Neuropsychological functioning remains stable (Lozano et al., 2008) or even improves (McNeely et al., 2008; Lozano et al., 2012) over time after chronic DBS in depressed patients, adding more safety to the technique. Whether or not those improvements are related to clinical changes are not clear, as disparate results have been reported and the number of studies specifically assessing cognitive functioning is very low.

2.3. The use of neuroimaging in Major Depressive Disorder

Neuroimaging techniques provide excellent opportunities for elucidating the anatomic correlates of MDD. PET, fMRI or resting state fMRI have enabled in vivo characterization of brain correlations of normal and pathological emotional states, treatment response/resistance or recurrent/chronic illness courses. Functional techniques assume that cerebral blood flow and neuronal activation are coupled, so by detecting associated changes in blood flow (blood-oxygen-level dependent (BOLD) contrast for fMRI or in regional glucose uptake (introducing an analogue of glucose molecule fluorodeoxyglucose (PET) neuronal activity will be indirectly measured.

Besides providing information about functioning, magnetic resonance imaging (MRI) techniques also analyse neuroanatomical data. Structural magnetic resonance imaging (sMRI) and diffusion MRI allow assessments of

brain morphology and morphometry by delimiting grey and/or white matter areas respectively. Likewise, MR spectroscopy allows the evaluation of presence and concentration of various metabolites of neurotransmission, which can indirectly provide in vivo helpful information about cellular integrity or loss.

2.3.1. Neuroimaging software tools

In order to process both functional and structural brain-imaging data, a great variety of software tools has been developed in the current years. Although most of them process different kind of images, each technique has its own gold standard software, facilitating the comparison of results among researchers. FMRIB software library (FSL) is widely used for task/resting fMRI and diffusion tensor imaging (DTI; Jenkinson et al., 2012), whereas Statistical Parametric Mapping (SPM) and FreeSurfer are typically employed for structural segmentation and grey/white matter volumetric analyses. The software used for all the works of this dissertation will be extensively explained in chapter 3.

2.3.2. Neuroimaging findings in Major Depressive Disorder

Advances in neuroimaging have produced a change in the direction of research of MDD. Depression is now understood as an illness that involves evident brain alterations. Structural and functional abnormalities occur principally in limbic and prefrontal regions. fMRI studies use experimental paradigms such as tasks of emotional or cognitive processing to engage regions that may be impaired. For its part, the relatively new approach of functional connectivity provides an additional understanding of the interactions among brain regions as a network. Likewise, sMRI research aims to investigate structural brain changes in MDD, its relationship with demographic and clinical variables and to assess the impact of medication. However, only few studies have taken into consideration patient's characteristics, possibly leading to the unresolved consensus in the literature of areas implied in the illness. Another possible contributor to the disparity of results is the use of pre-established region of interest (ROIs), which prevent for type I error while increase the type II, maybe missing to

report some important structures.

A recent meta-analysis of grey matter abnormalities associated with MDD (Atkinson et al., 2014) reported neuroimaging studies using both ROIs and whole-brain VBM methods, aiming to clarify to what extent the methodology used influence the findings. The whole brain analysis revealed volumetric grey matter reductions in 10 clusters across the brain: right ACG, right medial superior frontal gyrus (SFG), right DLPFC, bilateral orbitomedial prefrontal cortex (PFC), right inferior frontal gyrus (IFG) opercular and triangular part, bilateral insula, right claustrum and right putamen. When whole-brain analysis was combined with ROI analysis, clusters of grey matter reductions increased up to 18. New clusters included left ACG, left medial superior frontal gyrus, left superior frontal gyrus, left IFG triangular part and right rectus gyrus. Grey matter reductions not included in whole-brain analyses were bilateral parahippocampal gyrus, left thalamus and left postcentral gyrus. However, most of these findings do not account for subgroups (melancholic depression, TRD…) or do not take into consideration individual illness characteristics (i.e. depressive symptomatology scores, age at onset, duration of illness, comorbidities, etc.).

In this regard, researchers are now incorporating illness characteristics in neuroimaging studies. Ballmaier and colleagues (2008) studied hippocampal morphology in early and late MDD onset, finding more pronounced regional volume losses and memory deficits in late-onset depression. Results suggested a higher likelihood of late-onset MDD patients to develop cognitive impairment than those with an early-onset. In the same line, late-onset depressed patients showed more severe hyperintensity ratings in deep white matter than early onset and controls (Tupler et al., 2002). The hyperintensities of left hemisphere were significantly associated with older age onset patients, whereas those on the right hemisphere and left putamen were associated with melancholia in the depressed group. In a meta-analysis carried out by Bora and colleagues (2012), frontal and subcortical grey matter reductions were more prominent in those with late-life depression, with the most robust differences in thalamic volume. However, there are also studies, which find no differences between early and late MDD onsets. Hickie et al. (2005), for example, found reduced hippocampal volumes in older people with depression, both at early-onset and late-onset. Despite the relatively disparity of results, findings seem to

indicate a more prominent affectation in individuals who start the disease later in life. In any case, age at disease onset should be taken into account in MDD neuroimaging studies. Medication has also been seen to alter both brain function (Phillips et al., 2014) and structure (Lavretsky et al., 2005). In this regard, Bora and colleagues (2012) reported significantly smaller SgACG and orbitofrontal cortex in antidepressant-free samples compared to medicated patients, suggesting a neuroprotective effect of medication. Likewise, Huang et al. (2013) found smaller dentate gyrus volume in unmedicated MDD participants than in those who took antidepressant or than in control subjects, and lower cornu ammonis (CA1–3) than in control subjects. These findings highlight the importance of control for the potential effects of medication in MDD samples. Finally, in light of the above-mentioned observations, there is still the need to consider the effect that depression exerts on the brain. This does not only mean to compare patients with and without acute symptomatology between them, but to take into account illness duration, age at onset, medication or the number of previous MDEs altogether.

2.4. Predictors of clinical course

Recurrent depressive illness and chronicity remains one of the predominant problems when it comes to MDD treatment. The identification of predictors of therapeutic response is one possible approach to improve treatment efficacy. Eaton et al. (2008) reported that being female and carrying 1 or 2 short alleles of the 5-HT transporter gen were risk factors for depression onset. However, none of these variables were strong predictors of recovery or recurrence. In a recent systematic review, risk factors to develop a chronic depression were examined (Hölzel et al., 2011). Younger age at onset, longer duration of depressive episode and family history of mood disorders were identified as the strongest risk factors. These results, however, are difficult to generalize given that 40% of those patients were dysthymic and none of them suffered recurrent depressive disorder with incomplete remission between episodes. Thus, there is a clear lack of studies investigating individual predictors of the most common depressive trajectories.

2.4.1. Demographic and clinical predictors

Judd and colleagues have provided a great amount of literature regarding clinical characteristics with predictive capacity for relapse. One of the main risk factors is to suffer residual depressive symptoms. They reported (Judd et al., 1998) that symptomatic patients relapsed up to 5 times faster than asymptomatic ones. Afterwards, this result was supported (Judd et al., 2000), as patients with residual inter-episodic symptomatology had significantly more severe and chronic future courses, faster relapses, higher number of recurrences, shorter intervals between episodes and fewer symptom-free weeks during follow-up. In addition, residual symptomatology at time of recovery predicted early recurrences (Kanai et al., 2003) and increased the relapse risk (Lin et al., 1998; Mueller et al., 1999). History of depression is another risk factor for recurrence (Colman et al., 2011). Specifically, the rate of recurrence increases up to 60% after 5 years, 75% after 10 years and 85% after 15 years (Keller & Boland, 1998). In addition, experiencing several MDEs in the past seems to shorten the time to recurrence (Solomon et al., 2000). Regarding age at onset, results are disparate. Rao & Nammalvar (1977) observed that while the onset of depression before the age of 40 predisposed to recurrences, the risk of chronicity was more prominent in those patients who developed the illness after 40. These results partly explain the inconsistency of other findings regarding age at onset, as both poles (early and late) may entail some risks.

Psychosocial dysfunction it is also associated with a decrease in the likelihood of subsequent recovery (Solomon et al., 2008). In fact, for some patients, remission is only partial, as is not accompanied by a return to normal life and neither a real perception of well-being (Faravelli et al., 1986). Therefore, inter-episodic residual symptoms, past history of MDD and a poor social functioning are reasonable indicators of that the illness is still active and treatment should not be stopped. However, particularly for recovered patients, there are few factors with reasonable certainty to predict an individual's likelihood for recurrence (reviewed in Richards et al., 2011). Further investigation including biological measures like neuroimaging data seems essential to develop clinically useful biomarkers.

2.4.2. Neuroimaging predictors

Findings reported in the previous sections reveal how neuroimaging research has allowed characterizing functional and structural MDD abnormalities. However, it has failed to have significant impact on clinical practice. Thus, to translate these findings into clinical application; it is decisive to identify biomarkers for treatment response as well as for illness progression, helping to guide treatment strategies.

Recently, neuroimaging-based diagnoses and clinical predictions derived from different functional and structural techniques have gained the potential for clinical translation. The seminal PET study carried out by Mayberg and colleagues (1997), reported rostral ACG hypometabolism in non-responders to antidepressants while responders were hypermetabolic. A meta-analysis performed by Fu and colleagues including PET, fMRI and sMRI studies reported increased baseline activity in the ACG and medial prefrontal cortices as predictive of a higher likelihood of improvement. On the other hand, increased baseline activation in the right putamen and in right insula were associated with higher likelihood of a poor clinical response (Fu et al., 2013). McGrath and colleagues (2013) found that insula hypometabolism was associated with remission to cognitive behavioural therapy and poor response to escitalopram, while insula hypermetabolism was associated with remission to escitalopram and poor response to CBT. When a task is required (response to negative words), participants with the lowest pretreatment sustained subgenual ACG reactivity display the most improvement after cognitive therapy (Siegle et al., 2012). FMRI has also proven effective when discriminating chronic patients from those with better clinical trajectories (Schmaal et al., 2014) via neural activation to emotional faces. However, when the analysis was made via support vector machine (SVM) pattern classification, the approach was able to correctly classify patients and controls but failed to classify patients' clinical response (Fu et al., 2008). Hernández-Ribas et al. (2013), also used fMRI to predict clinical response to repetitive transcranial magnetic stimulation (rTMS), and found different patterns of activation in those patients with active stimulation.

As the underlying pathophysiology of MDD is unlikely to rely on a single area, the study of brain networks has become a necessary part of neuroimaging research. Different patterns of connectivity involving OFC have been found in non-responder MDD patients compared to treatment

responders (Lisiecka et al., 2011). Van Waarde and colleagues (2014) went a step further and identified a brain network that predicted MDD recovery, named dorsomedial PFC (including, DLPF, OFC, and posterior CG) with a sensitivity of 84% and specificity of 85%. Brain connectivity has also been studied via white matter tracts. DTI studies have reported that age and altered connectivity in the cingulum part of the cingulate and stria terminalis tract predicted patients' remission with an accuracy of 74% (Korgaonkar et al., 2014). In the same line, reduced anisotropy (indirect measure of white matter damage) in white matter lateral to the cingulate gyrus predicted poor treatment response in a sample with geriatric depression (Alexopoulos et al., 2002).

Focusing on grey matter structure, a recent publication (Pizzagalli, 2011) reviewed frontal and anterior cingulate gyrus volumes and their potential prognostic value in MDD. They reported greater GMV decline in right ACG and DLPFC in those individuals failing to remit during the next 3 years (Frodl et al., 2008). In addition, right ACC volumes predicted poor treatment response and more frequent hospitalization (Chen et al., 2007; Costafreda et al., 2009; Frodl et al., 2008; Gunning et al., 2009). A study by Costafreda and colleagues (2009) found that brain structural neuroanatomy predicted 88.9% of the clinical response prior the initiation of antidepressant treatment (fluoxetine). Clinical remission was predicted by greater grey matter density in right ACG (BA 32), left posterior CG (BA 31), left middle frontal gyrus (MFG, BA 6) and right occipital cortex (BA 19), whereas regions that predicted residual symptoms were bilateral OFC (BA 11), right SFG (BA 10) and left hippocampus. The structural neuroanatomy did not show a significant prediction of clinical remission to cognitive behavioural therapy. In the meta-analysis performed by the same group later on (Fu et al., 2013); right hippocampal volume was the structure more consistently predicting poorer treatment response. Finally, a study using support vector machine applied to grey and white matter correctly distinguished between non-refractory and refractory MDD patients with an accuracy of 69.57% and 65.22% respectively (Gong et al., 2011).

Therefore, although both clinical and neuroimaging data seem to provide useful information regarding treatment outcome and illness course, few are the studies that gather this information to optimize the prediction of depressive clinical outcomes. Longitudinal studies predicting patients'

relapses, recovery and treatment-resistance will offer definitive opportunities for progress.

2.5. References

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3. Hypotheses of the thesis

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The hypotheses raised for study in the present thesis are the following:

Hypothesis 1

Patients with MDD show structural brain abnormalities compared with healthy participants. The illness stage of MDD and its clinical characteristics have a distinct effect on grey matter volume.

Hypothesis 2

Functional brain modifications caused by DBS withdrawal in TRD patients may occur even before clinical changes when stimulation is stopped.

Hypothesis 3

TRD patients show impairments in neuropsychological functioning. Their cognitive performance improves after DBS of SCG.

Hypothesis 4

There are clinical characteristics with some potential to predict future illness courses. Structural brain data will increment the explained variance of the prediction of long-term clinical outcomes.

4. Objectives

4. Objectives

The objectives pursued in the present thesis are the following:

Objective 1

To investigate structural brain abnormalities at different stages of the illness and to determine the effect of clinical characteristics on brain grey matter volume.

Objective 2

To determine immediate cerebral metabolism changes during a short switch-off of electrical stimulation in implanted patients with TRD who had achieved clinical improvement after a period of chronic DBS.

Objective 3

To evaluate cognitive function of TRD patients before and after DBS of the SCG.

Objective 4

To examine the prognostic potential of clinical and sMRI data in the long-term clinical outcomes of MDD.

5. Methods

5. Methods

Study 1

A sample comprised by 66 right-handed individuals with MDD and 32 right-handed healthy controls underwent a specifically designed MRI protocol. Patients were split into three different groups: first (n=22) was comprised by patients with a high burden of illness and diagnosed of chronic depressive disorder, the second (n=22) included patients who had experienced three or more previous episodes of MDD and were euthymic at the time of scanning and third group (n=22) comprised individuals with a first episode (FE) of depression. 32 healthy subjects were also scanned and included in the analysis.

Study 2

Seven patients with TRD who had been previously implanted for DBS in SCG and had achieved clinical remission were included. After a period of clinical stabilization with chronic stimulation (9 months on average), two FDG-PET scans were acquired from each patient, in a 48 hours period. The first scan was done with the implants set to active stimulation ('on'), then the stimulator was turned off and a second scan was carried out after 48 hours of non-stimulation ('off'). Clinical ratings were performed before the first and the second scan by means of the HAMD-17 items.

Study 3

Eight individuals diagnosed of MDD resistant to pharmacological treatment were treated with the experimental therapy of DBS of SCG. They were assessed with a neuropsychological battery to determine their cognitive status before and after DBS implantation. In addition, a group of eight patients with a FE of MDD matched on age, gender and level of education, was also assessed. This group allowed us to control for possible practice effects and interference of acute symptoms on cognitive performance. Neuropsychological battery was comprised by test assessing memory (Rey Auditory Verbal Learning Test, RAVLT), executive functioning (Trail Making Test B, TMT-B; Verbal fluency, FAS; digit span backwards subtest of Wechsler Adult Intelligence Scale III, WAIS-III; Tower of London, TOL), language (Vocabulary subtest of WAIS-III and category test) and processing speed/attention (Digit span forward subtest of WAIS-III and TMT-A).

Study 4 (Under revision)

Sixty-six individuals with MDD who underwent a Magnetic Resonance Imaging (MRI) from September 2007 to July 2011 (Study 1) were followed up after an average period of five years and were fully interviewed again for the present study. Nine patients did not fulfil the entire assessment or they preferred not to participate on this occasion, and eight had been included in a DBS protocol (Study 2). The final sample included 49 patients divided into four different clinical outcomes: Recovery (patients who had not had any other MDE nor residual symptomatology since the baseline assessment), partial remission (patients without any other episode of major depression but some sub-clinical symptomatology such as anxiety, irritability, restricted affect or sleep disturbances), remission-recurrence (patients who had had more episodes of depression but always achieve interepisodic remission) and chronic depression (patients who had been depressed most of the time or had had more episodes of depression and suffered from inter-episodic residual symptomatology).

MRI data acquisition

MRIs, employed in Study 1 and Study 3, were obtained using a 3T Philips Achieva facility (software version 2.1.3.2), three-dimensional (3D) shortest echo scans (repetition time (TR)=6.7ms, echo time (TE)=3.2ms, 170 slices, voxel size (REC): 0.896×0.896×1.2 mm, image dimensions: 288×288×170; field of view: 256×256×204 mm, slice thickness: 1.2 mm). For each participant, high-resolution 3D-MPRAGE images were acquired (whole brain coverage), with a sagittal slice orientation, T1 contrast enhancement, flip angle: 88, grey matter as a reference tissue, acquisition matrix M×P=256×240 and turbo-field echo shots (TFE)=218. All technical procedures were carried out in the cluster of the Port d'Informació Científica (PIC) on Scientific Linux 5 (www.scientificlinux.org).

FDG-PET scans were performed on a Siemens ECAT EXAT HR+ PET/CT scanner in 3-D mode with a 15.8-cm axial field of view. Blood glucose measured before tracer injection was 96.93 mg/dl (mean). Scans were performed 30 min after intravenous injection of 7.78 mCi (on) and 7.99 mCi (off) of fluorine-18-fluorodeoxyglucose PET (18FDG-PET). The acquisition time was 20 min per position. Sixty-three slices 2.4 mm thick were acquired (matrix dimensions=128×128×63, voxel size=2.57×2.57×2.43 mm^3). Co-

registration of PET images was performed with a previous MRI T13D-MPRAGE acquired before DBS surgery (described above).

Data analyses

Demographics and clinical variables were analysed using the R statistical package version 2.10.1 for Windows (Study 1) and with the statistical package SPSS v.18 (Study 2, 3 and 4). Parametric and non-parametric tests were performed as appropriate. In general, t-test and analyses of variance (ANOVAs) were used for quantitative variables and χ^2 for categorical variables. For the fourth study (under revision), a regression model for ordinal variables (Polytomous Universal Model –PLUM–) with a hierarchical approach was used. Level of statistical significance was set at $p<0.05$ if no otherwise specified.

Brain data analyses (Study 1, 2 and 4)

Statistical Parametric Mapping

SPM software package has been designed for the analysis of brain data sequences, usually consisting on a series of images from different cohorts or time-series from same subject. SPM has different techniques to analyse a variety of images.

