Effects of remission and genetic variation on brain structure in treatment-resistant major depressive disorder: a prospective, longitudinal imaging study

Jennifer Phillips

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Department of Cellular and Molecular Medicine

Faculty of Medicine

University of Ottawa

ABSTRACT

Previous magnetic resonance imaging (MRI) studies have demonstrated brain atrophy in major depressive disorder (MDD) that is progressive with continuing illness and may be reversible with antidepressant treatment. What remains unclear is whether brain structure can be positively affected by pharmacological intervention even if patients fail to remit on the treatment. The primary aim of this thesis was to prospectively track changes in brain structure in patients with treatment-resistant depression while they underwent pharmacotherapy with the goal of attaining remission. There is evidence that gene variants associated with poorer antidepressant response also confer greater risk of volume reduction in the hippocampus. A secondary aim of the thesis was to investigate the effects of monoaminergic-related gene variants on hippocampal volume in patients and controls at baseline imaging. Outpatients with treatment-resistant MDD underwent structural MRI scans at baseline and after either 6-months of sustained remission or 12-months of failure to remit. Matched controls were scanned once to provide comparison data for patients' baseline scans. Participants also provided blood samples for genetic analyses. Imaging outcome measures included longitudinal changes in whole-brain volume, and gray matter volume and mean cortical thickness within specific cortico-limbic regions of interest (ROIs). Over follow-up, remitted patients had an increase in whole-brain volume, while nonremitted patients lost brain volume despite receiving more treatment strategies. Remitters and nonremitters also showed subtle changes in volume and thickness over time in several ROIs in opposing directions, with increasing hippocampal volume and cortical thickness in the rostral middle frontal gyrus and orbitofrontal cortex in remitters, and decreasing volume or thickness in these regions in

nonremitters. Genetic imaging analyses revealed that polymorphisms in certain norepinephrine- and serotonin-related genes have similar effects on hippocampal volume in patients and controls, while the serotonin transporter polymorphism differentially affects hippocampal volume in the presence of depression. Given the observations of volume increase in remitted patients and continuing atrophy in nonremitters, pharmacotherapy in the absence of sustained remission is likely insufficient to elicit structural recovery in depression. This finding is important since the restoration of brain structure in patients with treatment-resistant depression may have positive implications for their future prognosis.

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I. **Phillips, J.L.**, Batten, L.A., Aldosary, F., Tremblay, P., and Blier, P. (2012). Brain-volume increase with sustained remission in patients with treatment-resistant unipolar depression. Journal of Clinical Psychiatry. 73, 625-631.

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III. **Phillips, J.L.**, Batten, L.A., Tremblay, P., Aldosary, F., Du, L., and Blier, P. Impact of monoamine-related gene polymorphisms on hippocampal volume in treatment-resistant depression. Submitted to Acta Neuropsychiatrica.

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LIST OF ABBREVIATIONS

5-HT	serotonin
5-HTT	serotonin transporter
5-HTTLPR	serotonin transporter-linked polymorphic region
5-HT_{1A}	serotonin 1A receptor
5-HT_{2A}	serotonin 2A receptor
ACC	anterior cingulate cortex
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BA	Brodmann area
BDNF	brain-derived neurotrophic factor
COMT	catechol-O-methyltransferase
CRF	corticotropin-releasing factor
DA	dopamine
DARTEL	diffeomorphic anatomic registration through exponential lie algebra
DBS	deep brain stimulation
DLPFC	dorsolateral prefrontal cortex
DNA	deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
DTI	diffusion tensor imaging
ECT	electroconvulsive therapy
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional MRI of the Brain
FSL	FMRIB Software Library
GM	gray matter
$HDRS_{17}$	17-Item Hamilton Depression Rating Scale
HPA	hypothalamic-pituitary-adrenal

ICBM	International Consortium for Brain Mapping
IQ	intelligence quotient
LH	left hemisphere
MADRS	Montgomery-Åsberg Depression Rating Scale
MAOI	monoamine oxidase inhibitor
MANCOVA	multivariate analysis of covariance
MDD	major depressive disorder
MET	methionine
MNI	Montreal Neurological Institute
MPRAGE	magnetization-prepared rapid gradient echo
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NE	norepinephrine
NET	norepinephrine transporter
NIfTI	Neuroimaging Informatics Technology Initiative
NR	nonremitter
NRI	norepinephrine reuptake inhibitor
PBVC	percentage brain-volume change
PET	positron emission tomography
R	remitter
REDD1	regulated in development and DNA damage responses-1
RH	right hemisphere
ROI	region of interest
SCID-NP	Scheduled Clinical Interview for DSM-IV-Nonpatient Edition
SD	standard deviation
SE	standard error
SIENA	structural image evaluation using normalization of atrophy
SIENAX	structural image evaluation using normalization of atrophy cross-sectional
SNP	single nucleotide polymorphism

SNRI	serotonin norepinephrine reuptake inhibitor
SPM	statistical parametric mapping
SSRI	selective serotonin reuptake inhibitor
TE	echo time
TIV	total intracranial volume
TR	repetition time
VAL	valine
VBM	voxel-based morphometry
VNS	vagal nerve stimulation
WM	white matter

THESIS FORMAT

In accordance with the guidelines set forth by the Faculty of Graduate and Postdoctoral Studies, this dissertation is presented as a collection of manuscripts. The thesis includes three papers all derived from the same dataset stemming from a prospective, longitudinal imaging study involving a sample of patients with treatment-resistant depression and a matched healthy control group. A general introduction precedes the manuscripts and provides a review of the current literature concerning evidence of structural brain changes in major depressive disorder and outlines the objectives of the thesis research. Three original papers follow the general introduction, each with its own introduction, methodology and discussion sections. The thesis concludes with an overall discussion that summarizes and integrates the findings of the three papers and considers the relevance of the results in terms of future directions and clinical significance.