In the first study, voxel-based morphometry (VBM) approach was applied to study structural whole-brain characteristics. VBM is a method specifically designed to analyse T1-weighted images (Ashburner & Friston, 2000; Wright et al., 1995). VBM involves a voxel-wise comparison of GMV between groups of subjects. The procedure starts with the image tissue segmentation, which involves the generation of roughly (via rigid-body) aligned grey and white matter images. Afterwards subjects are aligned among them using a fast diffeomorphic image registration algorithm (DARTEL, Ashburner, 2007). DARTEL increased the inter-subject accuracy by modelling the shape of each brain using millions of parameters (three for each voxel). Moreover, it aligns grey matter among the images, while simultaneously aligns white matter. Finally, grey matter images are smoothed, spatially normalized and Jacobian scaled to Montreal National Institute (MNI) space. Once data are pre-processed, inferences can be made about the location of systematic differences within grey matter tissue. SPM uses statistics to identify differences in brain anatomy between groups of subjects, essentially

performing statistical tests across all voxels in the image to identify volume differences between groups.

SnPM provides an extended framework for non-parametric permutation/randomisation tests using the GLM and pseudo t-statistics for independent observations. It was used to carry on the PET analysis given the small sample size (Study 2).

FreeSurfer

In order to obtain total brain volumes of a series of structures affected in VBM analysis, specific software named FreeSurfer was used (http://surfer.nmr.mgh.harvard.edu/). The rationale for using this software was that it contains a fully automatic structural imaging stream to segment both cortical and subcortical brain areas. In order to segment cortical areas, FreeSurfer implements the pipeline “Surface-based Stream”, which consists of several stages (Dale et al., 1999; Fischl et al., 1999). First, the volume is registered with the Talairach atlas (Talairach & Tornoux, 1988), allowing FreeSurfer to compute seed points in later stages. The B1 bias field is estimated by measuring the variation in the white matter intensity. The main body of the white matter is used to estimate the field across the entire volume. Likely, white matter points are chosen based on their locations in Talairach space as well as on their intensity and the local neighbourhood intensities. The intensity within each voxel is then divided by the estimated bias-field at that location in order to remove its effect. The skull is stripped using a deformable template model. Voxels are then classified as white matter or something other than white matter based on intensity and neighbour constraints. An initial surface is then generated for each hemisphere by tiling the outside of the white matter mass for each one. This initial surface is then refined and nudged to follow the intensity gradients between the grey matter and CSF. The distance between the white and the pial gives us the thickness at each location of cortex (Fischl & Dale, 2000). Once cortical surfaces are delimitated, FreeSurfer use “Cortical Parcellation” technique (Fischl, 2004), which automatically assigns a neuroanatomical label to each location (vertex) on a cortical surface. This model is based on probabilistic information estimated from a manually labelled training set. This procedure includes both geometric information originated in the cortical model, and neuroanatomical convention, resulting in a complete labelling of cortical sulci and gyri.

In order to label subcortical tissue classes, a volume-based stream is used. The stream consists of five stages (Fischl et al., 2002). The first stage is an affine registration with Talairach space specifically designed to be insensitive to pathology and to maximize the accuracy of the final segmentation. An initial volumetric labelling follows this step. The variation in intensity due to the B1 bias field is corrected. Finally, a high dimensional nonlinear volumetric alignment to the Talairach atlas is performed. The last stage, labelling the volume, shared the same algorithm than in cortical labelling. Volume is labelled from a set of MRI images manually segmented (training set) mapped to a common space in a way that they achieve a point-by-point correspondence between the subject willing to be segmented and the training set (atlas). Each voxel is assigned a label based on three types of probabilities: its own spatial position, having neighbour voxels as reference and its intensity. This procedure is sequentially applied and ends when the segmentation remains unchangeable.

5.1. References

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6. Results of the thesis

6. Results of the thesis

6.1. Effects of illness duration and treatment resistance on grey matter abnormalities in major depression. (Published by Serra-Blasco et al., 2013. *Br J Psychiatry* 202:434-40)

6.1.1. Introduction

One of the major concerns regarding major depressive disorder is that it shows the tendency to become chronic (Rubio et al., 2011), with devastating consequences for patients such as a low quality of life, increased risk of mortality and elevated health and social costs. The pathophysiology of major depressive disorder at different stages of the illness is still unclear and the current neurobiological hypotheses exhibit some important weaknesses (Hasler, 2010). Predominant neurobiological models are based on the occurrence of neurotoxic and neurotrophic processes before and during the disorder, including changes in grey matter volume that have been observed in brain structures of patients with major depressive disorder (Du et al., 2012, Bora et al., 2012). Although the most replicated findings suggest losses of grey matter volume in frontolimbic areas (Sacher et al., 2012), other neuroanatomical systems may be involved in major depressive disorder. Such diversity would better mirror the psychopathological heterogeneity of this disorder. A recent meta-analysis (Kempton et al., 2011) has reported that patients with remitted major depressive disorder have a significantly larger hippocampal volume compared with patients who are currently depressed. However, other clinical variables (e.g. number of previous episodes, illness onset) did not seem to be relevant in relation to grey matter volume. The different imaging techniques used in previous studies, the heterogeneity of samples and the limited overlap of results across imaging paradigms make it difficult to reliably identify neuronal regions or networks consistently affected in major depressive disorder. In addition, the fact that crucial clinical characteristics such as duration of illness have not been considered could partly explain some of the inconsistencies regarding the structures affected. For example, volumetric differences may be less marked in the early stages of the illness and more pronounced in advanced stages. We hypothesise that the clinical

characteristics and the stage of the illness may affect the grey matter volume. The aims of this study were to investigate structural brain abnormalities at different stages of the illness and to determine the effect of clinical characteristics on brain grey matter volume.

6.1.2. Method

Participants

A total of 107 individuals were recruited for the present study, which is part of a bigger project investigating in vivo neuroimaging markers of clinical illness burden (Portella et al., 2011; de Diego-Adeliño et al., 2013) and who underwent an magnetic resonance imaging (MRI) protocol specifically designed for this study. Nine patients had to be excluded from the study for technical or clinical reasons. The final sample included 66 individuals with major depressive disorder (DSM-IV-TR criteria) (APA, 2000) from the out-patients' psychiatric service of the Hospital Sant Pau in Barcelona, Spain, and 32 control individuals. All patients were on medication at the beginning of the study. Given that all patients were receiving different treatment regimens, a medication load index was calculated by taking the current drugs at the time of scanning following the system code proposed by Sackeim (2001). The patients were split into three different groups. The first group (n=22, treatment-resistant/chronic group) consisted of patients with a high burden of illness, with a diagnosis of chronic depressive disorder, a last episode duration of more than 2 years, no response to several antidepressant strategies, a Thase–Rush Index (Thase and Rush, 1997) of treatment resistance, and a score above 14 on the Hamilton Rating Scale for Depression (HRSD; Hamilton, 1969) the second group (n=22, remitted–recurrent group) included patients who had experienced three or more previous episodes of major depressive disorder and were euthymic (HRSD <8) for the past 6 months. The third group (n=22, FE group) comprised individuals with a first episode of major depressive disorder. Thirty-two healthy controls (control group) were also included. The exclusion criteria for healthy participants were: lifetime psychiatric diagnoses, first-degree relatives with psychiatric diagnoses and clinically significant physical or neurological illnesses. Semi-structured interviews were carried out for all participants to collect demographics and clinical information by two experienced psychiatrists. Axis I comorbidity according to DSM-IV-TR criteria

was an exclusion criteria for all participants. Current depressive symptoms were assessed using the HRSD by experienced clinical researchers. All participating individuals were of a similar age (mean 46.86 years, SD=7.99) to avoid age-related variations in brain structures. The study was approved by the Research Ethics Committee of Hospital Sant Pau in Barcelona and was carried out in accordance with the Declaration of Helsinki. All participants gave informed and written consent after a full explanation of the study protocol.

MRI data acquisition and processing procedures

The MRIs were obtained using a 3T Philips Achieva facility (software version 2.1.3.2), three-dimensional (3D) shortest echo scans (repetition time (TR)=6.7ms, echo time (TE)=3.2ms, 170 slices, voxel size (REC): 0.8960.8961.2mm, image dimensions: 28862886170; field of view: 25662566204mm, slice thickness: 1.2mm). For each participant, high-resolution 3D-MPRAGE images were acquired (whole brain coverage), with a sagittal slice orientation, T1 contrast enhancement, flip angle: 88, grey matter as a reference tissue, acquisition matrix M6P=2566240 and turbo-field echo shots (TFE)=218. All technical procedures were carried out in the cluster of the Port d'Informació Científica (PIC) on Scientific Linux 5 (www.scientificlinux.org/).

VBM–DARTEL analysis

The voxel-based morphometry (VBM) analyses were performed using SPM8 (www.fil.ion.ucl.ac.uk/spm) in a MATLAB 7.6.0 environment. First, MRIs were segmented into grey matter, white matter and cerebrospinal fluid using a standard segmentation model in SPM8 (Ashburner and Friston, 2005). Second, grey matter templates were generated from the entire image data-set using the diffeomorphic anatomical registration and the exponentiated Lie algebra technique (DARTEL,; Ashburner, 2007). Afterwards, an initial affine registration of the grey matter DARTEL templates to the tissue probability maps was carried out to create warped images. Images were then modulated to guarantee that grey matter relative volumes were preserved following the spatial normalisation procedure. Finally, images were smoothed with an 8mm full-width at half maximum Gaussian kernel. Spatial pre-processing, smoothed, modulated, normalised grey matter data-sets were used to perform statistical analyses.

Cortical volume

Cortical surfaces were segmented using Freesurfer software for Linux (v.4.3.1, http://surfer.nmr.mgh.harvard.edu/) developed at the Martinos Center for Biomedical Imaging, to obtain the whole volume of every brain structure. Cortex volumes were obtained with the surface-based stream process, as described in detail in Fischl et al. (1999) and Dale et al. (1999). First, the MRIs are affine registered to the Talairach atlas (Talairach and Tournoux, 1988) and image intensity variations as a result of magnetic field inhomogeneities are normalised. Then, a skull stripping algorithm is applied (Ségonne et al., 2004) and the skull-stripped image is segmented into white and grey matter. Finally, the hemispheres are separated and the different surfaces are generated (white and grey). The distance between these surfaces gives the thickness at each location of the cortex (Fischl and Dale, 2000). Following generation of cortical models, surface inflation and the register to a spherical atlas, a parcellation of the cerebral cortex into parts based on gyrus and sulcus structure are executed (Desikan et al., 2006). The results of the cortical surface were verified by experts, and in some cases, manual modifications were applied to obtain more accurate results.

Total intracranial volume measures

Total intracranial volume was calculated in order to ensure that volume differences between participants were as a result of diagnosis instead of brain sizes. Given that two software tools were used, total intracranial volume was computed with both. To get the total intracranial volume provided by SPM8 (TIV_{spm}), the spm_get_volumes function was used, and segmented grey, white and cerebrospinal fluid of each rc* (registered and segmented) image was then summed up. In the case of the total intracranial volume provided by Freesurfer ($TIV_{FreeSurfer}$), values given by automatic segmentation of volume-based stream were used.

Data analyses

Demographics and clinical variables were analysed using the R statistical package version 2.10.1 for Windows (http://www.R-project.org). Voxel-based morphometry was calculated using the DARTEL algorithm in SPM8 to quantify structural brain volumes. Group differences in absolute grey matter volume were assessed using ANOVA with subsequent post hoc comparisons.

Absolute threshold mask was set at 0.2, as recommended by John Ashburner in an VBM Tutorial (Ashburner, 2010), and other parameters were left at their default values. An additional ANCOVA with the three groups (first-episode, remitted–recurrent and treatment resistant/chronic) was performed to control for the effect of medication load (included as a covariate). Significant effects were considered using a $P<0.05$, corrected for multiple comparisons with family-wise error (FWE) for both omnibus (no extent threshold) and post hoc (cluster extent threshold >100) whole-brain tests.

Since SPM8 does not provide absolute volumes of a given brain region, FreeSurfer brain segmentation was used to obtain the corresponding volumes of those areas that showed significant group effects (cluster level P-value set at <0.01). These values were then correlated with relevant clinical variables such as HRSD scores, duration of illness, age at onset, medication load and number of previous episodes. Given the number of comparisons, significance level for correlation analyses was set at $P=0.01$. In order to determine the percentage of volume decrease attributable to clinical variables, an additional linear regression was performed where x corresponded to clinical data and y corresponded to volumes of brain structures. The resulting y values were then divided by the interception of the regression model to get normalised values.

6.1.3. Results

Participants

A total of 98 participants entered the study. **Table 1** shows the demographic, clinical and treatment data of patients and healthy controls. No significant differences between groups were observed in the demographic characteristics. Differences in Hamilton Depression Rating Scale (HDRS) scores, age at onset, medication load and duration of illness were as a result of patients classification based on the stage of the illness and the inclusion criteria. The first-episode group had a significantly older age at onset than the remitted–recurrent or treatment- resistant/chronic group ($F=20.9$, d.f.=2,62, $P<0.0001$). However, this was a result of the age selection performed to minimise brain volume differences attributable to age. As expected, psycho- pharmacological treatments were unequally

distributed across patient groups ($F=10.2$, $d.f.=2,63$, $P<0.0001$). The treatment-resistant/chronic group were heavily treated, and frequently received concomitant treatment with other antidepressants, antipsychotics and/or stabilisers. There were no differences between groups with reference to TIVspm ($F=1.19$, $d.f.=3,94$, $P=0.32$) or TIVFreeSurfer ($F=1.65$, $d.f.=3,94$, $P=0.18$).

Table 1. Demographics, clinical characteristics and total intracranial volumes (TIV) provided by SPM8 and FreeSurfer for groups (upper side). Summary of treatment regimes are detailed in lower side of the table.

	Healthy Control (32)		First episode (22)		Remitted Recurrent (22)		Chronic (22)		F/ χ^2	p value
Characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Age	46	8.3	44	6.5	48	8.7	49	8	1.81	0.1501
Gender Male	9		7		2		4		4.19	0.24
Female	23		15		20		18			
Education										
Primary School	3		3		4		6		4.92	0.55
High School	9		8		6		4			
University	20		11		9		11			
HDRS[a,c,d,e]	2	1.7	16	6.5	4	5.2	21	4.6	94.12	< 0.0001
Age at onset [a,b]	NA	NA	43.5	6.6	29.7	11	27.4	8.4	20.90	<0.0001
Time evolution[a,b]	NA	NA	5.6	4.2	214.3	129	271.5	145	38.57	<0.0001
N of episodes[a,b]	NA	NA	1	0	4.56	4.2	6.2	6.5	7.98	0.0008
TIVspm (ml)	16176	2563	16701	1892	17233	1783	16402	1783	1.19	0.32
TIVFreeSurfer (ml)	11685	1699	12357	2301	11652	1562	11143	1685	1.65	0.18
Treatment										
Medication Load	NA		3.9	2.3	5.2	2.6	7.2	2.4	10.2	0.0001
Antidepressants										
SSRI or SSNRI	-		100%		75%		86%		5.27	0.3
TCA or MAOI[a,b]	-		0%		15%		36%		11.97	0.018
Others[b,d]	-		0.5%		0.5%		57%		13.13	0.011
Combination [a,b,d]	-		11%		20%		77%		26.84	<0.0001
No antidepressant	-		0%		10%		0%		6.4	0.17
Stabilizer	-		16%		20%		36%		5.09	0.28
Antipsychotic[b,d]	-		11%		10%		45%		12.64	0.013
Benzodiazepine	-		26%		30%		59%		8.38	0.08

[a] Significant differences between first-episode and remitted-recurrent patients
[b] Significant differences between first-episode and treatment-resistant/chronic patients
[c] Significant differences between first-episode and healthy controls
[d] Significant differences between treatment-resistant/chronic and remitted-recurrent patients
[e] Significant differences between treatment-resistant/chronic and healthy controls
HDRS=Hamilton Depression Rating Scale; SSRI=Selective Serotonine Reuptake Inhibitors; SSNRI=Selective Serotonine and Noradrenaline Reuptake Inhibitors; TCA=Tricyclic Antidepressant; MAOI= Monoamine Oxidase Inhibitors; Others=Noradrenaline Reuptake Inhibitors, Noradrenaline and Dopamine Reuptake Inhibitors, Tetracyclic antidepressants, Mirtazapine, Metilfenidate or Trazodone; Combination designs concomitant use of antidepressants with different mechanisms of action (e.g. SSRI with reboxetine). Stabilizer includes anticonvulsivants and mostly lithium. Antipsychotic comprehends mainly atypical antipsychotics associated with antidepressants.

VBM–DARTEL analyses (SPM8)

The ANOVA of the control, first-episode, remitted–recurrent and treatment-resistant/chronic groups showed a significant group effect in right superior frontal gyrus (Brodmann area, BA 8), left medial frontal gyrus (BA 6) and left cingulate gyrus (BA 24) ($F=11.10$, $d.f.=3,94$, $PFWE<0.05$, no extent threshold; **Table 2**). Post hoc contrast of the treatment-resistant/chronic group showed diminished grey matter volume compared with the control group ($t=4.75$, $d.f.=1,94$, $PFWE<0.05$, extent threshold $k>100$ voxels), in right superior frontal gyrus (BA 8/9), left cingulate gyrus (BA 24), bilateral medial frontal gyrus (BA 6/8 in left side and BA
10 in right side), left insula (BA 13), left inferior frontal gyrus (BA 44), left parahippocampal gyrus (BA 35), left transverse- temporal gyrus (BA 21) and left post-central gyrus (BA 40). Results are detailed in **Table 3**. No other reductions or increments survived FWE corrections. **Figure 1** represents the grey matter volume decreases in the treatment-resistant/chronic group compared with the control group. There was a tendency of volume decrease in the remitted group compared with the control group ($t=3.87$, $d.f.=1,94$, $P<0.0001$ (uncorrected)) in right superior frontal gyrus (BA 8), right anterior lobe of cerebellum (culmen) and left cingulate gyrus (BA 24). Similarly, the treatment-resistant/chronic group also displayed a decrease of grey matter volume in comparison with the first-episode group ($t=3.87$, $d.f.=1,94$,$P<0.0001$ (uncorrected)) in left pre-central gyrus (BA 4), left post-central gyrus (BA 40), left medial frontal gyrus (BA 6), right insula (BA 13), right transverse- temporal gyrus (BA 41), right inferior parietal lobule and left posterior cingulate (BA 30/31). Results are shown in **Table 4**.

Table 2. Location and peak significance of whole-brain GMV differences in ANOVA (omnibus test) carried out with SPM8. Anatomical region based on Talairach Atlas, t and Z scores, spatial extent in number of voxels (cluster size), voxel-level significance ($P<0.05_{FWE-Corr}$) of the cluster-level and MNI coordinates of the most significant voxel of each cluster are displayed. No extent threshold.

Anatomical region	*Test Value*		*Cluster size*	*Cluster-level*	*MNI coordinates**		
	F	*Z*			*x*	*y*	*z*
Right superior frontal gyrus (BA 8)	14.63	5.27	179	0.005	4	33	49
Left Cingulate Gyrus (BA 24)	12.66	4.89	30	0.009	-14	6	36
Left medial frontal gyrus (BA 6)	12.35	4.83	37	0.016	-10	-5	6

* The coordinates within each cluster were converted from Montreal Neurological Institute (MNI) spatial array to the stereotaxic array of Talairach and Tournoux (1988) using a nonlinear transformation (26).

Figure 1. Regions of smaller GMV in treatment-resistant/chronic patients vs healthy control subjects ($P\leq 0.05_{FWE-Corr}$). Results are presented as a "glass brain" and the MNI152 brain template (A) in render mode of left and right side (B1 and B2, respectively; sagittal view). Areas with a significant decrement of volume appeared in yellow superimposed in a canonical image named "*single_subj_T1.nii*"

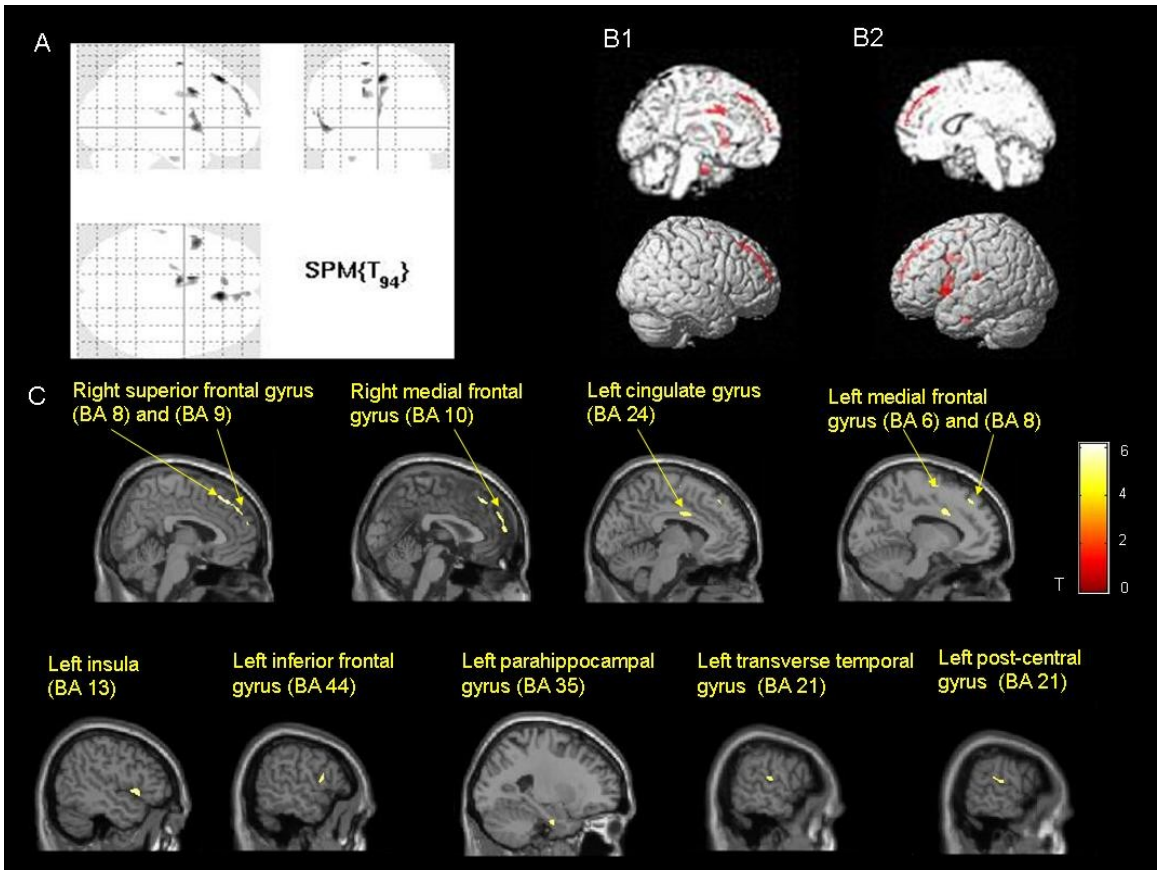


Table 3. SPM8 post-hoc whole-brain results of GMV differences between groups ($P<0.05_{FWE\text{-}Corr}$). Anatomical region based on Talairach Atlas, t and Z scores, spatial extent in number of voxels (cluster size) and MNI coordinates of the most significant voxel of each cluster are displayed. Extent threshold= 100.

Contrast / Region	*Test Value*		*Cluster size*	*Cluster-level*	*MNI coordinates**		
	t	*Z*			*x*	*y*	*z*
HC > FE	-	-	-	-	-	-	-
HC > RR	-	-	-	-	-	-	-
HC > TR/C	-	-	-	-	-	-	-
Right superior frontal gyrus (BA 8)	6.15	5.62	877	<0.001	5	34	49
Right superior frontal gyrus (BA 9)	5.33	4.97			5	51	36
Right medial frontal gyrus (BA 10)	5.31	4.96			2	60	17
Left cingulate gyrus (BA 24)	5.88	5.41	641	0.001	−14	7	36
Left cingulate gyrus (BA 24)	4.97	4.67			−6	−3	34
Left medial frontal gyrus (BA 6)	5.78	5.33	123	0.006	−11	−5	65
Left insula (BA 13)	5.69	5.26	767	0.002	−48	12	−0
Left inferior frontal gyrus (BA 44)	5.38	5.01			−56	9	13
Left medial frontal gyrus (BA 8)	5.30	4.94	192	0.011	−9	38	46
Left parahippocampal gyrus (BA 35)	5.06	4.75	180	0.022	−24	−10	−32
Left transverse-temporal gyrus (BA 21)	4.99	4.69	114	0.019	−59	−20	14
Left post-central gyrus (BA 40)	4.85	4.57			−61	−29	18
FE > RR	-	-	-	-	-	-	-
FE > TR/C	-	-	-	-	-	-	-
RR > TR/C							
HC < FE	-	-	-	-	-	-	-
HC < RR	-	-	-	-	-	-	-
HC < TR/C	-	-	-	-	-	-	-
FE < RR	-	-	-	-	-	-	-
FE < TR/C	-	-	-	-	-	-	-
RR < TR/C	-	-	-	-	-	-	-

HC, Healthy controls; FE, First Episode; RR, Remitted Recurrent patients; TR/C, Treatment-Resistant/Chronic patients. The coordinates within each cluster were converted from Montreal Neurological Institute (MNI) spatial array to the stereotaxic array of Talairach and Tournoux (14) using a nonlinear transformation (26).

Table 4. SPM8 post-hoc whole-brain results of GMV differences between groups ($p_{uncorrected}<0.0001$). Anatomical region based on Talairach Atlas, t and Z scores, spatial extent in number of voxels (cluster size) and MNI coordinates of the most significant voxel of each cluster are displayed. Extent threshold= 100.

Contrast / Region	*Test Value*		*Cluster size*	*Peak-level*	*MNI coordinates**		
	t	*Z*			*x*	*y*	*z*
HC > FE	-	-	-	-	-	-	-
HC > RR							
Right Superior Frontal Gyrus (BA 8)	4.88	4.6	239	<0.0001	4	31	51
Right Cerebellum (Culmen)	4.46	4.24	415	<0.0001	43	-40	-34
Left Cingulate Gyrus (BA 24)	4.04	3.87	164	<0.0001	-13	-26	31
HC > TR/C	Results not reported*						
FE > RR	-	-	-	-	-	-	-
FE > TR/C							
Left Precentral Gyrus (BA 4)	4.9	4.61	890	<0.0001	-58	-14	32
Left Medial Frontal Gyrus (Ba 6)	4.69	4.43	116	<0.0001	-7	-7	64
Right Insula (BA 13)	4.55	4.31	248	<0.0001	48	-16	3
Right Transverse Temporal Gyrus (BA 41)	4.5	4.28	216	<0.0001	61	-19	12
Right Inferior Parietal Lobule (BA 40)	4.44	4.22	109	<0.0001	59	-45	21
Left Posterior Cingulate (BA 30)	4.29	4.09	120	<0.0001	-10	-70	10
Left Posterior Cingulate (BA 31)	4.27	4.09	153	<0.0001	-8	-55	24
RR > TR/C							
HC < FE							
HC < RR	-	-	-	-	-	-	-
HC < TR/C							
FE < RR	-	-	-	-	-	-	-
FE < TR/C	-	-	-	-	-	-	-
RR < TR/C	-	-	-	-	-	-	-

HC, Healthy controls; FE, First Episode; RR, Remitted Recurrent patients; TR/C, Treatment-Resistant/Chronic patients.

*This contrast was not run given that it was already significant with $p<0.05_{FWE-Corr}$

Effects of medication

Mean values of the medication load index for each patient group are listed in **Table 1**. The ANOVA of the three groups with depression (first episode, remitted–recurrent and treatment- resistant/chronic) did not show significant differences between groups (F=15.12, d.f.=2,62, P>0.05, PFWE). The ANCOVA including the medication load as the covariate also failed to detect significant group effects (F=15.04; d.f.=2,62, P>0.05, PFWE).

Correlations between segmented brain volumes and clinical characteristics

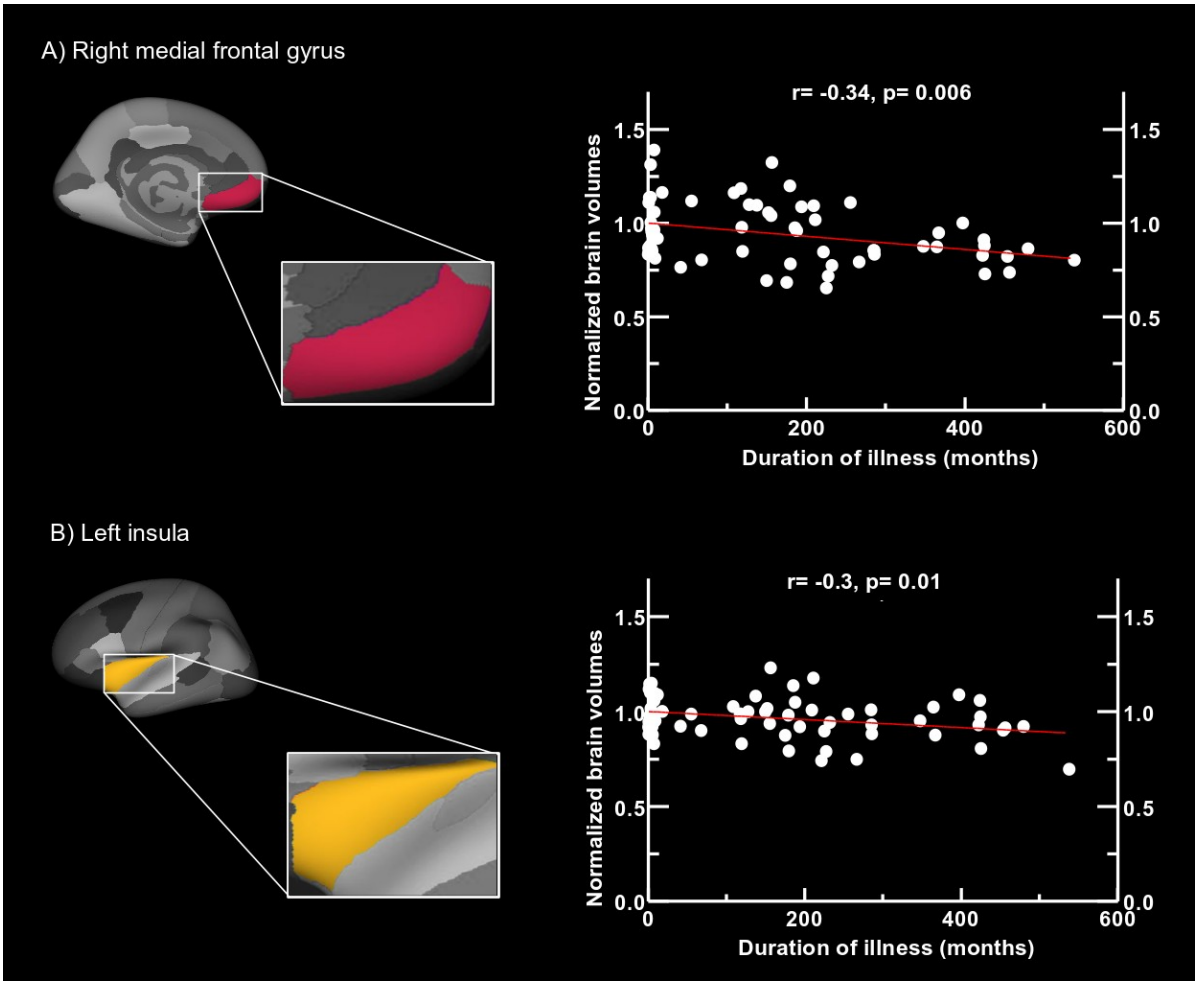
Table 5 displays absolute volumes of the segmented regions in ml (left anterior cingulate, right superior frontal gyrus, bilateral medial frontal gyrus and left insula). Group effects were only observed in right and left medial frontal gyri (F=4.2, d.f.=3,94, P=0.008 and F=3.52, d.f.=3,94, P=0.018 respectively) and left insula (F=3.19, d.f.=3,94, P=0.027). In post hoc analyses, individuals in the treatment-resistant/chronic group had less grey matter volume than those in the first-episode group in right medial frontal gyrus (P=0.011) and left insula (P=0.03). In addition, the chronic group also showed less volume than the remitted–recurrent group in both sides of medial frontal gyrus (right: P=0.02, left: P=0.01). Correlation analyses showed that duration of illness was significantly correlated with right medial frontal cortex (r=70.34, P=0.006) and with left insula (r=70.3, P=0.01; **Figure 2**). Linear regression analysis predicted 19% of grey matter volume reductions in right medial frontal gyrus and 11.4% in left insula. The rest of the clinical variables did not correlate with those areas showing significant volume reductions.

Table 5. Mean and standard deviation of FreeSurfer segmented volumes in ml of those areas which showed significant (p<0.01) less volume in treatment-resistant/chronic patients than in healthy controls (See **Table 3**).

Brain region	HC (32)	FE (22)	RR (22)	TR/C (22)
Left anterior cingulate	1588 (380)	1638 (350)	1733 (452)	1536 (403)
Right superior frontal gyrus	18097 (2381)	17988 (1868)	17927 (2756)	16689 (2347)
Right medial frontal gyrus	3969 (395)	4198 (679)	4160 (677)	3646 (585)
Left medial frontal gyrus	3628 (384)	3761 (687)	3891 (581)	3408 (41)
Left insula	5462 (526)	5857 (563)	5676 (685)	5365 (615)

HC, Healthy controls; FE, First Episode; RR, Remitted Recurrent patients; TR/C, Treatment-Resistant/Chronic patients. Values represented mean volumes (ml) of the brain area of the row with their correspondent standard deviation in parenthesis.

Figure 2. Left side displays medial (**A**) and lateral (**B**) sagittal view of FreeSurfer inflated cortical surfaces. In right side, significant correlations between normalized volumes of brain areas obtained by means of FreeSurfer segmentation (y axis) and duration of illness (x axis) are shown with regression line in red (n=64).



6.1.4. Discussion

The findings of the present study suggest that highly deleterious structural brain changes occur in patients exhibiting a more severe and chronic depressive disorder. Grey matter volume reductions in frontolimbic areas were observed in patients with long-lasting illness and with no response to treatment strategies, providing evidence of the implication of this neural circuitry in the changing pathophysiology of major depressive disorder. The observed differences were clearer when considering clinical variables related to the severity of the disorder. These findings suggest that grey matter abnormalities are directly correlated with past illness burden. The secondary analyses (using FreeSurfer) showed that individuals in the treatment-resistant/chronic group had smaller volumes in the segmented right medial frontal gyrus and left insula in comparison with those in the first-episode group, a result that was supported by the negative correlation between these two areas and duration of illness. This finding supports the potential risk of a history of severe illness on brain structures and the apparent brain preservation in the first stages of the illness. Moreover, the remitted–recurrent group showed bigger bilateral medial frontal gyrus volumes than the treatment–resistant/chronic group. This observation suggests a specific involvement of this area in maintaining depressive symptoms and refractoriness, and it is one of the targets for DBS in patients with depression that is treatment resistant (Mayberg et al., 2005). Previous studies reported that clinical outcome (response to antidepressant treatments) had a direct effect on grey matter volume in the prefrontal cortex of patients (Salvadore et al., 2011). Duration of illness has also been related to greater grey matter reductions (Bora et al., 2012). However, little attention has been paid to factors related to treatment non- response, whether this was as a result of a lack of response to the treatment strategy or whether patients experienced a more severe form of treatment resistance. Our findings revealed that only those patients with treatment-resistant/chronic major depressive disorder showed differences related to other clinical characteristics such as duration of illness, age at onset or number of previous episodes rather than to current symptomatology or medication load. The brain areas that seem to bear the deleterious effects of depression mainly coincide with those previously reported in patients whose condition was non-remitting: dorsolateral-prefrontal cortex, cingulate cortex, hippocampus, and medial prefrontal cortex (Salvadore et al., 2012).

In addition, a 7-year follow-up study (Soriano-Mas et al., 2011) reported that patients with slower recovery exhibited decreased volumes of left insula, hippocampus and lateral parietal cortex. Therefore, less grey matter volume in superior and medial prefrontal cortex, cingulate gyrus, insula and parahippocampal gyrus seem to be responsible for the persistence of depressive symptoms, hampering illness recovery.

In spite of the previous findings, the aetiology of brain volume decrease remains unclear. A review by Drevets (2004) identified elevations of glutamate transmission and cortisol hypersecretion in major depressive disorder and suggested that grey matter volume reductions in participants with current depression could be partly explained by interactions between elevated gluco- corticoid secretion and N-methyl-D-aspartate (NMDA)-glutamate receptor stimulation. Gold et al. (2002) also reported that the protective/ neurotrophic effects exerted by some antidepressant drugs may prevent and restore the volumetric alterations. However, an inadequate response to antidepressant strategies would most likely preclude these improvements and may even lead to a worsening as a consequence of sustained stress. These findings support the neurotoxic hypothesis, whereby a brain volume loss exists during the course of depressive illness, caused by glucocorticoid and glutamatergic toxicity, and a decrease in neurotrophic factors and neurogenesis (Soriano-Mas et al., 2011).

These possible neurotoxic effects cannot be investigated in our sample for two reasons: first, although the analyses took into account the effects of medication load it is not possible to know whether patients became resistant because of previous small grey matter volume or because of the toxicity associated with long- term medication. Second, the treatment-resistant/chronic group had not been followed up from the beginning of the illness. The participants with treatment-resistant/chronic disorder had been on long-term pharmacotherapy and had received more treatment combinations (as determined by medication load index) than the other groups of patients included. In any case, the impact of being exposed to antidepressant drugs would have not been beneficial and may have entailed greater impairment on the brain areas investigated. Unfortunately, there are few studies with drug-naive major depressive disorder samples. A recent study reported thinner cortical thickness in patients with depression with a late onset who were drug-naive compared with healthy controls (Lim

et al., 2012). The affected areas were located in frontotemporal and posterior cingulate cortex. Previous studies on patients who were drug-naive showed inconsistent results about which areas show decreases in grey matter volume, and many of these studies have been reported with uncorrected significance values. It is possible that in the case of treatment resistance, both factors, being depressed for a long period of time and not responding to antidepressant combinations, contribute to the apparent brain damage. Further studies are needed to clarify the effects of medication on grey matter volumes.