Chapter 1 presents a literature review of major depressive disorder, relevant neuroimaging findings, and contribution of genetic variation to structural imaging correlates and treatment response in patients. Additionally, this chapter contains a brief discussion of the imaging modalities employed to analyze the dataset.

Chapter 2 presents a manuscript entitled “Brain-volume increase with sustained remission in patients with treatment-resistant unipolar depression”. This paper was published in 2012 in The Journal of Clinical Psychiatry, 73(5), 625-631. The aims of this paper were (a) to prospectively examine whole brain volume changes in patients with treatment-resistant depression, comparing those who achieved sustained remission with

those who did not remit, and (b) to determine whether observed volumetric changes were more strongly associated with antidepressant treatment or clinical remission.

Chapter 3 presents a manuscript entitled "A prospective, longitudinal study of the effect of remission on cortical thickness and hippocampal volume in patients with treatment-resistant depression" which has been submitted for publication in The International Journal of Neuropsychopharmacology. As a follow-up to the paper presented in Chapter 2, this manuscript focuses on longitudinal structural changes in the same patient sample in specific cortico-limbic regions of interest. The aims were (a) to compare the patients' baseline imaging measures to a healthy matched control group, (b) to determine whether patients' baseline brain structure could predict their treatment response over follow-up, and (c) to determine how remission affected structural changes in the regions of interest over time.

Chapter 4 presents a manuscript entititled "Impact of monoamine-related gene polymorphisms on hippocampal volume in treatment-resistant depression". This article has been submitted for publication to Acta Neuropsychiatrica. The aim of this study was to examine the effect of variation in several monoaminergic genes on hippocampal volume in patients and controls at baseline imaging. The genetic polymorphisms were selected based on their potential association with treatment response in depression.

Chapter 5 summarizes and consolidates the findings of Chapters 2, 3, and 4. This general discussion describes the significance and clinical implications of the results and the strengths and limitations of the research described herein.

CHAPTER 1

General Introduction

1.1 Major depressive disorder

Major depressive disorder (MDD) is a severe psychiatric condition that affects 5-6% of the population in a twelve month period (Kessler et al., 2003) and has a lifetime prevalence of approximately 16% (Kessler et al., 2008). It is characterized by one or more major depressive episodes during which patients experience a cluster of clinical symptoms that may include depressed mood, loss of interest or pleasure, feelings of guilt, anxiety, and recurrent thoughts of death or suicide (American Psychiatric Association, 1994). Patients may also experience somatic and cognitive symptoms such as changes in appetite, sleep, and energy levels, and decreased concentration or difficulty making decisions. The current diagnostic strategy makes it possible that two individuals diagnosed with MDD may not share a single symptom in common. There is also a large degree of comorbidity between depression and several other psychiatric conditions including anxiety, post-traumatic stress disorder, and substance abuse (Curry et al., 2014). These factors demonstrate the heterogeneity of the disorder which can complicate its diagnosis and treatment (Goldberg, 2011).

Depression is associated with significant psychosocial impairment and is recognized as a leading cause of disability worldwide. In middle and high-income countries, unipolar MDD is the number one cause of burden of disease worldwide (measured in disability-adjusted life years) (World Health Organization, 2008). Individuals with MDD are at increased risk of medical comorbidities such as cardiovascular disease, and depression can negatively affect the outcomes of these co-occurring conditions (Benton et al., 2007). Depression also increases mortality by suicide (Nemeroff et al., 2001). There is a clear need to better identify and elucidate the

neurobiology underlying the pathophysiology of depression to improve diagnosis and treatment outcomes.

The exact causes of depression are unknown but several hypotheses have been proposed to explain how various mechanisms contribute to the disorder. Among these are hypotheses relating to genetic vulnerability, stress exposure, altered neurotrophin levels, and abnormalities of neurotransmission. Epidemiologic studies have revealed that depression has a strong genetic component with heritability estimates ranging from 40-50% (Fava and Kendler, 2000; Sullivan et al., 2000). Depression does not solely result from genetic influences; instead the interaction between genetic predisposition and environmental factors likely contributes to its development. Perhaps the most important environmental factor associated with increased vulnerability for the development of depression is stress.

The brain reacts to acute and chronic stress through activation of the hypothalamic-pituitary-adrenal (HPA) axis. Neurons in the paraventricular nucleus of the hypothalamus synthesize corticotropin-releasing factor (CRF) in response to stress. CRF then stimulates the synthesis and release of adrenocorticotropic hormone from the anterior pituitary, which then stimulates glucocorticoid synthesis and secretion from the adrenal cortex (for review see Smith, 2006). Glucocorticoids inhibit further HPA axis activation through feedback effects on the hypothalamus and pituitary (Keller-Wood and Dallman, 1984). The activity of the HPA axis is controlled by several brain pathways that mediate the stress response. In particular, the hippocampus, with its high concentration of glucocorticoid receptors, is an important site for glucocorticoid feedback inhibition of the HPA axis (Jacobson and Sapolsky, 1991). There is evidence of hyperactivity of the HPA

axis in MDD (Naughton et al., 2014). Less inhibitory control over the corticotrophin-releasing cells of the hypothalamus leads to an increase in the amount of circulating glucocorticoids. At high levels, glucocorticoids can have adverse effects on brain structure and may contribute to long-lasting cellular alterations in the hippocampus such as impaired neurogenesis and dendritic retraction (Campbell and MacQueen, 2004). The excitotoxic damage which follows prolonged exposure to glucocorticoids may be implicated in the hippocampal atrophy reported in depression (Sapolsky, 2000a; McEwen, 2005).