Limitations of the study

This is a cross-sectional study and therefore the harmful effects of depression on grey matter volume could not be evaluated. Nevertheless, two different types of post-processing software were used to test our hypothesis about the impact of illness burden on brain structures. Both found similar differences within the medial frontal gyrus confirming our hypothesis. In addition, all main results were strictly corrected for multiple comparisons. The present study may also be limited by the older age at onset of patients with a first episode, which might cast doubt about the representativeness of this sample. Although there was no significant relationship between age at onset and brain volumes, a later onset has been associated with a better prognosis in major depressive disorder (Souery et al., 2007). Nonetheless, this sample of individuals in the first-episode group is similar in age to the other investigated patients, providing a good comparison group to control for illness burden, and minimising the confounding effects of age-related changes in brain structures. Additionally, the grouping of patients performed in this study offers the possibility to compare patients with depression at different and well-defined stages of illness. Finally, our findings may be limited by the lack of a treatment washout period, although withdrawing antidepressant treatment to severely ill patients would constitute an ethical issue. Moreover, treatment regimens differed among groups: the treatment- resistant/chronic group, in particular, received combined treatments more frequently. Treatment effects on grey matter have not been well established yet but some evidence have suggested that antidepressant drugs may even attenuate volume decreases after successful treatment and remission (Salvadore et al., 2011; Arnone et al., 2012). Nevertheless, we included an index of medication load in VBM

ANCOVA with no changes in the results. In conclusion, frontolimbic areas were reduced in the individuals who were the most severely depressed, namely those in the treatment-resistant/chronic group. The insula and the medial frontal gyrus are the most affected brain regions, which may underlie the varying pathophysiology of major depressive disorder. Further research is needed to investigate the preservation of these brain structures, known to play key roles in regulating endocrine, autonomic, behavioural and emotional responses.

6.1.5. References

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6.2. Immediate cerebral metabolic changes induced by discontinuation of deep brain stimulation of subcallosal cingulate gyrus in treatment-resistant depression. (Published by Martín-Blanco, Serra-Blasco et al., 2015. *J Affect Disord*, 1;173:159-62.)

6.2.1. Introduction

Up to 33% of patients with major depressive disorder do not reach full remission after four sequenced pharmacological treatments (Rush et al., 2006), and some of these patients might experience disabling adverse effects or might not even improve with electroconvulsive therapy (ECT; Kellner et al., 2006; Dierckx et al., 2012). For this reason, alternative therapies for patients with treatment-resistant depression (TRD) are currently being tested, such as deep brain stimulation (DBS). This technique consists in high-frequency electrical stimulation of stereotaxically implanted electrodes in certain brain regions, such as the subcallosal cingulate gyrus (SCG) (Mayberg et al., 2005; Lozano et al., 2008; Holtzheimer et al., 2012; Lozano et al., 2012; Puigdemont et al., 2012; Merkl et al., 2013), the ventral capsule/ventral striatum (VC/VS) (Malone et al., 2009) or the nucleus accumbens (NAc) (Schlaepfer et al., 2008; Bewernick et al., 2010). Findings of DBS for TRD have shown promising outcomes, as most of the studies describe good remission rates (from 42% to 58% in SCG DBS) and tolerability appears to be high (Riva-Posse et al., 2013). Although the exact mechanism underlying DBS efficacy in TRD
is unknown, it is thought to modulate nerve transmission in cortico-striatal-thalamo-cortical loops (Mayberg, 2009). To shed light on this mechanism, studies on SCG DBS have used positron emission tomography (PET) to compare brain activity after chronic stimulation with pre-treatment baseline (Mayberg et al., 2005; Lozano et al., 2008). Mayberg et al. (2005) reported a reduction of blood flow in Cg25, adjacent frontal cortex (BA11), anterior insula, hypothalamus and medial frontal cortex (BA10), plus an increase in prefrontal dorsolateral (BA9/46), premotor region (BA6), par- ietal region (BA40), and dorsal anterior (BA24) and posterior (BA31) cingulate, after 6 months of stimulation (see a schematic representation in Fig. 1A). Lozano et al. (2008) obtained similar results with the same stimulation length:

decreases of glucose metabolism in orbital (BA11), medial frontal cortex (BA 10/9/8) and insula, and increases in lateral prefrontal cortex (BA 11/47, BA 46/10/9), parietal (BA 40), anterior midcingulate (BA 24), and posterior cingulate areas (BA 23). In both studies changes at 3 months were restricted to medial and orbital frontal decreases. Overall, these findings show that chronic DBS modifies brain activity at key structures, and these changes may occur gradually. Whether these metabolic changes persist when turning stimula- tion off is unknown but clinical worsening has been reported within initial weeks of stopping stimulation in patients with chronic SCG DBS (Mayberg et al., 2005; Holtzheimer et al., 2012). Functional brain modifications caused by DBS withdrawal may occur even before clinical changes when stimulation is stopped, however there are no studies exploring this hypothesis. The aim of this study was to determine immediate cerebral metabolism changes during a short switch-off of electrical stimulation in implanted patients with TRD who had achieved clinical improvement after a period of chronic DBS.

6.2.2. Methods

Participants

Seven patients with TRD who had been previously implanted for DBS in SCG in Hospital de la Santa Creu i Sant Pau and had achieved clinical remission were included. Remission was defined as a fall of the HAMD-17 mean score below a cut-off of 8. Inclusion criteria for DBS can be found elsewhere (Puigdemont et al., 2012). All patients gave informed consent to participate in the study and did not receive any economic retribution. The study was approved by the hospital ethical committee and the Agencia Española de Medicamentos y Productos Sanitarios (Spanish regulatory drug agency).

Procedure

After a period of clinical stabilization with chronic stimulation (9 months on average), two FDG-PET scans were acquired from each patient, in a 48 hours period. The first scan was done with the implants set to active stimulation (‘on’), then the stimulator was turned off and a second scan was carried out after 48 hours of non-stimulation (‘off’). Afterwards, the stimulator was turned on again. Stimulation para- meters were specific for each patient and were kept the same before and after the brief

discontinuation (in particular, each patient achieved clinical stabilization with these parameters): subject 1: 0–1+,4–5+,5 V, 180 ms, 135 Hz; subject 2: 0–2+,4–6+,3.5 V, 180 ms, 135 Hz; subject 4: 0–1+,5–6+,4 V, 150 ms, 135 Hz; subject 5: 1–2+,6–7+,3.5 V, 180 ms, 135 Hz; subject 6: 0–1+,4–5+,5 V, 210 ms, 135 Hz; subject 7: 0–2+,4–6+,3.5 V, 120 ms, 135 Hz; subject 8: 1–2+,5–6+,5 V, 210 ms, 135 Hz. Details of such values can be referred elsewhere (Puigdemont et al., 2012). Pharmacological treatment was not modified during this 48 h period. Clinical ratings were performed before the first and the second scan by means of the Hamilton Depression Rating Scale_17 items (HAMD-17). Patients were fully advised of the whole procedure during the trial.

PET imaging

FDG-PET scans were performed on a Siemens ECAT EXAT HR+ PET/CT scanner at Hospital del Mar in Barcelona, in 3-D mode (Biograph; Siemens Medical Solutions Inc., software version 6.5.9.1) with a 15.8-cm axial field of view. Blood glucose measured before tracer injection was 96.93 mg/dl (mean). Scans were performed 30 min after intravenous injection of 7.78 mCi (on) and 7.99 mCi (off) of fluorine-18-fluorodeoxyglucose PET (18FDG-PET). The acqui- sition time was 20min per position. Sixty-three slices 2.4mm thick were acquired (matrix dimensions=128×128×63, voxel size=2.57×2.57×2.43mm^3).

Co-registration of PET imageswas performed with a previous MRI T13D-MPRAGE acquired before DBS surgery. MRI T13D-MPRAGE scans were obtained using a 3T Philips Achieva facility (software version 2.1.3.2) at Hospital de la Santa Creu i Sant Pau in Barcelona, with three-dimensional shortest echo scans (TR=6.7ms, TE=3.2ms, 170 slices, voxel size (REC)=0.89×0.89×1.2mm^3, matrix dimensions=170x288x288; field of view=204x××56×256 mm^3, slice thickness¼1.2 mm). Data was acquired in a sagittal slice orientation, T1 contrast enhancement, flip angle 8º and grey matter as a reference tissue, ACQ matrix M×P=256x240 and TFE shots=218. Image post-processing comprised the following steps. PET images were manually reoriented at the same anatomical space that their corresponding T1-weighted MRI scan, using ITK-SNAP (Yushkevich et al., 2006) and SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL) implemented in Matlab 7.6.0 (The Mathworks Inc, Natick, Mass). The rest of the process was carried out with SPM8. Firstly, PET

images were co- registered to the T1s. T1 images were spatially normalized to the standard space and the normalization parameters were then applied to the PET images. Finally, PET images were smoothed using an 8mm FMHW Gaussian kernel. The storage, management and post-processing of the images were carried out in the cluster of the Port d'Informació Científica (PIC).

Statistical analyses

Statistical differences between the two PET conditions (‘on’ vs. ‘off’) were assessed using a statistical non-parametric tool for SPM8 (SnPM8; Nichols and Holmes, 2002). An absolute threshold masking of 0.2 and an implicit mask were used (exclusion of voxels with zero values in any of the subjects). Global nuisance effects were accounted by including the global covariate as a nuisance effect in the general lineal model (ANCOVA), obtaining a pseudo-t. Pseudo-t: variance smoothed with FWHM [10×10×10] mm3. Two different contrasts were evaluated [Off<On and Off>On] to identify clusters meeting $p<0.01$ for brain regions that reached expected cluster size in SnPM8, resulting in a pseudo t-map. HAMD-17 scores in ‘on’ and ‘off’ conditions were compared using paired t-test so as to determine changes in clinical symptoms.

6.2.3. Results

Demographics and clinical characteristics

The sample was composed of 5 females and 2 males, with a mean age at onset of illness of 24.9 year (SD=5.3). Five of these patients had melancholic characteristics. Four patients were married and three single, and the average schooling was of 12.5 year (SD=3.9). At the time of surgery for DBS the mean age was 47.4 year (SD=11.3) and the HAMD-17 mean score was 21.3 (SD=2.4). After surgery and onset of stimulation, it took an average of 5.2 months (SD=4.8) to get clinical remission and stabilization, defined as a maintenance of the remission for at least three months. HAMD-17 mean score before the first PET was 6.0 (SD=2) and before the second one was 6.42 (SD=2.9); therefore, no significant changes on depressive scores between the ‘on’ and ‘off’ conditions were observed.

PET findings

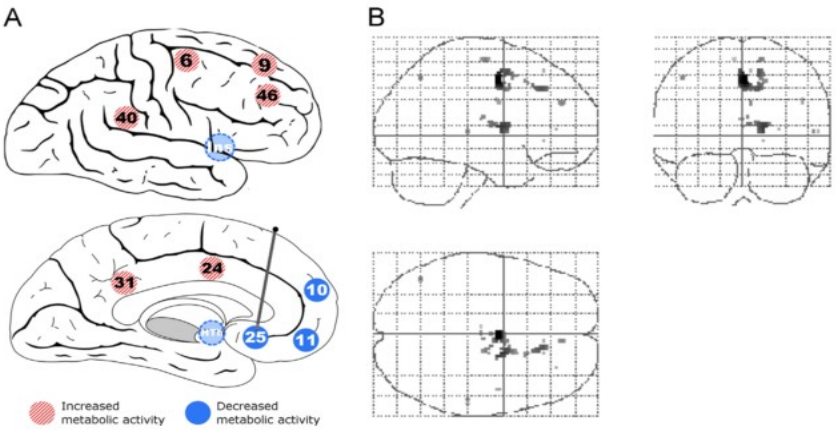
The resulting pseudo-t values are displayed in **Table 1**, with brain locations reported as x, y, and z coordinates in Montreal Neurological Institute space with approximate Brodmann's areas identified by anatomical regions into Talairach space. Group analyses showed local cerebral metabolism decreases (pseudo-t=−3.09, p=0.007) in dorsal anterior cingulate (Broadmann Area, BA24), premotor region (BA6) and in the putamen, but no increases, when stimulation was stopped (**Fig. 1B**).

Table 1. Location and peak significance of PET activity in On condition relative to Off condition using SnPM8. Anatomical region based on Talairach Atlas (Talairach, 1988), Pseudo-t, spatial extent in number of voxels (cluster size), voxel-level significance (po0.01) of the highest significant peak and MNI coordinates of each peak are displayed.

Anatomical region	Cluster size	Pseudo-t	Whole- brain	MNIcoordinates[a]		
				x	y	z
Right cingulate gyrus (BA* 24)	44	3.09	0.0078	2	−4	46
Right putamen	20	2.15	0.0078	16	0	8
Right cingulate gyrus (BA 24)	16	1.82	0.0078	12	2	52
Right medial frontal gyrus (BA 6)	16	1.80	0.0078	12	28	40

*BA: Broadmann area
[a] The coordinates localizing the peak voxel t-value within each cluster were converted from Montreal Neurological Institute (MNI) spatial array to the stereotaxic array of Talairach and Tournoux (Talairach and Tournoux, 1988).

Figure 1. A: schematic representation of brain metabolic changes after 6 months of deep brain stimulation of the subcallosal cingulate gyrus in treatment resistant depression (as measured by Mayberg et al., 2005). Numbers indicate Brodmann's areas; Ins=Insula; Hth=Hypothalamus. B: SnPM8 results of Off<On contrast showing decreased metabolic activity in BA24, BA6 and putamen when stimulator was turned off.



6.2.4. Discussion

This is the first study to investigate immediate cerebral metabolism differences between ‘on’ and ‘off’ stimulation in patients with TRD implanted for SCG DBS. Our results confirmed the existence of early regional glucose metabolism changes, which were strikingly independent of clinical variations due to the 48-hour switch-off. Specific differences consisted of a decreased brain metabolism in dorsal anterior cingulate (BA24), premotor region (BA6), and putamen when the stimulation was stopped. These are only some of the areas showing decreased activity during depressive states and increased activity after chronic SCG stimulation (Mayberg et al., 2005; Lozano et al., 2008; see a schema in Fig. 1A). Therefore, our results suggest that SGC DBS discontinuation could lead to immediate changes in medial components of depression network which might progressively spread to nearby and remote areas – such as SCG or dorsolateral prefrontal cortex – when electrical discontinuation is longer. The SCG represents a key point in the network of brain regions involved in mood regulation. Animal studies have shown that this area shares reciprocal connections with other regions of the orbitomedial prefrontal cortex, as well as the amygdala, subiculum, accumbens, ventral tegmental area, substantia nigra, raphe, locus ceruleus, brainstem nuclei, and hypothalamus (Drevets et al., 2008). As DBS affects several neural structures, such as myelinated axons and to a lesser extend cell bodies (Vedam-Mai et al., 2012), it is possible that the orthodromic and antidromic axonal stimulation of SCG may affect neuronal transmission in a large number of the structures connected to this area. A recent case report of patient-specific tractography activation (Lujan et al. 2013) suggests that a critical mass of cortical, sub-cortical and cingulate pathways, mentioned above, needs to be activated to obtain therapeutic benefits. In consequence, focal and distal effects may be inferred after DBS of the SCG, which may take place following a temporal sequence, as suggested by our findings. Despite the described metabolic changes, no apparent clinical shifts were observed in our sample neither during the 48 h of inactive stimulation nor after switching on the stimulator. Consistently, data from previous studies have shown that clinical benefits of chronic SGC DBS persist temporally after stimulation discontinuation.

Mayberg et al. (2005) described a two-week delay in the emergence of a subtle worsening after cessation of the stimulation in a patient who had achieved robust and sustained remission with chronic DBS. Holtzheimer et

al. (2012), by contrast, reported progressive but steeper exacerbations during the first 2 weeks in 3 patients after single-blind DBS switch-off. Moreover, data from a double-blind crossover study with the same sample described here (Puigdemont et al., 2012), showed that depressive relapses did not appear upon stopping the neurostimulator but mostly within the first month. All these observations suggest that metabolic and clinical consequences of DBS discontinuation may not occur simultaneously; the former may appear earlier in specific regions of the depressive brain network (i.e. in hours or days), while clinical benefits may persist for a few weeks. Several limitations to this study should be addressed. The small sample size may cast doubt on the findings. Nevertheless, previous PET studies of DBS, based in limited numbers of patients, have reported fairly consistent results with those presented herein (Mayberg et al., 2005; Lozano et al., 2008). In addition, data were analyzed with the non-parametric tool of the SPM, which is stricter than other parametric tools. DBS for TRD is still an experimental treatment and has a very specific target population, which limits access to large samples. The design did not include a single-blinded condition or a control group, but PET imaging analyses were performed by blinding the conditions of stimulation. Lastly, while it could be argued that HAM-D may not be sensitive enough to capture subtle clinical variations in such a brief switch-off period and possibly, self-reported scales would have been more adequate to objectify them, although no major issues were reported by any patient during clinical interviews. In conclusion, our results show that DBS discontinuation produces immediate effects on metabolism of specific brain regions involved in depression, which precede clinical manifestations. These results provide more data on the complex dynamics and still unclear mechanisms underlying DBS efficacy in TRD.

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6.3. Cognitive functioning after deep brain stimulation in subcallosal cingulate gyrus for treatment-resistant depression: An exploratory study (Published by Serra-Blasco et al., 2015. *Psychiatry Res* 225:341-346.)

6.3.1. Introduction

There are a wide variety of pharmacological and psychotherapeutic interventions that have proven efficacious in the treatment of MDD. However, as many as 30% of patients treated with antidepressants fail to respond, and around 50% do not achieve a complete and sustained recovery, suffering further relapses (Fava, 2003; Holtzheimer & Mayberg, 2011). Individuals who fail to respond to more than two psychopharmacological treatments are suffering from TRD. ECT has demonstrated efficacy for TRD patients (Sienaert, 2011), but it is often accompanied by memory disturbances and relatively high relapse rates (Rasmussen, 2002). Other treatments such as transcranial magnetic stimulation (rTMS) or vagal nerve stimulation (VNS) appear to have limited efficacy (Kennedy and Giacobbe, 2007). Ablative techniques like anterior cingulotomy, anterior capsulotomy, subcaudate tractotomy and limbic leucotomy are more invasive and their efficacy is far from being established (Nuttin et al., 2014). As reviewed by Dougherty and Rauch (2007) only few studies have explored their cognitive effects, raising the conclusion that cognitive deficits are among the more serious adverse events although relatively infrequent and usually transient. In any case, impairment of cognitive functions has to be taken into consideration when facing these latter treatment strategies, as it may contribute to worse long-term functional outcomes and may be cumulative over the course of the illness (Beblo et al., 2011). DBS has become a potential therapeutic alternative to treat TRD patients. Different promising brain targets are being investigated. One of them is the SCG, which has yielded an average of 68% of response and 44% of remission rates in five different studies (Anderson et al., 2012). To date, some of these previous studies have also investigated the cognitive effects of DBS for TRD, reporting cognitive safety of the nucleus accumbens (Grubert et al., 2011) and SCG stimulation (McNeely et al., 2008; Holtzheimer et al., 2012; Bogod et al., 2014; Moreines et al., 2014).

However, these previously cited studies did not have a control group, and, if they did (Moreines et al., 2014), it was not followed over time. As concluded in a recent review (Bergfeld et al., 2013), DBS seems to be cognitively safe in most of the psychiatric diseases and, particularly, in TRD. However, the authors also point out the necessity of more studies adding further evidence to give support to these findings that in turn overcome some of the limitations of previous works. In the present study, a comprehensive battery of neuropsychological tests was used to evaluate the main cognitive domains affected in depression, and a control group of patients with a first episode (FE) of depression, instead of a group of healthy controls, was included. The rationale for using such a group of patients was the intention to control for practice effects and at the same time, the effects of acute symptomatology during evaluations.

We previously reported (Puigdemont et al., 2012) clinical outcomes during the first year of DBS in eight TRD patients, in which half of the sample showed full remission and most of them had responded after 1 year of chronic stimulation, supporting its validity as a new therapeutic strategy for TRD. Electrodes were implanted bilaterally in the SCG (Broadman areas 24–25). The objective of the present exploratory study is to investigate cognitive effects of chronic stimulation of SCG in this sample. We hypothesized that cognitive performance would improve after DBS of Cg25 in TRD patients.

6.3.2. Method

Participants

Eight individuals diagnosed of MDD according to DSM-IV-TR criteria were selected to be intervened for DBS in SCG. Participants had to be resistant to pharmacological treatment, at least in stage IV of the Thase–Rush scale (Thase and Rush, 1997), (i.e. on average, 9.8 different drug tryouts), and with lack of efficacy of ECT or partial response to its maintenance. Admission score on the 17-item HDRS (Hamilton, 1967) had to be >18. Before being implanted, they were assessed with a comprehensive neuropsychological battery to determine their cognitive status. Four out of eight received maintenance ECT, (but it was stopped 2 weeks before study entry). A group of eight patients with a FE of MDD matched on age, gender and level of education, was also assessed in order to control for possible

practice effects and interference of acute symptoms on cognitive performance. To be eligible as a FE, patients had to be newly diagnosed from an episode of MDD, following DSM-IV-TR criteria, with a HDRS score above 14. Exclusion criteria for both groups included: Axis I comorbidity according to DSM-IV-TR criteria; acute, serious or unstable comorbid neurological or medical illness; current or past non-affective psychotic disorder; severe personality disorder and current or unstable remitted substance abuse or dependence (except nicotine). Pharmacological treatment could not have been changed during the previous month of study commencement. Deficits on neuropsychological performance of all patients were characterized using normative databases for Spanish samples (CIBERSAM, Banco de Instrumentos y Metodologías en Salud Mental). Current depressive symptoms were measured with HDRS. All participating individuals were of a similar age [mean: 46.4 years (S.D. 9.1)] to avoid age-related variations in cognitive functioning. All patients were recruited from the Psychiatry Department of Hospital de la Santa Creu i Sant Pau from Barcelona.

Informed consent

The study was approved by the Research Ethics Committee of Hospital Sant Pau in Barcelona and the Agencia Española de Medicamentos y Productos Sanitarios (Spanish regulatory drug agency) and was carried out in accordance with the latest version of the Declaration of Helsinki. All subjects gave informed and written consent after a full explanation of the study protocol.

Neuropsychological assessment

Neuropsychological tests covered four cognitive domains: Memory which was assessed by means of the RAVLT, using the number of words recalled in the first trial, total number of words after all trials and delayed recall; Executive Functioning, through the TMT-B, Verbal fluency (FAS), the Digit Span backwards subtest of WAIS-III and the TOL; Language was assessed by means of the Vocabulary subtest of WAIS-III and the Category test (total number of animals named); and finally, Processing Speed and attention were evaluated via the Digit Span forward subtest (WAIS-III), Digit Symbol Coding subtest (WAIS-III) and TMT-A. Standardized neuropsychological tests are described in detail by Strauss (2006) neuropsychological manual and

validated Spanish versions of test involving verbal material have been used (RAVLT, FAS, Vocabulary and Digit Span). The Tower of London is a non-standard test widely used to evaluate planning functions (Van den Heuvel et al., 2003; Unterrainer et al., 2004; Wagner et al., 2006), and the version used in this study overcomes the ceiling effects of other versions (Portella et al., 2003). In order to control for practice effects, parallel forms were used when available (i.e., RAVLT).

Pharmacotherapy

TRD patients had been on medication for more than 2 years previous to the study inclusion, while FE patients were treated for the very first time with an SSRI (and benzodiazepines when required). Table 1 displays detailed information of medications for each group of patients at time 1 and time 2. A composite measure of medication load was estimated for each patient in the two assessments (Hassel et al., 2008; de Diego-Adeliño et al., 2013), which is based on Antidepressant Treatment History Form (Sackeim, 2001). This index was then used to examine associations of medication load and cognition.

Statistical analyses

Demographics and clinical variables were analyzed with the statistical package SPSS v.18 using t-test and analyses of variance (ANOVAs) for quantitative variables and χ^2 for categorical variables. Level of statistical significance was set at $p<0.05$. Neuropsychological scores were transformed to T scores (mean=50, S.D.=10) based on normative data for Spanish samples with the exception of the Tower of London test. The longitudinal analysis was performed with repeated measures multivariate ANOVAs which were carried out for each cognitive domain to analyze group and time effects. Before carrying out the MANOVAs, normal distribution and homocedasticity were checked by means of Shapiro–Wilk's test (for small samples) and Levene's test. Most of the requirements to apply MANOVA were assumed, as only few variables did not show normal distribution or equality of variances. Further post-hoc analyses were performed to determine univariate effects of time and group. Subsequently, paired t-test of those tests showing time effects was carried out for each group separately. Level of statistical significance was set at $p<0.05$. To explore whether neuropsychological domains (those showing time effects in the

MANOVAs) were associated with clinical and pharmacological variables, Spearman correlations analyses of the whole sample were carried out. In order to reduce the number of correlations, a single index of each cognitive domain was obtained by averaging standardized scores and criterion for significance was set at $p<0.05$.

6.3.3. Results

As can be observed in **Table 1**, groups were equally distributed regarding gender, age, marital status and years of schooling. HDRS score did not differ between groups at time 1 although TRD patients had a higher score. Medication load was statistically different, as TRD patients received further medication at time 1 and at time 2 than FE patients, but there were no significant changes in medication over time within groups nor in type of medication (McNemar's $p>0.8$) neither in medication load index (FE $t(7)=0.15$, $p=0.9$; TRD $t(7)=-0.31$, $p=0.8$). **Table 2** displays t-standardized scores of all neuropsychological tests at time 1 and time 2 for TRD and FE patients. Repeated measures MANOVAs showed different patterns for each cognitive domain. Memory did not show a significant interaction of time × group ($F(12,2)=0.60$, $p=0.626$) but showed significant main effects of group ($F(3,12)=4.47$, $p=0.025$) and time ($F(3,12)=9.95$, $p=0.001$), where FE patients rated higher than TRD patients and the two groups improved over time in all tests (see **Figure 1**): First trial ($F(1,14)=5.62$, $p=0.033$), Total Recall($F(1,14)=4.64$, $p=0.049$) and Delayed Recall ($F(1,14)=11$, $p=0.005$). Paired t-tests between first and second assessments, carried out separately for each group, showed that TRD patients significantly improved on delayed memory ($t(7)=-3.04$, $p=0.02$). In light of the findings, an additional analysis was performed to test possible cognitive improvements due to stopping maintenance ECT at study entry. A backward stepwise regression model was carried out. Medication load, age, performance on memory at time 1and maintenance ECT (dichotomized to have received it or not) were included as predictive variables. The model was significant ($F(4,6)=36.8$, $p=0.027$), and it explained 96% of variance in which all variables except ECT were significantly predictive. FE patients also displayed a better performance on Total Recall ($F(1,14)=10.53$, $p=0.006$) and Delayed Recall ($F(1,14)=5.17$, $p=0.039$) than TRD patients with no significant differences on first recall ($F(1,14)=3.41$, $p=0.086$). Language domain did not show significant

interactions nor main effect of time, but a main effect of group ($F(2,13)=5.83$, $p=0.016$), where FE patients had better performance than TRD in the Category test ($F(1,14)=11.51$, $p=0.004$; **Figure 1**) with no differences in vocabulary test ($F(1,14)=0.496$, $p=0.49$). The two other cognitive domains, executive function and processing speed, did not show any main effects nor significant interactions. Correlations between clinical variables (medication load, HDRS) and a composite score of memory measures were performed. Spearman correlation analyses showed a significant negative relation between medication load and memory composite score at time 1 ($\rho=-0.767$; $p=0.001$) but not at time 2($\rho=-0.481$, $p=0.059$). **Figure 2** displays scatter plots for significant correlation analyses for the whole sample (red best fit line), and values are differently coloured by group (black dots for TRD patients; white dots for FE patients) with their corresponding best fit lines (no stats were calculated per group due to small sample size). HDRS scores did not show any significant relation with memory performance neither in first nor second assessment, while the correlation between HDRS change scores and Memory change scores was significant ($\rho=-0.633$, $p=0.009$).

Table 1. Demographics and clinical characteristics of the sample.

	FE[a] (n=8)	TRD[b] (n=8)	t/χ^2	p
Gender (F/M)	6/2	6/2	0	1.00
Age	46 (7)[c]	47 (11)	0.001	0.98
Marital status			5.94	0.11
Single	3	4		
Married	1	4		
Divorced	3	0		
Widowed	1	0		
Years of education	13 (2.82)	13.1 (3.8)	0.083	0.94
Time between evaluations	14.70 (2.90)	16.64 (7.84)	0.650	0.527
Age at surgery	NA[d]	47.4		-
Age at MDD[e] onset	45 (7)	25 (5)	34.61	0.00007
Length of current episode (year)	0.011 (0.005)	6.3 (1.8)	9.89	<0.00001
Duration of illness (total months)	6(5)	231.4 (124)	4.46	0.007
Previous suicidal attempts (n)	NA	8		-
N. of previous episodes	NA	5.5 (3.7)		-
N. of previous hospitalizations	NA	7.5 (5.5)		-
HDRS[f]				
Time 1	16.8 (6)	22.3 (4.5)	4.52	0.052
Time 2	6.5 (9.2)	6.25 (5.3)	0.004	0.948
Medication				
Time 1				
Antidepressant	100[g]	100	-	-
Anxiolitic	25	87.5	6.9	0.020
Anticonvulsant	0	50	5.3	0.038
Lithium	25	12.5	0.41	0.5
Antipsychotic	0	62.5	7.23	0.013
Medication load	2.8 (1.8)	6.4 (1.3)	22.5	0.0003
Time 2				
Antidepressant	87.5	100	1.07	0.5
Anxiolitic	12.5	87.5	9	0.005
Anticonvulsant	12.5	62.5	4.27	0.059
Lithium	0	25	2.29	0.233
Antipsychotic	0	75	9.6	0.003
Medication load	2.6 (1.5)	6.6(2.4)	15.5	0.001
Remitted patients at *Time 2*	6/8	7/8	0.410	0.522

[a] First Episode
[b] Treatment-resistant depression
[c] Mean (standard deviation)
[d] No applies
[e] Major Depression Disorder
[f] HDRS= Hamilton Depression Rating Scale
[g] Percentage of patients taking the drug

Table 2. Neuropsychological scores tests at *time 1* and *time 2* assessments reported in T scores if no otherwise specified.

	Time 1		Time 2	
	FE[a] (8)	TRD[b] (8)	FE (8)	TRD (8)
Neuropsychological Tests				
Memory				
RAVLT[c] - First trial	43.1(13)	35.9(8)	50.6(11.2)	41.6(13.9)
RAVLT - Total	46.5 (12.1)	31.1 (6.4)	51.8 (9.3)	36.7 (13.2)
RAVLT - Delay	47.4 (12.4)	33.6 (9)	52.2 (5.3)	43.6 (13.5)
Executive functioning				
Tower of London (Raw scores)	29.6 (8.8)	31.4 (6.8)	33.2 (8.2)	32.3 (8.1)
Digit backward subtest (WAIS[d]-III)	56.3(4)	43.7(10.3)	47.7(19.1)	44.3(8.2)
Verbal Fluency	44.4 (9)	39.3 (9.1)	46.8 (6.9)	38.1 (11)
Trail Making Test B	52.7 (7.8)	29.5 (13.9)	52.2 (9.2)	37.1 (20.3)
Language				
Category Test	50.5 (9.4)	38.4 (4.5)	58 (8.5)	41.1 (10.6)
Vocabulary	52.5 (9)	51.6 (4.6)	56 (9.3)	50.9 (8.5)
Processing speed/ Attention				
Trail Making Test A	41.17 (9.9)	23.9 (10.7)	35.3 (17.1)	19.7 (15.4)
Digit forward Subtest (WAIS-III)	53.2(8)	46.8(11.5)	39.2(24.2)	48.9(10.5)
Symbol Digit	48.1 (4.2)	47 (6.9)	66 (10.7)	45.7 (7.8)

[a] First episode patients
[b] Treatment-resistant depressed patients
[c] Rey Auditory Verbal Learning Test
[d] Wechsler Adult Intelligence Scale

Figure 1. Mean T-scores for Rey Auditory Verbal Learning Test subtests and for category test performed by patients with treatment-resistant depression (triangles) and patients with first episode of MDD (circles) at *time 1* and *time 2* assessments. Gray background band indicates the range of T-Scores considered normal. Statistics correspond to MANOVA main effects of time and/or group (*) and to the paired t-test carried on in each group (†).

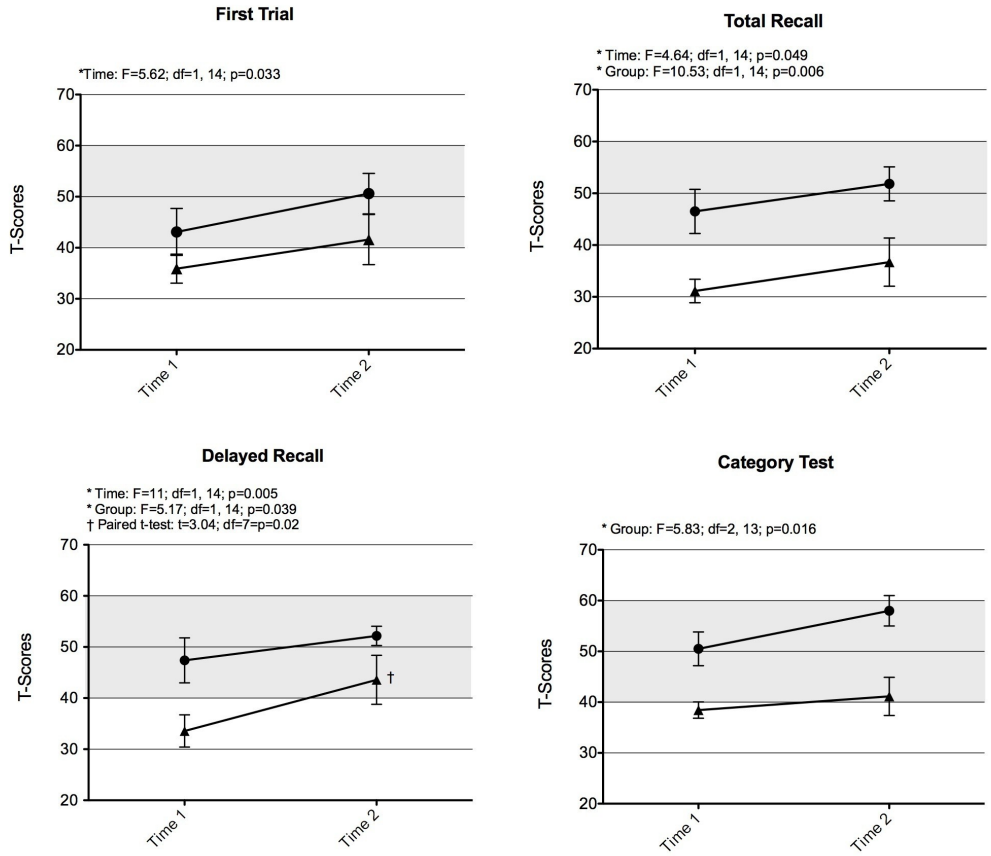
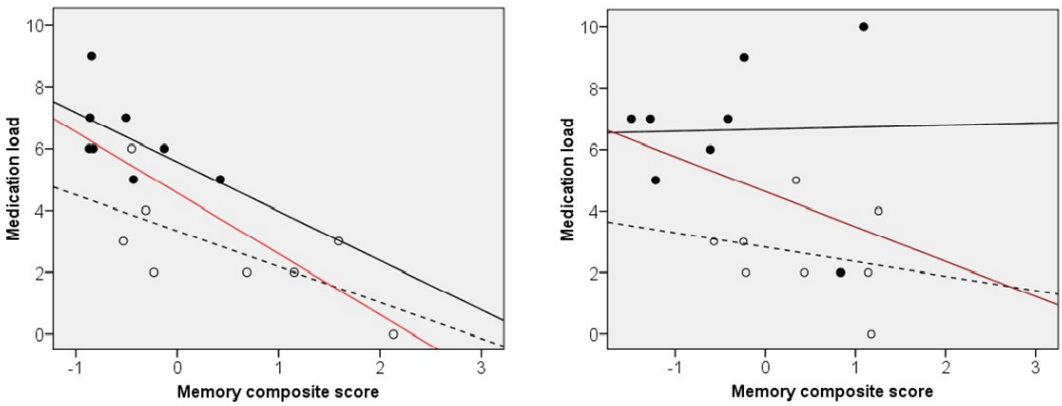


Figure 2. Correlations between cognitive performance and medication load at *time 1* and *time 2* for patients with treatment-resistant depression (black dots and solid line) and patients with first episode of MDD (white dots and dotted line). Red lines refer to correlations reported in the text (including all patients).



6.3.4. Discussion

The present data give support to previous findings, showing that DBS surgery and continuous stimulation of SCG appear to be well tolerated for TRD patients, with no negative effects on neuropsychological function. It is worth mentioning that patients severely impaired before DBS intervention showed memory improvement, although such improvement was observed in both groups. These findings provide further and promising evidence of the cognitive safety of DBS for TRD. Previous works have also reported no neuropsychological impairment after SCG chronic stimulation and others have even found improvements in memory (Moreines et al., 2014; Bogod et al., 2014) and in frontal skills (Holtzheimer & Mayberg, 2011). In particular, Moreines and colleagues reported long-term immediate recall improvements, though without controlling for practice effects. Taking into account that both groups (TRD and FE) were responders in the second assessment, one might think that memory functioning would have been boost by treatment response, regardless the particular therapy, as also proposed by Bogod and colleagues, although the correlation between the clinical and memory changes was negative, not supporting this idea. Alternatively, although the interaction of group by time was not statistically significant, it appeared that delayed evocation improvement seemed to be higher in TRD than in FE patients. One hypothesis is that DBS of the SCG could have bettered memory processes by means of the so-called neural jamming effect (Lozano et al., 2008), which states that DBS may regulate and correct pathological activity in the neural circuit being stimulated, by suppressing spontaneous neural signalling of the altered processes observed in depression. This effect might modify transmission via neuron flood out, which could in turn, “normalize” information flow within distal neural pathways such as the hippocampus and the temporal regions (Mori et al., 2005). Therefore, taking into account that DBS stimulation enables changes in areas distal to the place of stimulation via white matter tracts (Johansen-Berg et al., 2008), it is plausible that memory performance could have been enhanced by the indirect stimulation of the mentioned areas. The relation found between amount of medication and performance on memory tests might suggest harmful effects of medication when there is no clinical response, which might reverse when treatment is successful. In the same

line, previous studies (McNeely et al., 2008; Grubert et al., 2011; Holtzheimer et al., 2012) also concluded that the amount of medication was not a relevant factor for cognitive changes over time.