The stress-related effects described above are also thought to lead to reductions in neurotrophin levels, particularly brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain (Huang and Reichardt, 2001; Martinowich and Lu, 2008). The neurotrophin hypothesis of depression supposes that a decrease of BDNF plays a major role in the neurobiology of depression and that its restoration may represent a critical mechanism underlying antidepressant efficacy (Duman and Monteggia, 2006; Groves, 2007). Neurotrophins regulate neuronal formation and plasticity in the adult brain (Thoenen, 1995; Kafitz et al., 1999; Huang and Reichardt, 2001). BDNF is widely expressed in the hippocampus where it plays an important role in supporting the survival of existing neurons and encouraging the growth and differentiation of new neurons and synapses (Conner et al., 1997). Animal studies have shown that BDNF modulates hippocampal plasticity and hippocampal-dependent learning and memory (Lu and Gottschalk, 2000). Given its diverse roles, alteration in BDNF function may lead not only to defects in neuronal maintenance, survival and plasticity, but also to detrimental effects on hippocampal function and ultimately lead to the development of depressive symptoms

(Groves, 2007). In contrast, numerous studies have shown that chronic antidepressant treatment can increase BDNF expression and hippocampal neurogenesis suggesting a mechanism through which antidepressants may exert their therapeutic effects (Martinowich et al., 2007).

The monoamine hypothesis, which theorizes that underactivity of serotonin, norepinephrine and dopamine underlies the origin and maintenance of depressive symptoms, has dominated depression research for over fifty years (Hindmarch, 2002). Following the discovery that monoamine-targeting drugs alleviate depressive symptoms (Schildkraut, 1995; Slattery et al., 2004), all classes of medications that have been developed to treat depression target monoamine neurotransmission (Elhwuegi, 2004). Such treatments however remain suboptimal (Massart et al., 2012) and about two-thirds of patients with major depression fail to remit with a first standard treatment (Nemeroff, 1998). The primary limitation to the monoamine hypothesis is that it fails to account for any of the alternate mechanisms now believed to be associated with the etiology of depression. There are likely interactions between the various factors thought to be associated with the pathogenesis of depression, including those described herein (genetic vulnerability, HPA axis dysfunction, neurotrophins, and neurotransmitter abnormalities), and additional mechanisms not reviewed here (such as circadian system abnormalities and neurodegenerative and inflammatory factors) (McClung, 2013; Ogłodek et al., 2014).

1.1.1 Treatment of depression

Major depressive disorder is commonly treated using medication and/or psychotherapy. In terms of pharmacological treatment, as discussed above, the

monoaminergic system has been the major focus for antidepressant development and primary drug targets include monoamine transporters, receptors, and degradation enzymes. The oldest antidepressants are the monoamine oxidase inhibitors (MAOIs) and the tricyclic antidepressants. MAOIs work by inhibiting the enzymatic breakdown of norepinephrine, dopamine and serotonin increasing their availability. Tricyclic antidepressants increase the concentrations of norepinephrine and/or serotonin in the synapse by inhibiting the norepinephrine and serotonin transporters. Today, the first-line antidepressant drugs prescribed to MDD patients are the selective serotonin reuptake inhibitors (SSRIs), and selective serotonin norepinephrine reuptake inhibitors (SNRIs) that specifically target the serotonin and/or norepinephrine transporters. These medications have more focused effects on monoaminergic receptors and lack significant affinity for muscarinic and histamine receptors and therefore have fewer side effects compared to tricyclics. Despite their improved side effect profile however, these drugs have not been shown to have greater efficacy relative to older antidepressants (Millan, 2006).

Overall, most patients have relatively poor response and remission rates to SSRIs and SNRIs (Machado et al., 2006; Trivedi et al., 2006). Patients who have an inadequate response or no response to antidepressants given in monotherapy, can be treated using various combination or augmentation strategies. In the combination approach, the initial antidepressant is combined with another antidepressant, typically of a different class, which can improve remission rates in patients (Blier et al., 2009). An augmentation strategy enhances response to the primary antidepressant by augmenting its effects using other drugs with different mechanisms of action or targets (Fava, 2009). Commonly used

augmentation agents in depression include lithium, atypical antipsychotics, and triiodothyronine (T3), among others (Carvalho et al., 2007; Chang et al., 2013). In addition to these strategies using existing medications, new multi-target antidepressant drugs are currently in development (Connolly and Thase, 2012; O'Leary et al., 2014). Furthermore, in recent years, the anesthetic ketamine has been shown to be effective in the treatment of major depressive disorder (aan het Rot et al., 2012; Fond et al., 2014). In addition to these pharmacological treatments, there are various non-pharmacological, neuromodulatory strategies reserved for treating patients resistant to remission. These treatment options include electroconvulsive therapy (ECT), vagal nerve stimulation (VNS), and deep brain stimulation (DBS) (Kennedy and Giacobbe, 2007).

1.1.2 Treatment resistance

Only one third of depressed patients treated with an antidepressant at adequate dosage for a sufficient duration will achieve complete remission (Tranter et al., 2002). The remaining two thirds, left with no response or significant residual symptoms, may have some degree of treatment resistance. Treatment-resistant depression is commonly defined as failure to respond to several courses of optimally administered antidepressant treatments (often of different classes) for adequate durations (Fava, 2003). Although treatment-resistant patients represent only 15-30% of depressed patients, the costs associated with their treatment accounts for over half of the total treatment costs for MDD (Petersen et al., 2001). The most significant contribution to this increased burden is the recurrence of depressive episodes with increasing frequency and severity in resistant

patients (Greden, 2001). Longitudinal studies have shown that the longer patients remain depressed, the less likely they are to attain remission (Keller et al., 1992).