Our data also shows that there is no relation between HDRS and cognitive tests, which agrees with previously cited studies, pointing out that cognitive impairment might be more related to illness per se than to a given mood state. Differences in HDRS scores between the two groups at time 1 may account for this lack of relation. Interestingly, when looking at the relation between neuropsychological and clinical changes, a significant value came up, which might also give support to the idea that cognitive impairment is more related to the response to treatment (along the illness) than to static depressive symptoms. Regarding group effects, TRD showed lower performance on RAVLT and Category Tests than FE patients. Given the lack of correlation between the task scores and HDRS scores, it is possible that such a difference is mirroring the neuropsychological profile of TRD per se, providing an interesting finding that enriches the literature on this field.

There are many methodological issues that should be considered to better interpret the findings. The relative small sample size makes difficult to generalize the present results, but the similarity of samples among the few studies published to date adds valuable information of cognitive preservation after DBS of SCG. A non-parametric statistical approach would have been more adequate given the limited sample size, but no other real options exist to test the main hypothesis of the present study, and one has to be aware of the exploratory nature of the present findings. In the same line, although cognitive performance was tested by cognitive domains, four different MANOVAs were carry out. In any case, the inclusion of a comparison group, which was also followed-up, goes a step further in the investigation of cognitive effects of DBS. TRD patients received ECT before DBS implantation, which is a potential confounding factor given its known effects on cognition (Semkovska et al., 2011). However, previous maintenance ECT was not a significant predictor of delayed memory improvement, and the long-term benefits of stopping ECT are far from being established (Verwijk et al., 2012). The findings reported here are promising and provide more evidence about chronic stimulation of SCG, which does

not impair cognitive functioning and even more seem to ameliorate memory retrieval. Given the exploratory nature of this study, future research with larger samples and double-blind on–off stimulation will be necessary to confirm cognitive safety and find out the underlying mechanisms leading to cognitive improvement.

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6.4. Naturalistic course of major depressive disorder predicted by clinical and structural neuroimaging data: a 5-year follow-up. Serra-Blasco et al. (Under revision, *Br J Psychiatry*).

6.4.1. Introduction

The episodic nature of MDD is well established (DSM-IV-TR). After a first episode of depression, half of patients are likely to relapse. Of these, 35% will experience recurrent episodes and 15% will have an unremitting, chronic disease course (Richards, 2011). No reliable markers are available at present to help mental health professionals determine whether or not a given patient is likely to present another episode in the future or to develop resistance to treatment. Residual symptoms and chronic disease negatively impacts the psychosocial functioning of patients with MDD and, in turn, their quality of life (Papakostas et al., 2004; Rapaport et al., 2005). Residual symptomatology (Judd et al., 1998), number of lifetime MDD episodes (Solomon et al., 2000), and duration of illness and symptom severity (Keller et al, 1992) have all been associated with a high risk for earlier relapses as well as with chronicity. Although useful, clinical symptoms alone might not be sufficient to predict clinical course, (Eaton et al., 2008) and their combination with biological markers appear to offer a promising approach to this problem.

MDD is widely associated with impairment of the brain regions involved in emotional processing such as a reduction in the limbic and prefrontal regions and functional dysregulation of these same areas (Atkinson et al., 2014). Several of the clinical characteristics of MDD, including illness duration (Serra-Blasco et al., 2013), number of previous MDD-episodes (Yucel et al., 2008), and symptom severity (Diego-Adeliño, 2013) appear to be related to brain abnormalities. Researchers have started to investigate which of those brain alterations are caused by the disease burden and might therefore serve as neurobiological predictors of treatment-outcome. A recent meta-analysis (Fu et al., 2013) demonstrated that certain functional and structural brain correlates can predict the probability of response to treatment in depression. However, as that study points out, there is a lack of

studies combining clinical and neuroimaging data to predict the long-term outcome of depressed patients.

Given the context described above, we hypothesized that the addition of structural brain data to a predictive model of disease course in MDD would improve the model's capacity to predict long-term clinical outcomes. Therefore, the main aim of this study was to combine demographic, clinical, and sMRI variables to assess their combined ability to predict the naturalistic course of MDD and long-term depressive symptomatology at 5 years of follow up.

6.4.2. Method

Participants

Sixty-six right-handed individuals with MDD who underwent an (MRI) from September 2007 to July 2011 (Serra-Blasco et al., 2013) were followed up after an average period of five years and were fully interviewed again for the present study (in our outpatient service). The study was approved by the Research Ethics Committee of the Hospital Sant Pau in Barcelona and was carried out in accordance with the Declaration of Helsinki. All participants gave informed and written consent after a full explanation of the study protocol and agreed to be contacted the future. Nine patients did not fulfil the entire assessment or preferred not to participate, and eight were excluded due to participation in a separate study (deep brain stimulation) (Puigdemont et al., 2012). As a result, the final sample included 49 patients from the Psychiatric Department of the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain. Of these 49 participants, 19 had been diagnosed with a first-episode MDD at the time of the initial MRI, while 20 had previously experienced three or more MDD-episodes, and ten had TRD. These pre-existing illness categories were taken into account for the current analyses, with this variable denominated “clinical stage”.

Outcome measures

Information collected from participants at baseline interviews included the following: age, gender, education level, marital status, age at illness onset, duration of illness, and number of previous MDD episodes. Depressive

symptoms were assessed with the 17-item HDRS (17, 18). Each item on the questionnaire is scored on a 3 or 5 point scale, depending on the item. Finally, to collect the amount of medication, a composite measure of medication load —which reflects dose and variety of medications taken (de Diego-Adeliño, 2013; Hassel et al., 2008)—was estimated for each patient. This measure is based on Antidepressant Treatment History Form developed by Sackeim (2001). At the follow-up assessment, information collected at baseline were updated.

Clinical outcomes (CO)

An experienced psychologist from the Department of Psychiatry conducted follow-up interviews with all the participating patients. The clinical interview was based on the Life-Chart Manual for Recurrent Affective Illness (for Clinicians Retrospective; LCM-C/R, Roy-Byrne et al., 1985). In addition, the information collected was double-checked with the hospital database and the treating psychiatrist. Patients were asked about whether they had had more episodes of depression since the MRI scan, and if so, if they had achieved remission after that last episode. In addition, they were asked to report the presence or not of subclinical symptomatology during remission periods. After gathering all the compiled information, patients were then divided into 4 different CO categories:

- Recovery (Recov): patients without any other episode of major depression or residual symptomatology since the baseline assessment.
- Partial remission (PartRem): patients without any other episode of major depression but who did present some sub-clinical symptomatology such as anxiety, irritability, restricted affect or sleep disturbances.
- Remission-Recurrence (RemRec): patients who experienced addition episodes of depression but were always able to achieve inter-episodic remission.
- Chronic Depression (ChronDep): patients who had been depressed most of the time or experienced additional episodes of depression and had inter-episodic residual symptomatology.

MRI data acquisition

The MRIs were obtained using a 3T Philips Achieva facility (software version 2.1.3.2), three-dimensional (3D) shortest echo scans (repetition time (TR)=6.7ms, echo time (TE)=3.2ms, 170 slices, voxel size (REC): 0.896×0.896×1.2 mm, image dimensions: 288×288×170; field of view: 256×256×204 mm, slice thickness: 1.2 mm). For each participant, high-resolution 3D-MPRAGE images were acquired (whole brain coverage), with a sagittal slice orientation, T1 contrast enhancement, flip angle: 88, grey matter as a reference tissue, acquisition matrix M×P=256×240 and turbo-field echo shots (TFE)=218. All technical procedures were carried out in the cluster of the Port d'Informació Científica (PIC) on Scientific Linux 5 (www.scientificlinux.org/).

Voxel-based morphometry (VBM) DARTEL analysis

The VBM analyses were performed using SPM8 (www.fil.ion.ucl.ac.uk/spm) in a MATLAB 7.6.0 environment. First, MRIs were segmented into grey matter, white matter and cerebrospinal fluid using the "New Segment" option. Second, grey matter templates were generated from the entire image data set using DARTEL. DARTEL works by aligning grey matter among the images, while simultaneously aligning white matter. Afterwards, "Normalise to MNI" uses the resulting 'u_rc1" files (which encode the shapes), to generate smoothed, spatially normalised and Jacobian scaled grey matter images in MNI space. Spatial pre-processing, smoothed (8mmFWHM), modulated – preserving amount-, normalised grey matter data-sets were used to perform statistical analyses.

Brain volume (FreeSurfer)

To obtain the whole volume of every brain structure, volumes of brain areas showing differences in whole-brain VBM ANOVA were segmented using Freesurfer software (v.4.3.1, http://surfer.nmr.mgh.harvard.edu/), developed at the Martinos Center for Biomedical Imaging. Cortex volumes were obtained with the surface-based stream process, while subcortical volumes were obtained through automated labelling of the brain volume.

Statistical analyses

Demographics and clinical variables were analysed with the SPSS statistical package version 22. Parametric and non-parametric tests were performed as appropriate. Kendall's tau-c correlation was calculated to test clinical stability during the five-year period. In order to determine specific brain areas involved in the course of the disease, a whole-brain VBM ANOVA with the 4 COs as between-subject factor was carried out using SPM8 by applying the general linear model (GLM). Interactions with age and duration of illness at the time of MRI, as well as time-lag between MRI and follow-up assessment were entered as covariates into the analysis. Then post-hoc comparisons were carried out (i.e., Recov>PartRem, Recov>RemRec, Recov>ChronDep, PartRem>RemRec, PartRem>ChronDep, RemRec>ChronDep). Scans were absolute thresholded for masking at 0.2 to exclude the influence of any non-grey matter tissue. As no previous regions of interest were specified beforehand, significant effects were considered using an uncorrected $p<0.001$.

To test the incremental predictive capacity of sMRI, a hierarchical approach was used; SPSS ordinal regression procedure (Polytomous Universal Model –PLUM–, i.e., an extension of the general linear model to ordinal categorical data) was run to predict COs (ordered variable) in two different blocks: the first block included demographic and clinical data, and the second block included demographic, clinical and neuroimaging data. Additional hierarchical stepwise multiple linear models were applied for depressive severity (using follow-up HDRS scores). Colinearity diagnostics were taken into account for linear models [Tolerance<0.1 and VIF *(variance inflation factor)* >10 were indicative of high correlation between predictors that could lead to poor estimations of coefficients]. All the variables included as predictors in PLUM and the linear regression models came from baseline data (i.e., at the time of the MRI scan): gender, education, marital status, age at illness onset, duration of illness, number of previous MDD-episodes, HDRS, clinical stage and GMV.

6.4.3. Results

Demographic and clinical measures

Table 1 shows the demographic, clinical and treatment information of the sample at baseline and at the 5-year follow-up evaluation. Current sample characteristics are also displayed separately for the COs. One-way ANOVA or Kendall's tau-c analyses showed that age, gender, level of education and marital status were equally distributed among groups. Of the clinical variables, HDRS was significantly different ($F(3,40)=18.55$; $p<0.0001$), with the ChronDep group presenting higher scores than any of the other outcome groups. Duration of illness also differed among COs ($F(3,45)=3.157$, $p=0.034$), although none of the post-hoc comparisons reached significance. There were no significant differences in age at illness onset or in the number of previous MDD-episodes. In terms of clinical stability, Kendall's tau-c correlation showed a significant relation (Kendall's tau-c=0.356; $p=0.002$) between clinical stage at baseline and current CO. The majority of chronic patients at baseline were still categorized as chronic in the 5-year assessment, while the other baseline classifications (FE and remitted-recurrent patients) were equally distributed across COs.

With regard to treatment (**Table 1**), significant differences in medication load as a global measure ($F(3,41)=4.21$), $p=0.011$) were found. The post-hoc analyses showed that the ChronDep group had higher scores on that index versus patients in the Recov ($p=0.034$) and PartRem ($p=0.044$) groups. Prescriptions for anxiolytics and antidepressants –TCA/MAOIs– were significantly higher in ChronDep patients (up to 80%) versus the other groups (Kendall's tau-c=0.419, $p=0.001$ for anxiolytics and Kendall's tau-c=0.248, $p=0.035$, for TCA/MAOIs). No significant between-group differences were observed for SSRI or NRI, nor for other antidepressants or combinations thereof. Similarly, no significant differences in the use of stabilizers and antipsychotics were seen among the various COs.

Table 1 Demographics, clinical and treatment characteristics of the sample at baseline and at follow-up assessment. The four last columns provide information of all variables for each clinical outcome.

	Baseline (n=49)	Follow-up (n=49)	Clinical Outcome at Follow-up			
			Recov	PartRem	RemRec	ChronDep
Demographics						
Age, years: mean (s.d.)	47.78(7.7)	53.09(7.6)	51.47(6.8)	50.08(7)	53.27(8.1)	55.02(7.9)
Gender, *n*						
Male	11	–	3	2	3	3
Female	38	–	7	5	11	15
Education, *n*						
Primary school	9	–	0	2	2	5
High shcool	15	–	4	3	6	2
University	23	–	5	2	6	10
Marital Status, *n*						
Single	10	8	1	2	2	3
Married	27	26	6	4	7	9
Divorced	9	11	1	1	5	4
Widow	1	2	1	0	0	1
Time Baseline-follow-up	–	5.35(1.3)	5.47(1.3)	5.11(1.3)	5(1.2)	5.67(1.2)
Clinical stage (Baseline), *n*						
First MDD-Episode	19	–	5	2	9	3
Remission-Recurrence	20	–	5	5	4	6
Chronic Depression	10	–	0	0	1	9
Clinical characteristics						
HDRS,[a]	14.7(10.2)	5.7(5.4)	1.7(2.1)	3.43(1.9)	3.17(2.7)	11(5)
Age at onset, years	35 (11.7)		38.2(11.7)	34.8(8.6)	41(9.6)	31.6(11.8)
Duration of illness,[b]	139.2(151.2)	202.1(154.3)	135.2(88.7)	203.5(14)	147.6(148.2)	281.1(166.3)
Number of MDD-Episodes	3.6(4.9)	4.5(5.2)	2(1.2)	2.6(1.1)	6.1(7.6)	5.4(4.9)
Treatment						
Medication Load,[c]	3.25(1.8)	3.62(3)	1.89(2.3)	1.71(1.7)	3.92(3.4)	5.19(2.6)
Antidepressants (%)	98	61.7	13.8	10.3	31	44.8
SSRI or SNRI	81.6	54.5	12.5	8.3	37.5	41.7
Tricyclic antidepressants or monoamine oxidase inhibitors [d]	12.2	13.6	0	16.7	0	83.3
Others	14.3	6.8	33.3	0	0	66.7
Combination	24.5	18.2	12.5	0	50	37.5
No Antidepressant	2	34.1	33.3	20	26.7	20
Stabilisers (%)	77.6	18.2	12.5	12.5	12.5	62.5
Antipsychotics (%)	14.3	9.1	25	0	0	75
Anxiolytics,[e] (%)	61.2	22.7	0	10	10	80

Recov, Recovery; PartRem, partial remission; RemRec, remission recurrence; chrondep, chronic depression; s.d., standard deviation; MDD, major depressive disorder; Hamilton Depression Rating Scale; SSRI, selective serotonin reuptake inhibitors; SNRI, selective serotonin–noradrenaline reuptake inhibitors.

a ChronDep differed from Recov, PartRemand RemRec $p<0.0001$

b ChronDep differences in ANOVA $p=0.034$. Post-hoc analyis did not reach significance.

c ChronDep differed from Recov and PartRem $p=0.011$

d ChronDep differed from Recov, PartRem and and RemRec $p=0.035$

e ChronDep differed from Recov, PartRem and and RemRec $p=0.001$

Structural imaging analyses

Whole-brain VBM ANOVA showed a significant main effect of group –CO– ($F(3,33)=6.88$; $p_{unc}<0.001$, cluster extension≥50), as can be seen in **Table 2.** Post-hoc analyses showed greater GMV in Recov compared to RemRec patients ($t(1, 33)=3.36$; $p_{unc}<0.001$, cluster extension≥100) and to ChronDep ($t(1,33)=3.37$; $p_{unc} <0.001$, cluster extension≥100). PartRem patients showed larger brain volumes than RemRec ($t(1,33)=3.37$; $p_{unc} <0.001$, cluster extension>100) and ChronDep patients ($t(1,33)= 3.36$; $p_{unc}<0.001$ cluster extension≥100). Finally, RemRec had larger volumes than ChronDep in left middle temporal gyrus ($t(1,33)=3.36$; $p_{unc}<0.001$, cluster extension≥100). There were no GMV differences between Recov and PartRem patients. These results are summarized in **Figure 1.**

Regression models

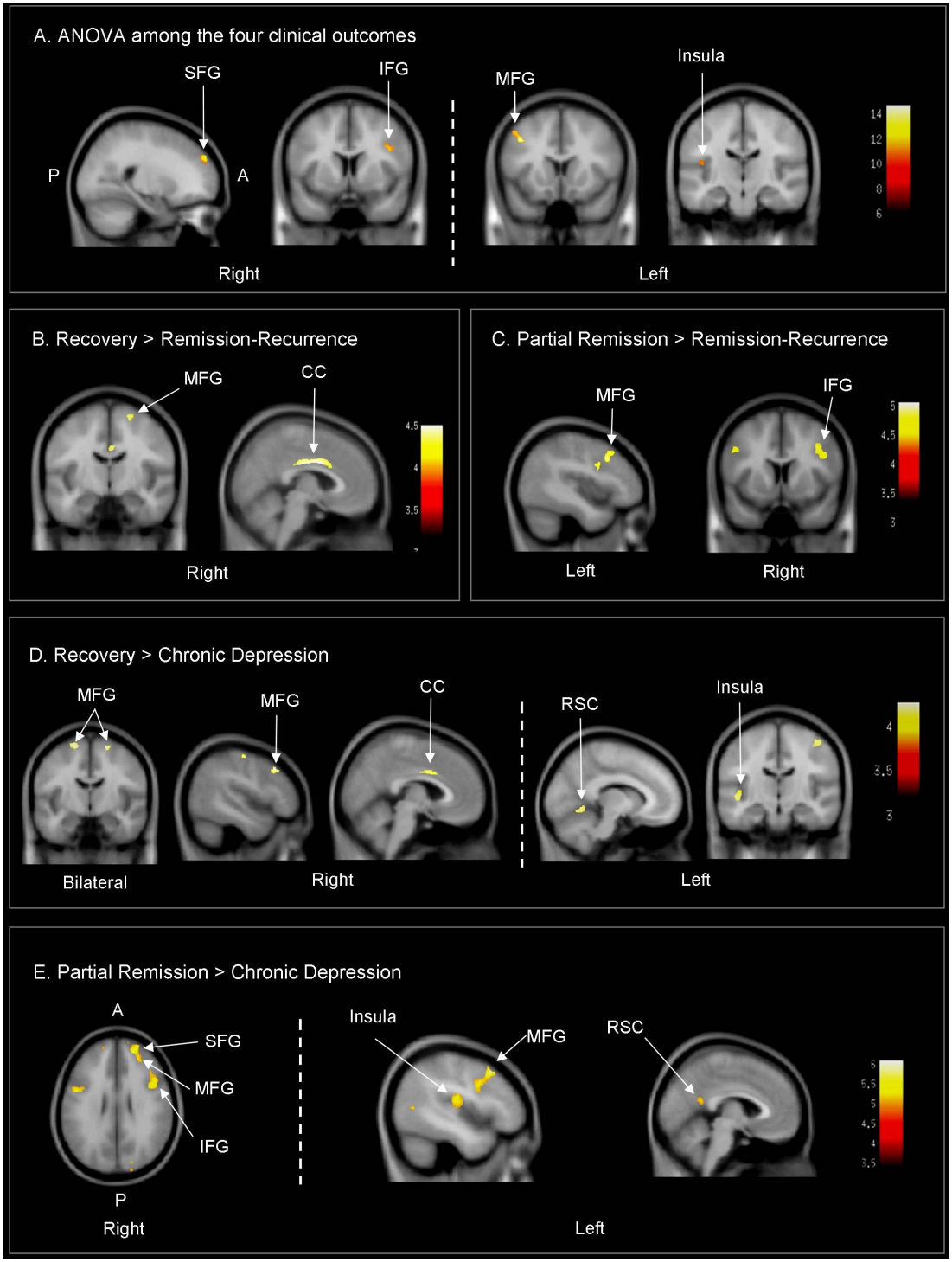
To reduce the number of brain areas in the regression analyses, we included only the brain areas that required at least one of the following criteria: a) included in the whole-brain ANOVA and in one posthoc comparison, b) showed a difference in at least 3 posthoc comparisons, or c) included in at least 2 posthoc comparisons but known to be key brain structures in the pathophysiology of MDD (23, 24). As a result, the brain areas that met these criteria were as follows: left/right middle frontal gyrus, right superior frontal gyrus, right inferior frontal gyrus, right ACG, left insula, and retrosplenial cingulate gyrus (see **Table 3**).