Unfortunately, it is difficult to determine in advance whether a patient will respond to pharmacotherapy. In fact, there does not seem to be any reliable clinical predictors that can accurately anticipate pharmacotherapy-resistance (Serretti et al., 2009). At present, the most reliable predictor of treatment-resistance in depression is a history of previous non-response (Mathew, 2008). At least some of the variability in response to treatment in major depressive disorder is thought to have a genetic basis (Serretti et al., 1998, 2005; Maier and Zobel, 2008; Kato and Serretti, 2010). Currently, there is an ongoing search for biomarkers for pharmacotherapy resistance involving genetic investigations and prospective neuroimaging (El-Hage et al., 2013; Wise et al., 2014).

1.2 Structural brain imaging in depression

Functional imaging data derived primarily from positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have identified several key brain regions thought to be involved in major depressive disorder. Mayberg (1997, 2003) proposed an integrative model of altered limbic-cortical dysfunction in depression that involves the hippocampus, lateral and medial prefrontal cortices, orbitofrontal cortex, subgenual and rostral anterior cingulate cortex, and thalamus (Seminowicz et al., 2004). Cross-sectional structural magnetic resonance imaging (MRI) studies have consistently shown regional brain volume reductions in patients with MDD compared to healthy individuals in many of these same regions. Meta-analytic techniques have been used to

identify the most common brain structures reported in such studies. They have primarily localized volumetric abnormalities in depression to frontal, limbic, and striatal areas. Koolschijn et al. (2009), Kempton et al. (2011), and Arnone et al. (2012) conducted meta-analyses of 64, 101, and 143 volumetric MRI studies respectively, and each reported significant volume reductions in patients with unipolar depression relative to controls in the prefrontal, orbitofrontal, and anterior cingulate cortices, hippocampus, putamen, and caudate nucleus.

Most of the articles included in the above-mentioned meta-analyses used region of interest (ROI) approaches that focused on particular brain regions using manual tracing techniques. Automated volumetric methods such as voxel-based morphometry (VBM) (Ashburner and Friston, 2000) allow regionally unbiased assessment of brain tissue composition across the entire brain without a priori selection of ROIs. Meta-analyses of VBM studies comparing MDD patients and healthy controls have identified gray matter (GM) reduction in the anterior cingulate cortex (ACC) as the most consistent finding. Lai (2013) conducted a meta-analysis on 20 VBM studies and found GM reductions among patients solely in the bilateral ACC, while Bora et al. (2012) reported GM reduction in the bilateral rostral ACC as the most replicable finding in their meta-analysis of 23 VBM studies. This second study also identified GM reductions in the dorsolateral and dorsomedial prefrontal cortices, the latter especially in patients who had experienced multiple depressive episodes (Bora et al., 2012). Two additional meta-analyses of 20 and 6 VBM studies respectively also reported reduced GM volume in cortical and limbic regions; specifically, the ACC, middle and inferior frontal gyri, hippocampus and

thalamus (Du et al., 2012), and the dorsomedial frontal cortex, paracingulate cortex, and amygdala (Sacher et al., 2012).

Recently, several studies have examined the thickness of the cerebral cortex in depression using vertex-based analyses (where cortical thickness values are compared at each vertex across the whole brain). These studies have largely reported reduced cortical thickness in frontal regions in patients compared to matched controls. Specifically, focal cortical thinning has been reported in the orbitofrontal cortex (Järnum et al., 2011; Tu et al., 2012; Grieve et al., 2013; Na et al., 2014), dorsolateral prefrontal cortex (van Tol et al., 2014), and rostral middle frontal gyrus (Tu et al., 2012; Na et al., 2014). While fewer affected brain regions have been reported in surface-based cortical thickness analyses in depression, the data mostly implicate the same frontal brain regions identified with volumetric approaches.

Diffusion tensor imaging (DTI) has been used to characterize white matter (WM) microstructural integrity in patients with MDD. Meta-analysis of 11 DTI studies reported decreased fractional anisotropy (a measure reflecting the structural integrity and organization of tissue) in the white matter bundles connecting cortical and limbic brain areas (Liao et al., 2013). Fang et al. (2012) using anatomical connectivity (tractography) and machine learning approaches found the most discriminating features to separate MDD patients from controls was abnormal WM tracts in cortical-limbic networks (especially the frontal-limbic network). Collectively, these structural imaging studies provide evidence of gray and white matter changes in cortico-limbic brain regions in patients with MDD. Accordingly, the following structures were chosen for inclusion in region of interest analyses within this thesis: the hippocampus, dorsolateral prefrontal

cortex, anterior cingulate cortex, and orbitofrontal cortex. These brain regions and their purported role in MDD are further reviewed below.

1.2.1 Review of key areas affected in depression

1.2.1.1 The role of the hippocampus in depression. Through connections with diverse cortical areas including the prefrontal cortex, anterior thalamic nuclei, amygdala, basal ganglia and hypothalamus, the hippocampus forms part of an anatomical network involved in mood regulation (Soares and Mann, 1997). The hippocampus also plays a role in the control of learning and memory (Fanselow, 2000; Squire et al., 2004) and the regulation of the HPA axis (Wingenfeld and Wolf, 2014), both of which are altered in depression (Shah et al., 1998; Hickie et al., 2005; Swaab et al., 2005).

The hippocampus is a highly plastic structure that is sensitive to stress and vulnerable to atrophic changes (Campbell and MacQueen, 2004). In fact, acute, chronic stress in humans can result in atrophy of the hippocampal formation (Duman, 2004). Reduced volume of the hippocampus is the most replicated finding in the structural imaging literature in depression (Campbell et al., 2004; Videbech and Ravnkilde, 2004; Koolschijn et al., 2009; McKinnon et al., 2009; Arnone et al., 2012). Meta-analyses have estimated the magnitude of bilateral hippocampal atrophy to be 8-15% in MDD patients compared to healthy individuals (Campbell et al., 2004; Videbech and Ravnkilde, 2004). The hippocampus is rich in glucocorticoid receptors and elevated glucocorticoid levels are often seen in severely depressed patients (Checkley, 1996). The excitotoxic damage that follows prolonged exposure to glucocorticoids is thought to result in long-lasting cellular alterations in the hippocampus that may affect its structure (Campbell and

MacQueen, 2004). Such effects include cell death, dendritic shrinkage, decreased levels of neurotrophins within the hippocampus and decreased neurogenesis (Santarelli et al., 2003). Direct evidence of a relationship between increased glucocorticoids and decreased hippocampal volume has been clearly shown in rats (Watanabe et al., 1992) and nonhuman primates (Sapolsky et al., 1990). Chronic antidepressant treatment affects hippocampal plasticity (D'Sa and Duman, 2002) and studies have shown that decreased neurogenesis caused by stress exposure can be reversed by almost all antidepressant treatments (Malberg et al., 2000). This reversal can, in some cases, halt or reverse hippocampal atrophy in animals (Alonso et al., 2004; Fuchs et al., 2004). Regardless of their underlying mechanisms, the structural modifications observed in the hippocampus in depression may have implications for patients' clinical course.

1.2.1.2 The role of cortical regions in depression. Functional and structural imaging suggests abnormalities of the cortical gray matter in major depression. MRI studies in patients with MDD have shown loss of GM volume across a range of cortical brain regions, including the lateral and medial prefrontal cortices, the anterior cingulate cortex and orbitofrontal cortex (Ballmaier et al., 2004; Grieve et al., 2013). A recent study evaluated the magnitude of GM volume reductions in MDD patients relative to controls in frontal regions and reported 27% decrease in volume in the dorsolateral prefrontal cortex (DLPFC), 10% decrease in the medial prefrontal cortex, and 11% decrease in the ACC (Grieve et al., 2013). Findings of reduced volume in imaging reports are supported by evidence from postmortem studies which suggest neuronal and glial cell loss, reduced

neuronal size, and cortical thickness in the DLPFC, orbitofrontal cortex, and ACC (Ongür et al., 1998; Rajkowska et al., 1999; Cotter et al., 2001, 2002).

These frontal brain regions are involved in emotional modulation through the inhibition of limbic activity (Hariri et al., 2000). Patients with MDD have been shown to have abnormal responses in fMRI studies in frontal regions, including hypoactiviation of the DLPFC during working memory tasks (Fitzgerald et al., 2006; Korgaonkar et al., 2013). A meta-analysis of fMRI studies showed that abnormalities in the lateral frontal regions in particular appear to be related to the depressed state, as activity in these areas in depressed patients tends to normalize with treatment (Graham et al., 2013).

There is also of evidence of functional abnormality and volume reduction in the ACC in depression. An early PET study of the ACC demonstrated increased resting blood flow in the prefrontal cortex of currently depressed patients but not recovered patients, suggesting an association between activity in this region and disease state (Drevets et al., 1992). Mayberg et al. (2005) further localized this functionally abnormal area to the subgenual ACC (Brodmann area 25). There have since been consistent reports of increased resting state activity in this region during depressive episodes and reduced activity following antidepressant treatment (Seminowicz et al., 2004). This finding is the basis for the use of the subgenual ACC as a target for deep brain stimulation in treatment-resistant patients (Mayberg et al., 2005). Furthermore, smaller GM volume in the ACC has been reported specifically in treatment-resistant patient populations (Machino et al., 2014).

A recent whole-brain fMRI and VBM study found functional and structural abnormalities in the subgenual ACC in MDD patients (Rodríguez-Cano et al., 2014).

Findings included reduced volume of the subgenual ACC and lack of deactivation in this region during cognitive task performance (Rodríguez-Cano et al., 2014). This latter finding supports involvement of the subgenual ACC in the dysfunction of the default mode network in depression. Reduced volume of the ACC has been reported in meta-analyses of studies using both region of interest (Hajek et al., 2008; Arnone et al., 2012), and VBM approaches (Bora et al., 2012; Du et al., 2012). A recent large imaging study comparing MDD patients to controls found reduced volume of the ACC among patients yet failed to find between-group differences in ACC cortical thickness (Grieve et al., 2013).

Research has demonstrated reduced volume and abnormal glucose metabolism and cerebral blood flow in the orbitofrontal cortex in MDD patients relative to controls (Drevets, 2007). Along with the DLPFC, fMRI data confirms a role for the orbitofrontal cortex in the regulation and processing of emotion (Golkar et al., 2012). Like the other cortical regions discussed above, meta-analyses have identified the orbitofrontal cortex as an area of gray matter reduction in patients with MDD (Koolschijn et al., 2009; Kempton et al., 2011; Arnone et al., 2012; Bora et al., 2012). Given its distinct connections with the limbic system, including the ACC (Ongür and Price, 2000), volume changes in both the orbitofrontal cortex and ACC suggest abnormality of the cortico-limbic network in major depression (Grieve et al., 2013).