Table 2. Voxel-based morphometry results of ANOVA among clinical outcomes and post hoc contrasts, $P_{unc}<0.001$.

Contrast	Brain area	BA	*Test value*		*Cluster size*	Peak level	MNI coordinates[a]		
			F / t	*Z*			x	y	z
ANOVA	L middle frontal gyrus*	8/9	14.71	4.53	311	<0.001	−40.5	14	37
	R superior frontal gyrus*	9	11.80	4.10	393	<0.001	23.4	53	34
	L inferior parietal lobule	40	8.94	3.57	51	<0.001	−31.5	−43	48
	R inferior frontal gyrus*	9	8.65	3.51	123	<0.001	40.5	7	26
	L inferior frontal gyrus	9	8.04	3.37	93	<0.001	−48.6	2	28
	L insula*	13	7.72	3.30	67	<0.001	−44.1	−19	14
Rec>RemRec	R anterior cingulate gyrus*	24/23	4.50	3.95	1249	<0.001	4.5	1	31
	R middle frontal gyrus*	6	4.05	3.62	119	<0.001	19.8	−10	64
PartRem>RemRec	L middle frontal gyrus*	9	5.06	4.32	433	<0.001	−41.4	16	36
	L precentral gyrus	6	4.5	3.94	562	<0.001	−47.7	2	28
	R inferior frontal gyrus*	9	4.27	3.78	518	<0.001	41.4	6	26
	R precentral gyrus	9	4.11	3.67	-	<0.001	36.9	8	34
	R middle frontal gyrus*	9	3.77	3.41	-	<0.001	49.5	17	31
Rec>ChronDep	L middle temporal gyrus	39	4.28	3.79	778	<0.001	−53.1	−73	16
	R middle frontal gyrus*	6	4.27	3.78	135	<0.001	18.9	−8	64
	L superior temporal gyrus	38/13	4.19	3.73	661	<0.001	−40.5	6	−18
	L middle temporal gyrus	21	3.64	3.31	-	<0.001	−54	1	−12
	R middle frontal gyrus*	9	4.08	3.64	159	<0.001	48.6	25	41
	L middle frontal gyrus*	6	4.07	3.63	268	<0.001	−18.9	−8	67
	R precentral gyrus	4	3.96	3.55	475	<0.001	42.3	−20	59
	L cerebellum, culmen	-	3.89	3.5	293	<0.001	−9.9	−58	−7
	L posterior cingulate*	30	3.56	3.25	-	0.001	−3.6	−55	1
	L insula*	13	3.81	3.44	242	<0.001	−45.9	−16	−5
	L inferior frontal gyrus	47	3.80	3.43	106	<0.001	−18.9	23	−17
	R anterior cingulate gyrus*	24	3.63	3.31	140	<0.001	4.5	1	31
PartRem>ChronDep	L middle frontal gyrus*	8/9	6.10	4.96	2115	<0.001	−40.5	14	37
	R superior frontal gyrus*	9	5.75	4.75	1382	<0.001	23.4	53	34
	R middle frontal gyrus*	10	4.02	3.60	-	<0.001	27	41	28
	L superior frontal gyrus	9/10	5.38	4.53	1084	<0.001	−27	34	47
	R postcentral	3	5.31	4.48	110	<0.001	32.4	−38	47
	R inferior frontal gyrus*	9	4.81	4.16	1683	<0.001	41.4	7	25
	L postcentral	3	4.72	4.10	146	<0.001	−29.7	−42	48
	L middle temporal gyrus	37	4.63	4.04	239	<0.001	−40.5	−65	7
	L insula*	13	4.57	3.99	996	<0.001	−44.1	−19	14
	R insula	13	4.25	3.77	392	<0.001	42.3	11	11
	R superior frontal gyrus	10	4.01	3.59	181	<0.001	15.3	62	24
	R inferior frontal gyrus*	44/45	3.90	3.51	291	<0.001	50.4	17	12
	L retrosplenial cingulate*	30	3.85	3.47	207	<0.001	−4.5	−53	18
	R middle temporal gyrus	21	3.84	3.46	356	<0.001	59.4	−26	−18
	R inferior temporal gyrus	20	3.77	3.41	-	<0.001	58.5	−37	−20
	R superior temporal gyrus	38	3.73	3.38	176	<0.001	43.2	10	−16
	L cerebellum, culmen	-	3.70	3.36	467	<0.001	−41.4	−42	−30
	R cunneus	19	3.66	3.33	122	<0.001	18	−85	29
	L middle frontal gyrus*	6	3.64	3.31	144	<0.001	−28.8	−8	49
RemRec>ChronDep	L middle temporal gyrus	21	3.66	3.33	133	<0.001	−45.9	8	−30

BA, Brodmann area; L, left; R, right; Recov, Recovery; PartRem, partial remission; RemRec, remission recurrence; chrondep, chronic depression. [a]Anatomical region based on Talairach Atlas, F/t and Z scores, spatial extent in number of voxels (cluster size) and Montreal Neurological Institute (MNI, x y z) stereotaxic coordinates of the most significant voxel of each cluster are displayed. ANOVA extent threshold: 50; Post-hoc comparisons: 100. [b]The coordinates within each cluster were converted from MNI spatial array to the stereotaxic array of Talairach and Tournoux (33) using a non-linear transformation. * Areas included in the regression analyses.

Figure 1 Grey matter volume differences represented in axial brain slices of an avg152T1 SPM8-template. Colour bars represent an F (ANOVA) or a t (post-hocs) value of each analyis.



ANOVA, Analysis of variance; A, anterior; P, posterior; SFG, superior frontal gyrus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; CC, cingulate cortex; RSC, retrosplenial cingulate cortex.

Table 3. Mean and standard deviation (SD) of FreeSurfer segmented volumes (in ml) of those areas that fulfilled one of the three criteria to enter the regression analysis.

Brain region	Mean (SD)			
	Recov (n=10)	PartRem (n=7)	RemRec (n=14)	ChronDep (n=18)
Left middle frontal gyrus	4888.3(758)	5476.6(538.5)	4557(1028.5)	4437.4(699.8)
Right middle frontal gyrus	4992.8(818.1)	5149.6(683.2)	4560.29(756.1)	4157.1(847.15)
Right superior frontal gyrus	18085.5(1568.8)	18454.9(1974.8)	16954.4(2380)	16635.4(2634.8)
Right inferior frontal gyrus	3255.4(483.2)	3623.3(315.4)	3247.4(405.1)	3151.72(733.9)
Right anterior cingulate gyrus	2168(548.9)	1931.7(373.1)	1808.5(371.4)	1845.4(431.1)
Left insula	5903.1(398.9)	6080.3(1084.9)	5571(754.1)	5478(796.8)
Left RSC gyrus	2525.7(441)	2401.7(425.4)	2269.4(388.7)	2250.8(536.5)

SD, standard deviation; Recov, Recovery; PartRem, partial remission; RemRec, remission recurrence; chrondep, chronic depression. RSC= Retrosplenial cingulate gyrus

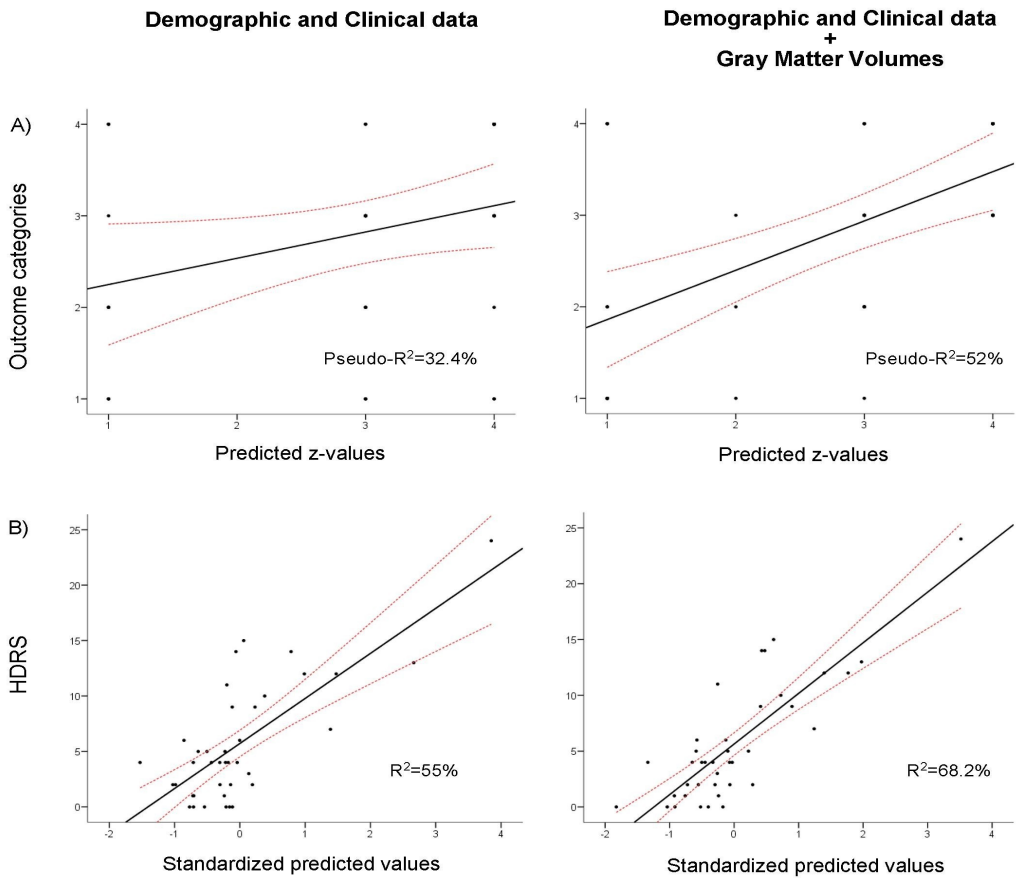
Clinical outcomes prediction by demographic, clinical and sMRI data

The overall ordinal regression model was significant ($\chi2=15.1$; df=3; p=0.002, with a pseudo-R2 of 0.324) while the goodness-of-fit statistic (deviance Chi-square) was not significant ($\chi2=91.928$, df=105, p=0.82). Parameter estimates and related statistics for the individual variables indicated that baseline HDRS (0.122; 95%CI, 0.025-0.219; p=0.014), baseline number of previous MDD-episodes (0.825; 95%CI, 0.098-1.553; p=0.026) and clinical stage at baseline (1.253; 95%CI, −0.015-2.521; p=0.053) were the included regressors. Pseudo-R2 significantly increased to 0.52 when brain GMV was added to the model ($\chi2=27.79$; df=6; p<0.001; deviance $\chi2=84.78$, df=117, p=0.98). Parameter estimates included: baseline duration of illness (0.007; 95%CI, 0.0001-0.14; p=0.044), HDRS (0.14; 95%CI, 0.03-0.25; p=0.013) and number of previous MDD-episodes (0.762; 95%CI, −0.117-1.641; p=0.089), together with right inferior frontal gyrus (0.002; 95%CI, 0.000057-0.003; p=0.042), anterior cingulate (−0.003; 95%CI, −0.005-−0.001; p=0.005) and right middle frontal gyrus (−0.001; 95%CI, −0.002-0.003; p=0.066). **Figure 2A** compares the explained variances and best-fit lines slopes between the two models.

HDRS prediction by demographic, clinical and sMRI data

The linear regression model for HDRS (follow-up assessment) explained 55% of the variance (R2=0.55; F(4,33)=10.088; p<0.001), in which baseline education (β=1.98; p=0.029), duration of illness (β=0.015; 0.012), HDRS (β=0.172; p=0.017) and the number of previous MDD-episodes (β=0.894; p=0.001) were significant factors. When GMV data were included, the explained variance significantly increased [F-change(df1=2, df2=31)=4.79, p=0.015] up to 68.2% (R2=0.682; F(7,30)=9.195; p<0.001; Figure 2B); the factors included in this model were baseline education (β=1.56; p=0.067), marital status (β=−1.92; p=0.042); duration of illness (β=0.019; p=0.002), HDRS (β=0.15; p=0.02), number of previous MDD-episodes (β=0.84; p<0.001); left retrosplenial cingulate cortex (β=0.003; p=0.082) and right anterior cingulate cortex (β=−0.005; p=0.005). Multi-colinearity had no effect on coefficient estimations (T>0.2 and VIF<4.4).

Figure 2. Correlations (A) and linear regression analyses (B, C) between outcome measures (axis Y) and regression predicted z values (axis X). Black solid lines show the best-fit line and red dotted lines the confidence intervals.



6.4.4. Discussion

The findings presented here show that predicting long-term clinical outcomes in MDD can be significantly improved by adding structural brain data to standard clinical and demographic variables. As expected, certain clinical variables, including longer illness duration, higher HDRS scores at scanning time and greater number of previous MDD episodes, were predictive of worse outcomes. Adding structural brain data to these clinical variables increased the predictive capacity of the model: the addition of GMV of the right anterior cingulate and right inferior frontal gyrus volumes increased the models' explanation of variance by 20%. The combined models, which gather brain, demographic, and clinical characteristics of patients, can also predict depressive symptoms at five years of follow-up. Moreover, this combined set of data provides a much wider perspective of illness course at any given moment, regardless of whether this was the first episode of depression or the patient had suffered multiple previous episodes.

Our findings support the results of some previous studies that have found that worse clinical characteristics (number of previous depressive episodes, symptom severity and longer illness trajectories) lead to poorer clinical courses (Colman et al., 2011). Thus, clinicians should use these clinical features as indicators of future relapses. To improve the prediction of illness course, our data show that clinical information might be complemented with brain characteristics, which represent the biological substrate of illness progression. Similar neural correlates related to treatment response have also been found in previous studies (Korgaonkar et al., 2014). Irion and colleagues (2013) found that effective 5HT antidepressant treatment was associated with increased cerebral perfusion in the anterior cingulate cortex and the medial prefrontal cortex after 12 weeks. Similarly, Lisiecka and colleagues (2011) found higher OFC connectivity with other OFC areas, as well as with left motor areas, in responders to antidepressant drugs. None of these previous studies, however, provide a prognostic value of the course of the illness, with the exception of a work by Frodl and colleagues (Frodl et al., 2008). Those authors found that patients with recurrent depression who had a smaller hippocampal volume and previous depressive episodes experienced a worse

clinical outcome within the first 3 years after an acute depressive episode. Their findings suggested that small hippocampal volumes might be a vulnerability factor for poor treatment response when considered together with certain clinical characteristics (previous MDD episodes). Our results support this idea, providing information on the value of combing brain data and clinical information to better predict the probable course of the illness. A revision carried out afterwards (MacQueen, 2009) supported the involvement of hippocampus plus added ACG volumes as sMRI predictors of poor outcomes. Our results did not show differences in hippocampal volumes among COs, but it did in the anterior cingulate. The right anterior cingulate gyrus (rACG, BA 24) was the variable that best explained the extra variance: higher GMV values correlated positively with better CO and lower HDRS scores at 5-years. In the same line, Frodl and colleagues (Frodl et al., 2008b) reported that patients with larger ACG showed better clinical outcomes than those with smaller ACG. This finding adds evidence to several studies that have shown the anterior cingulate to be a key brain structure for MDD pathophysiology, with a potential involvement in treatment response. In this regard, Mayberg and colleagues (1997) found that it was the rostral anterior cingulate (BA25) which was specifically involved, instead of BA24. One possible explanation may be that, in our study, the rostral cingulate did not meet the criteria for inclusion in the regression models; thus, we cannot be certain that this region is unrelated with long-term COs. A recent study by Schmaal and colleagues (2014) also used MRI (both structural and functional) to predict the naturalistic course of depression, but only fMRI was able to differentiate clinical trajectories, where reduced activity in prefrontal regions was found in chronic patients. Therefore, MRI seems to provide a complement in the prediction of long-term COs in depression, as our findings suggest that the combination of patient clinical and brain characteristics may help reveal the factors implicated in the course of the illness.

Limitations

The study has some methodological issues that deserve a comment. The sample was limited in size, although it covered different clinical trajectories of MDD. A bigger sample would have been desirable, not only to increase

the statistical power, but to be able to run the models in half of the sample, which could have allowed the model testing. Therefore, future studies are needed to test the predictability of the models. Another aspect relies on the fact that clinical interviews were performed retrospectively, yielding to memory biases that could have had an undesired impact on information collection. For this reason, patients' history was double checked with databases of the hospital. Patients' baseline differences regarding illness stage should be considered when facing conclusions of the results, as one third of the sample were already diagnosed as chronic at scanning. In any case, the characteristics of the sample allow the observation of diagnostic stability of the worst clinical outcomes (i.e. treatment- resistant or chronic). Finally, those patients classified as first episodes at baseline did not display the common age at onset, as they were in their forties, which could affect the generalizability of the results. Given the nature of the study, however, the impact on the findings appears to be minimal.

In conclusion, findings presented in this study suggest that structural neuroimaging data combined with clinical data in MDD could improve predictive models of long-term COs. In addition, this information is also able to predict depressive symptomatology. Future research should be directed at integrating neuroimaging, clinical and genetic data to identify all the potential factors involved in determining the course of MDD. The value of sMRI is that it can be easily implemented and it requires minimal or no patient's interaction. Finally, the fact that patients were at different stages of their illness, allow our results to be generalized to MDD patients not only in their first depressive episode, but at any stage of the disease.