1.2.2 Association between brain structure and clinical features

There is clear evidence of structural differences in patients with MDD relative to healthy controls. What remains to be discussed is whether these structural abnormalities

precede or follow the onset of depression. In other words, do the volume reductions in patients confer vulnerability for depression, or does the observed atrophy result from ongoing depressive processes? In fact, there is consistent support for larger regional brain volume reductions among patients with multiple previous depressive episodes, longer illness durations, and treatment nonresponse.

Volume reductions in the hippocampus, basal ganglia, orbitofrontal cortex, and ACC appear to be more pronounced in MDD patients with persistent forms of illness (i.e. those with multiple episodes, repeated relapses, and longer illness durations) (Lorenzetti et al., 2009). A meta-analysis of VBM studies revealed greater GM volume decrease in the rostral ACC and dorsomedial frontal cortex in MDD patients in association with longer duration of depression (Bora et al., 2012). Yucel et al. (2008) reported reduced volume of the subgenual ACC only in MDD patients with 3 or more episodes of untreated depression. Treadway et al. (2014) reported cortical thinning of the medial prefrontal cortex and reduced volume of dentate gyrus in association the number of prior depressive episodes in a large sample of medication-free patients versus healthy controls. Similarly, meta-analyses have showed smaller hippocampal volume among patients with longer illness durations (McKinnon et al., 2009; Kempton et al., 2011), and multiple depressive episodes (Videbech and Ravnkilde, 2004). The results of these latter imaging studies are supported by postmortem findings of decreased total volume of the hippocampal formation with increasing duration of illness in patients with recurrent or chronic MDD (Cobb et al., 2013). Despite these findings however, a meta-analysis of 7 MRI studies also reported decreased hippocampal volume in first-episode MDD patients compared to controls (Cole et al., 2011a). This finding lends support to the hypothesis

that smaller hippocampal volumes may increase vulnerability for depression but does not negate the convincing evidence of a mediating effect of illness burden on regional brain volume changes in patients.

Inadequate pharmacological response can lead to poorer outcome for patients, less likelihood of remission, and greater relapse risk (Judd et al., 1998; Fava, 2003). As reviewed above, continuing depression is associated with progressive atrophic changes yet it is not clear to what extent brain structure at treatment initiation predicts a patients' response to treatment. Frodl and colleagues (2004, 2008a) reported that MDD patients with smaller hippocampal volumes at hospitalization experienced a more chronic course of depression and suffered more relapses than those with larger hippocampi over one- and three-year follow-up periods. Such findings of smaller hippocampal volume predicting poorer response to treatment have since been replicated (Kronmüller et al., 2008; MacQueen et al., 2008; Hoogenboom et al., 2013). Pretreatment hippocampal volume has also been associated with speed of treatment response in patients (Soriano-Mas et al., 2011; Sheline et al., 2012).

In terms of cortical brain regions, smaller volumes of the rostral middle frontal gyrus (Hoogenboom et al., 2013), subcallosal/orbitofrontal cortex (Sämann et al., 2013), and thinner posterior cingulate gyrus (Järnum et al., 2011) have been associated with poorer outcome among MDD patients. Functional imaging studies have identified the importance of pretreatment rostral ACC activity in predicting antidepressant response (reviewed in Pizzagalli, 2011). Consistent with these findings, structural studies have revealed better response in patients with increased cortical depth in the subgenual ACC (Coryell et al., 2005), greater GM volume in the right rostral ACC (Costafreda et al.,

2009), and increased WM connectivity between the subgenual ACC and limbic regions (Korgaonkar et al., 2014). Greater gray matter volume in the ACC has also been associated with a faster rate of treatment response (symptom improvement) in patients (Chen et al., 2007).

Evidence suggests an accumulation of atrophic changes over time in untreated or nonresponsive patients. Reduced brain volume in such patients at treatment initiation may impede treatment response which in turn may lead to further brain volume loss. This apparent cycle of treatment nonresponse and atrophic changes may need to be targeted in order to improve outcomes in depression. The relationships between brain structure and illness burden suggest that volumetric changes may be state-related and perhaps amenable to treatment. Longitudinal imaging studies are required to determine how structural modifications change over time and how they are affected by treatment response and remission.

1.2.3 Longitudinal changes in brain structure

Cross-sectional studies in major depressive disorder have revealed brain structure differences in patients according to current disease state. Larger prefrontal GM volume has been reported in remitted versus currently depressed patients (Yucel et al., 2009; Li et al., 2010). Further, a meta-regression of cross-sectional studies reported larger hippocampal volumes in remitted patients compared to nonremitted patients (Kempton et al., 2011). It is possible that, as has been shown with treatment response, the brain structure of patients who go on to achieve remission differs at treatment initiation compared to nonremitters. Alternatively, the effects of antidepressant treatment or the

alleviation of depressive symptoms through remission may lead to reversal of atrophic changes in remitted patients. Investigation of these possibilities requires prospective follow-up imaging with longitudinal studies.

There have been relatively few longitudinal imaging studies conducted in major depression to date and several of the recently published reports have examined adolescent (Whittle et al., 2014), and geriatric patient populations (Hou et al., 2012; Zannas et al., 2013; Elbejjani et al., 2014). Frodl et al. (2008b) in a prospective longitudinal VBM study revealed a depression-specific pattern of GM volume decline in patients relative to healthy individuals in several of the same areas identified as affected in cross-sectional imaging studies of depression. Namely, patients showed more GM loss over 3-year follow-up compared to controls in the hippocampus, ACC, and prefrontal cortex. The authors also reported differences in patients who achieved remission over follow-up compared to those who failed to remit, with remitted patients showing less GM volume decline over time in the abovementioned regions (Frodl et al., 2008b). These findings suggest that gray matter changes during depressive episodes are progressive yet their severity differs according to patients' clinical course.

There have been numerous findings of increased brain volume or cortical thickness in individuals treated with various pharmacotherapies. Increases have been reported for whole brain volume, as well as cortical, and hippocampal regions. Examples of GM changes with treatment include a 4% total GM volume increase in lithium-treated bipolar patients (Moore et al., 2000; Sassi et al., 2002), and a 3% increase in atypical antipsychotic-treated schizophrenia patients (Garver et al., 2005). Järnum et al. (2011) found an increase in orbitofrontal cortical thickness over 6-month follow-up in

antidepressant-treated patients with MDD. The fact that their sample contained both remitters and nonremitters suggests that these specific changes may be driven by antidepressant therapy rather than clinical outcome.

Hippocampal volume increase following antidepressant treatment has also been reported. Bremner et al. (1995) first described a 4.6% increase in hippocampal volume in paroxetine-treated patients with post-traumatic stress disorder. In major depression, there have been a number of studies that have found hippocampal volume increase following relatively short-term SSRI treatment (Malykhin et al., 2010; Schermuly et al., 2011; Arnone et al., 2013), however, other studies have failed to find changes in hippocampal volume after treatment durations of 7 months (Vythilingham et al., 2004) and 1 year (Frodl et al., 2004). The patient sample from the latter study was followed for an additional 2 years and a modest increase in hippocampal volume was detected in the subgroup of patients who took antidepressants continuously during the entire 3-year follow-up period (Frodl et al., 2008a). Finally, Ahdidan et al. (2011) reported hippocampal volume differences between a group of MDD patient and controls at baseline imaging but after an 11-year follow-up period, the patient sample (all in remission) no longer differed from the control group indicating that volume abnormalities had normalized.

Antidepressants are thought to exert their effects on brain volume through neurotrophic and neuroprotective actions (Hunsberger et al., 2009). The biological mechanisms that underlie brain volume changes during pharmacotherapy however remain unclear. Antidepressant treatment results in increased numbers of adult-born neurons in the dentate gyrus (part of the hippocampus). Studies in rats and nonhuman

primates support a role for antidepressants in affecting neurogenesis and driving volume increase in the hippocampus (Hajszan et al., 2005; Wu et al., 2014). There have been reports from human postmortem studies of increased dentate gyrus granule cell and glial cell numbers (Cobb et al., 2013), and increased dentate gyrus neural progenitor cell numbers (Boldrini et al., 2009, 2012) in hippocampal sections from antidepressant-treated MDD subjects. A recent imaging study that specifically investigated volume changes in hippocampal subfields, found larger dentate gyrus volume in antidepressant-treated MDD patients compared to unmedicated patients (Huang et al., 2013).

The preceding literature review demonstrates that brain atrophy in major depression is progressive with continuing illness, yet it may be reversible through antidepressant treatment. A number of reports of regional brain volume increases only in remitted patients suggest a mediating role for clinical outcome on longitudinal volume changes. It is not known whether pharmacotherapy-driven brain volume recovery is possible even in patients who fail to respond to the treatment. Addressing this question is one of the major goals of this thesis.

1.3 Imaging genetics in depression

Genetic susceptibility plays an important role in the development of major depressive disorder (Harvey et al., 2007). However, the genetic associations in complex and heterogeneous disorders such as depression are typically modest, difficult to replicate, and often associated with subtle phenotypic correlates (Goltser-Dubner et al., 2010; Flint and Kendler, 2014). A specific gene may be implicated as a candidate for genetic association with depression through knowledge of the gene's functional impact

on some aspect of the disorder's pathophysiology. The search for candidate genes in depression has mainly included genes involved in monoaminergic neurotransmission and neurotrophic processes (Levinson, 2006; López-León et al., 2008). The candidate gene approach involves comparison of allele or genotype frequencies of nucleotide sequence variations in potential candidate genes in patients with depression versus healthy individuals (case-control association). This approach requires homogeneous samples and large study populations to provide sufficient statistical power to detect association effects (Lohmueller et al., 2003). The inherent heterogeneity and complexity of major depression has led to the search for genetic association between proposed candidate genes and intermediate phenotypes (Gottesman and Gould, 2003) such as antidepressant responsiveness and functional or structural imaging correlates.

Imaging genetics describes how individual genetic variation influences brain structure or function. It involves examination of the effect of common variations in a gene sequence that impact gene function (i.e. polymorphisms) on selected imaging phenotypes. Such an approach is coveted as chemical, functional or structural characteristics of the brain may more closely reflect the underlying mechanistic consequences of a polymorphism compared to examining associations between gene variants and broad clinical symptom clusters or diagnostic categories (Meyer-Lindenberg and Weinberger, 2006). Key imaging phenotypes commonly investigated in depression include receptor binding (especially for pharmacological-related polymorphisms), gray matter volume and density measurements (particularly in cortico-limbic regions such as the hippocampus, amygdala, and anterior cingulate cortex), functional MRI measures (such as amygdala reactivity to emotional stimuli), and functional and structural

connectivity (Glahn et al., 2007; Frodl et al., 2008c). Imaging genetics studies in depression require the selection of a candidate gene that may be associated both with the disorder and with the structural or functional alteration under investigation.