6.4.5. References

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7. General Discussion

7. General Discussion

The current thesis provides evidence of structural brain abnormalities present in patients suffering from more severe and chronic depressive courses. The main finding is an important GMV reduction in frontal, limbic and temporal regions. Such grey matter reductions correlate negatively with duration of depressive illness, suggesting a toxic effect of long-lasting depression on brain integrity. Fronto-temporo-limbic alterations of chronic patients are also observable through slow processing speed and in deficits in memory and executive functioning. The use of the promising strategy DBS for patients with chronic depression has shown that this therapy does not impair even more the cognitive dysfunction, but it improves the performance in some domains. Therefore, the deep stimulation can be considered a cognitively safe alternative for patients who do not respond to conventional treatments. As regard to its mechanisms of action, the brain metabolism changes observed when stimulation was inactivated, help to disentangle the rationale behind its efficacy. Finally, by gathering all these findings, we have observed that structural data added to other clinical and demographic information permits the prediction of clinical trajectories along time. These are promising results in the search of early biomarkers to prevent patients to end up suffering chronic courses.

The impaired brain areas identified throughout the works of this thesis have been involved in the neurocircuitry of mood disorders (Price & Drevets, 2010). Cortical areas like inferior/middle frontal gyrus and anterior cingulate cortex displayed structural abnormalities in treatment-resistant patients, and seem to have an added value to clinical data in predicting future illness trajectories. Several reviews (Atkinson et al., 2014; Drevets et al., 2008) and meta-analyses (Bora et al., 2012; Koolschijn et al., 2009) have reported such prefrontal regions to be smaller in MDD patients, while functional results during acute episodes and in remission show more complex patterns (Phillips et al., 2003). Whereas anterior cingulate and inferior frontal gyrus are hyperactivated during depressive episodes, more medial regions show hypoactivity, and this pattern reverses after depression recovery. Interestingly, our findings showed smaller volumes of left anterior

cingulate and inferior frontal gyrus in TRD compared to controls, but not when compared with other illness stages (firs-episode or remittent-recurrent). In the same line, these areas did not discriminate the different long-term outcome. A similar pattern was observed in the left insula; it showed less GMV in TRD patients compared to healthy controls and to FE patients, and its volume correlated negatively with illness duration. However, in the follow-up study, although left insula appear diminished in those patients with a chronic course, it was not predictive of illness trajectories. This limbic area has already been found to discriminate between depressed patients and healthy controls, especially with functional studies (Guo et al., 2011; Veer et al., 2010). However, left insula volumes were correlated negatively with number of previous MDD episodes (Soriano-Mas et al., 2011) and with illness duration (Salvadore et al., 2011). Therefore, volumes of insular and prefrontal cortices, apart from discriminating healthy controls and depressed patients, appear to become more affected as illness progresses. A high density of type 1 and type 2 corticotropine-releasing hormone receptors (Sánchez et al., 1999) have been found across these specific areas. In addition, left hemisphere lesions enhance sympathetic autonomic arousal and corticosterone response to stress (Drevets, 2000). It is reasonable to think that the close relation of these cortical areas with cortisol release (given the hyperactivity of the HPA axis in MDD) is mediating the cellular loss along illness progression, augmenting the occurrence of new episodes or diminishing the chances of recovery. Our findings would give some support to the kindling hypothesis proposed by Post et al. (1982).

Another area that is able to differentiate individuals with and without MDD and is related to long-term clinical outcomes is the right middle frontal gyrus. This region had less grey matter volume in TRD patients than in healthy controls, but also than in first-episode and remittent recurrent patients. In addition, right MFG had an added value predicting long-term clinical trajectories. Given its implication in cognition and behavioural processes commonly impaired in MDD (i.e. voluntary behaviour (Nchev et al., 2005) or social cognition (Amodio et al., 2006) and its activity changes after antidepressant treatment (Fitzgerald et al., 2008), one might think that right MFG has a main role mediating the origin and perpetuation of MDD.

Likewise, right-sided ACG (BA 24) displayed bigger volumes in recovered MDD patients, being the most significant indicator of better clinical outcomes and lower long-term HDRS scores, even more than clinical characteristics. These results are compatible with findings of PET studies of treatment response to antidepressant drugs. Mayberg et al. (2000) found that patients who improved after fluoxetine administration also experienced metabolic increases in right ACG (BA 24b) among other cortical areas (prefrontal, parietal, anterior and posterior cingulate gyrus). Likewise, our PET study showed metabolism decreases in right dorsal ACG (BA 24), premotor region (BA 6) and putamen when DBS stimulation of SCG was turned off. Right dorsal anterior cingulate cortex also showed glucose metabolism increases (among other prefrontal and parietal areas) in MDD male patients who successfully responded to paroxetine (Kennedy et al., 2001). A plausible hypothesis is that MDD patients who successfully respond to treatment, increase ACG activity preventing the damaging effects of cortisol on neuron cells. Therefore, a greater right ACG might have a protective effect against the more severe illness courses.

TRD patients' brain damage is also observable in their neuropsychological functioning. Interestingly, cognitive deficits of more severe depressed patients rely on some of the structures showing GMV reductions, like left prefrontal cortex (superior, middle, inferior), left anterior (BA 24) or left parahippocampal gyrus. Semantic memory, which was impaired TRD patients, depends of multiple brain areas distributed across cortical and subcortical regions, (reviewed in Hart et al., 2007) and it is crucial, together with autobiographical memory, to remember the past and imaging the future (Irish & Piguet, 2013). Memory improved in TRD patients more than in FE (although not significantly) after each respective antidepressant treatment. Thus, it is proposed that DBS, by the stimulation of ACG, which connects with different cingulate subregions and temporal areas (Medford & Critchley, 2010), may have induced this memory improvement. Interestingly, the areas reported to decrease their metabolism when DBS of SCG is stopped (named ACG, premotor region and putamen), have been reported to have a role in semantic memory (Wiggs et al., 1999), making plausible that the memory improvement observed were partly caused by DBS. Regarding executive functioning, and specifically

cognitive flexibility, TRD patients showed greater impairment than FE patients. Moreover, right middle frontal gyrus was more decreased in TRD than in FE, and was the most predictive area of long-term outcomes. Thus, cognitive flexibility impairment might be due in part, by such structural impairment, which may persist in TRD condition. Interestingly, TMT-B showed a not negligible (although not statistically significant) improvement in TRD patients after DBS. However, FE patients remained stable, suggesting an effect of ACG stimulation via its PFC connections (Enatsu et al., 2015). By contrast, planning was generally affected in both groups of depressed patients. Such executive function does not rely on the right middle frontal gyrus, but on strong relationships within ACG and DLPFC (Unterrainer et al., 2004; van den Heuvel et al., 2003; Wagner et al., 2006). Strikingly, this cognitive function did not improve after treatment neither in FE nor in TRD patients, suggesting a more profound and long-lasting impairment. Such damage might be independent not only of mood state, but also of illness stage. Finally, processing speed appeared severely impaired in TRD patients' performance (TMT-A) and in the average range in FE patients, showing no improvement after treatment. According to our results, a meta-analysis by McDermott & Ebmeier (2009) reported significant correlations between depression severity and processing speed among other cognitive domains. From our findings, it is difficult to establish a clear relation between processing slowness and cortical structures. One explanation could be that information processing gets worse as the illness progresses, or alternatively, that those patients with more severe manifestations of the disorder need more aggressive treatments that in turn, worsen speed processing. However, other authors have not found relation between depression and processing speed (Jungwirth et al., 2011) and such cognitive function is hardly mentioned in the recent study carried out by Beblo and colleagues (2011) reviewing the neuropsychology of affective disorders. Such disparity of results may have several causes. As TMT-A is sensitive to a variety of neuropsychological impairments (Bowie & Harvey, 2006), lower scores on TMT-A are sometimes reported to be measuring visual-motor sequencing (Paradiso et al., 1997) and sometimes processing speed (Sheline et al., 2006; Story et al., 2008). In turn, some studies conclude processing speed alteration when symbol digit, which

rather measure psychomotor functioning, is administered (Loo et al., 2010; Sheline et al., 2006). Overall results point to a clear slowing of cognitive processing in depressed individuals, which would be more prominent in patients with a higher burden of disease. However, specific variables mediating this effect need further research (Herrera-Guzmán et al., 2009; Salthouse, 1996; Sheline et al., 2006).

Taking together, findings of the current thesis support the hypothesis of the damage that depression exert on brain structure and function. This impairment is revealed by structural neuroimaging data, as grey matter volumes appear highly affected in patients at the most severe illness stages. Furthermore, it seems to be specific combinations of brain structure and illness characteristics, which may ease a worst illness trajectory. The brain areas with a predictive capacity have an scarce overlap with those impaired on TRD patients. This result suggests that those areas suffering changes as illness progress (possibly more sensitive to neurochemical and neuroendocrine effects) are different to those areas predisposing patients to worse long-term Cos. Intriguingly, right dorsal anterior cingulate, the brain area with more predictive capacity of future clinical outcomes, also show metabolic decreases when DBS is stopped. On the other hand, the observed brain affectation is also obvious at a neuropsychological level. Depressed patients, independently of their depression severity, exhibit deficits in the executive function of planning, which remains after antidepressant treatment (both pharmacological and DBS). These results taking together give support to the hypotheses of Phillips and colleagues (2003), which state that MDD structural abnormalities could be associated with the impairments found in executive function, hindering the regulation of emotional behaviour and, in return, perpetuating depressed mood and anhedonia. On their side, TRD patients display a broader affectation comprising fronto-temporo limbic grey matter volume reductions and memory, executive, and processing speed dysfunctions. These findings lead to think that TRD patients may constitute a variant of MDD condition not only clinically, but also neurobiopsychologically, which must to be taken into account when studying the pathophysiology of disease.

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8. Conclusions

8. Conclusions

In this thesis neurobiological and cognitive characteristics of major depressive disorder have been discussed. A list of the main conclusions derived from the present work follows:

- Gray matter abnormalities observed in depressed patients are associated with illness duration, but not with medication patterns.
- There are negative long-lasting effects of MDD on grey matter structure.
- Patients with severe depression present smaller grey matter volumes than healthy controls in frontotemporolimbic areas.
- DBS discontinuation produces immediate effects on metabolism of prefrontal and cingulate regions, which precede clinical manifestations.
- DBS do not worse cognitive functioning of TRD patients, but improve memory performance.
- Memory improvement is more related to treatment response than to static depressive symptoms.
- Structural brain data constitute an added value to clinical patient information as predictor of long-term MDD clinical outcomes.

9. Future goals

9. Future goals

a) A complete characterization of patients with a first episode of depression with a long-term follow-up, including neuropsychological assessment, personality traits, stressful life events, genetics and neuroimaging. Such longitudinal study would more accurately answer the question of which brain deficits help to precipitate the illness and which are as consequence of it. This information is critical to find out new treatment strategies as well as to prevent recurrences.

b) To investigate the effect of first line antidepressant treatments (drugs and psychotherapy) on brain MDD patients' structure and function. An important next step is to validate the classification models in completely independent data.

c) To study the effect of somatic therapies such as DBS on brain structure and function, in order to truly understand the mechanisms underlying their efficacy in TRD.

10. Summaries

10. Summaries

NEUROTOXICITAT DE LA MALALTIA DEPRESSIVA:

ESTUDI DE NEUROIMATGE I NEUROPSICOLOGIA

(Resum de la tesi)

Introducció

El trastorn depressiu major (TDM), caracteritzat per sentiments de tristesa i/o apatia, malestar físic i dèficits cognitius, presenta un 50% de recaigudes i un 20% de cronificació. Les teories etiològiques més integradores inclouen característiques estructurals i funcionals cerebrals com a part de la patofisiologia del trastorn. Així, postulen que les alteracions de matèria gris i els dèficits cognitius facilitarien la recurrència/cronifiació del episodi. No obstant, les àrees afectades són inconsistents entre els estudis, dificultant la caracterització d'un model patofisiològic i complicant la millora dels tractaments disponibles. A més, els tractaments per als pacients resistents als tractaments (DRT) són escassos, afectant generalment la cognició, i dificultant una complerta recuperació. Així, es requereixen noves vies d'estudi centrades en la prevenció de la recurrència/cronificació dels pacients a través de la detecció de variables predictores individuals que optimitzin les opcions terapèutiques.

Objectius

E1: Investigar l'afectació de matèria gris cerebral en diferents estadis de la malaltia depressiva i determinar l'efecte de les característiques clíniques en el seu volum.

E2: Determinar els canvis metabòlics cerebrals que ocorren al aturar l'estimulació cerebral profunda (ECP) del cortex cingulat anterior subgenual (CASg) en pacients amb DRT.

E3: Avaluar les funcions cognitives en pacients amb DRT abans i després de l'ECP del còrtex CASg).

E4: Examinar el potencial predictiu de les dades clíniques i de neuroimatge estructural en el curs del TDM.

Mètodes

Es compara el volum de matèria gris (VMG) entre 66 pacients amb TDM en diferents estadis de la malaltia amb 32 controls sans a través de la tècnica de morfometria basada en el vòxel. També es correlacionen els VMG amb les variables clíniques associades a cada pacient (E1). Els 66 pacients es contacten als 5 anys i es divideixen en 4 grups segons l'evolució clínica que han tingut (n=49). Es fa un anàlisi de regressió amb les dades clíniques i de neuroimatge basals com a variables predictores del curs observat (E3). Finalment s'administra una bateria neuropicològica abans i després de l'estimulació cerebral profunda del CAsg en pacients amb DRT, agafant com a grup control pacients amb un primer episodi de TDM (E2). A més, se'ls realitza una tomografia per emissió de positrons després d'un període d'estabilitat clínica amb el neuroestimulador on versus off.

Resultats

L'anàlisi principal mostra un efecte significatiu del grup en el VMG del gir frontal superior dret, frontal medial i cingulat esquerre. Els pacients amb DRT exhibien menor VMG frontotemporal. El VMG en l'insula dreta i el còrtex prefrontal medial dret correlaciona negativament amb la duració de la malaltia (E1). En el quart estudi s'observa que el VMG afegeix un 20% de variància a les dades clíniques a l'hora de predir el curs clínic dels pacients, essent el gir cingulat anterior l'àrea amb més poder predictiu. Aquesta àrea, a més, mostra un descens en la seva activitat metabòlica quan s'apaga el neuroestimulador en pacients amb DRT que havien respòs clinicament (E2). Per últim, l'ECP no empitjora cap funció cognitiva en aquests pacients i l'avaluació neuropsicològica mostra una millora mnèsica posterior a l'intervenció (E3).

Conclusions

Els pacients amb DRT tenen menors VMG frontotemporolimbics, els quals estan associats amb la durada de malaltia, però no amb la medicació, suggerint efectes nocius del trastorn depressiu per se en la matèria gris. A més, el VMG tindria un valor afegit a la informació purament clínica a l'hora de predir la resposta clínica dels pacients a llarg termini. La part dorsal del còrtex cingulat anterior dret sembla esta estretament relacionada amb la resposta al tractament. Finalment, les troballes en l'estudi neuropsicològic

donen suport a la seguretat cognitiva de l'estimulació cerebral profunda del cingulat anterior, contribuint en la seva implantació com a alternativa terapèutica pels pacients amb DRT.

Limitacions

El seguiment de primers episodis respondria millor si les reduccions de VMG són resultat de la depressió o si ja estaven presents d'un inici, incidint en el curs del trastorn. Quant a l'estudi longitudinal, la diferència en l'estadi del TDM basal podria ser un factor confusor. En l'estudi neuropsicològic i metabòlic dels pacients intervinguts caldria tenir en compte la reduïda mida de la mostra així com la retirada de teràpia electroconvulsiva pre-implantació.

NEUROTOXICITY OF MAJOR DEPRESSIVE DISORDER:

NEUROIMAGING AND NEUROPSYCHOLOGICAL STUDY

(Abstract)

Introduction

Major depressive disorder (MDD) is characterized by feelings of sadness and/or apathy, physical disturbances and cognitive impairment. After the first episode, 50% of patients relapse and up to 20% become chronic. Current aetiological theories, which consider brain structure and function as part of MDD pathophysiology, postulate that structural alterations and cognitive impairments would ease recurrence and chronicity. However, the brain areas implied are inconsistent throughout studies, hindering the characterization of MDD pathophysiological models and slowing the finding of new treatments. In addition, therapeutic strategies for patients with treatment resistant depression (TRD) are scarce and generally have a negative impact on cognition, preventing them from a complete recovery. Thus, new studies determining individual variables predicting clinical trajectories such as chronicity are needed.

Objectives:

E1: To investigate structural brain abnormalities at different stages of the illness and to determine the effect of clinical characteristics on brain GMV.

E2: To determine the cerebral metabolism changes during a switch-off of electrical stimulation in implanted patients with TRD who had achieved clinical improvement.

E3: To evaluate cognitive function of TRD patients before and after DBS of the SCG.

E4: To examine the prognostic potential of clinical and sMRI data in the long-term clinical outcomes of MDD.

Methods

Voxel-based morphometry (VBM) was used to compare 66 MDD patients at different illness stages with 32 healthy controls. GMV were also correlated with patients clinical characteristics (E1). 66 MDD patients were contacted at 5 years after MRI scan and split in 4 groups depending on their clinical trajectories during that time (n=49). Regression analysis with clinical and neuroimaging data as predictive variables and clinical outcomes as dependent variable was carried out (E4). Finally, a neuropsychological battery was administered before and after DBS of subgenual cingulate gyrus (SCG) in TRD patients, with a control group of first episode patients (E3). In addition, clinically stable TRD patients underwent a positron emission tomography (PET) analysis comparing active versus inactive DBS (E2).

Results

VBM showed a significant group effect in right superior frontal gyrus, left medial frontal gyrus and left cingulate gyrus. Patients whose condition was treatment resistant/chronic exhibited the smallest volumes in frontotemporal areas. Longer illness duration was negatively correlated with decreases in right medial frontal cortex and left insula (E1). Fourth study showed that GMV explained a 20% more of variance when joined to clinical

characteristics predicting long-term clinical outcomes. Anterior cingulate gyrus was the area adding more value to the prediction. In addition, such cingulate area showed a metabolic decrease in TRD patients who were clinically stable when the stimulation was stopped. Finally, neuropsychological assessment of TRD patients show no impairment of cognitive functioning after DBS, but a memory improvement (E2).

Conclusions

Frontotemporolimbic areas were smaller in the patients with severe depression and were associated with duration of illness, but not with medication patterns, suggesting negative effects of long-lasting MDD on grey matter. In addition, GMV may demonstrated an added value to clinical information of depressive patients in terms of predicting their long-term clinical outcome (E4). Right dorsal anterior cingulate gyrus seems to be closely related to treatment response (E2). Finally, neuropsychological performance of patients after DBS (E3) supported the cognitive safety of this new technique adding a valuable information for its future implementation as a therapeutic alternative for TRD patients.

Limitations

A longitudinal study would be more appropriate to ascertain whether volume reductions in chronic patients are a result of enduring MDD effects or the cause of a more severe disorder. Regarding the follow-up study, differences in illness stage at baseline could lead to confusion. The results of second and third study should be interpreted cautiously given the small sample size and the fact that some TRD patients received ECT before implantation.


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