1.3.1 Selection of candidate genes for inclusion in thesis

In the search for candidate genes in depression, the most promising findings have come from genes in monoaminergic systems, genes involved in the synthesis, transport, signal transduction and degradation of the monoamine neurotransmitters (serotonin, norepinephrine and dopamine). All known classes of antidepressant drugs target the monoamine system (Mann, 2005). Not surprisingly, several single nucleotide polymorphisms (SNPs) have been identified in genes encoding monoaminergic transporters, receptors and enzymes that appear to be associated with both major depression and antidepressant responsiveness. Genes were selected for inclusion in the present study based on their involvement in the mechanism of action of antidepressant drugs and previous evidence of their potential association with treatment response in MDD. Among these were genes encoding monoamine transporters: *SLC6A4* (serotonin transporter), *SLC6A2* (norepinephrine transporter); serotonin receptors: *HTR1A*, and *HTR2A* (serotonin 1A and 2A receptors, respectively); and a monoamine metabolic enzyme: *COMT* (catechol-O-methyltransferase). In addition to these monoamine-related genes, a neurotrophic gene was also examined in the thesis: *BDNF* (brain-derived neurotrophic factor).

Despite evidence of an immediate presynaptic response in serotonergic neurons to acute antidepressant treatment, the 2-3 week therapeutic latency of monoamine-

potentiating antidepressants suggests that monoamine deficits alone do not fully explain the pathophysiology of MDD (Duman et al., 1997). Increasingly, research has begun to focus on the role of growth factors. As previously described, BDNF has been implicated in both the origin and course of depression as well as in the therapeutic mechanisms that underlie its treatment (Duman and Monteggia, 2006; Groves, 2007).

1.3.2 Selection of hippocampal volume as imaging phenotype

Structural neuroimaging measures have the potential to be used as intermediate phenotypes as they provide quantitative, replicable data that may be sensitive to the subtle brain changes that occur in psychiatric conditions. An appropriate imaging phenotype is one which is consistently reported, highly heritable, and thought to be related to the proposed genes under investigation (Gottesman and Gould, 2003; Glahn et al., 2007; Winkler et al., 2010). In this thesis, hippocampal volume was the selected phenotype examined for the imaging genetics analyses. As reviewed in the preceding section, a vast number of volume-based imaging studies have found widespread anatomical changes throughout the brain in depression. One of the most consistent findings is smaller volume of the hippocampus among patients. Despite overall findings of hippocampal volume reduction in MDD patients by meta-analyses (Campbell et al., 2004; Videbech and Ravnkilde, 2004), when all studies comparing patients and controls are considered, about half have failed to find differences in hippocampal volume (Frodl et al., 2008c). This inconsistency may be due in part to the heterogeneity of the patient samples studied since various clinical factors have been shown to affect hippocampal volume (McKinnon et al., 2009). Equally likely however, this discrepancy could result

from genetic variation as studies suggest that genetic factors may modulate stress-related changes in hippocampal volume (Frodl et al., 2008c).

Several studies have reported that genetic factors account for 50-80% of the variance in the volume of the subcortical structures in the brain (Wallace et al., 2006; Kremen et al., 2010; den Braber et al., 2013). Hippocampal volume itself has been shown to be heritable and to have significant region-specific genetic contributions (Rentería et al., 2014). These factors suggest that hippocampal volume is a suitable target to study genetic effects on brain morphology. Furthermore, Winkler et al. (2010) showed that volume-based imaging techniques are appropriate for imaging genetic studies which examine subcortical regions.

1.3.3 Review of selected single nucleotide polymorphisms

The imaging genetic analyses included in this thesis examine the effects of single nucleotide polymorphisms (SNPs) in the following candidate genes: *SLC6A4*, *SLC6A2*, *HTR1A, HTR2A*, *COMT*, and *BDNF*. For each SNP, there has been published evidence of its association with major depression, data to suggest potential association of its risk allele with treatment response, and in some cases, evidence of an effect of the SNP on hippocampal volume. The selected polymorphisms are described in detail in the following sections of this dissertation.

1.3.3.1 Serotonin transporter polymorphism. The main mechanism of action of some of the most commonly used antidepressants, particularly SSRIs, is to block the reuptake of serotonin (5-hydroxytryptamine or 5-HT) at the transmembrane protein called the

serotonin transporter (5-HTT) (Blakely et al., 1994; Tsai and Hong, 2003). A functional polymorphism in the gene encoding 5-HTT (*SLC6A4*), the serotonin transporter-linked polymorphic region (5-HTTLPR, rs25531), involves the insertion (long (L) variant) or deletion (short (S) variant) of 44 base pairs in the promoter region (Collier et al., 1996; Heils et al., 1996). The S allele is associated with reduced transcriptional activity of the 5-HTT promoter, diminished serotonin activity, and has half the 5-HTT expression in the basal state compared to the L allele (Lesch et al., 1996). Two forms of the long variant have been discovered, for the A>G SNP within the long allele, A-allele carriers (L_A) have high mRNA levels, while G-allele carriers (L_G) have low mRNA levels and are functionally similar to carriers of the low-expressing S allele (Hu et al., 2005).

The short variant of 5-HTTLPR has been shown to confer vulnerability to the development of depression especially in the presence of stressful life events (Karg et al., 2011; Sharpley et al., 2014). 5-HTTLPR is also thought to affect treatment response in MDD. To date several meta-analyses have investigated the effect of the low-expressing S allele on treatment response and remission. Consistent with the S and L_G allele inhibition of the binding of antidepressants to the transporter, meta-analyses have reported poorer and more delayed response to SSRIs among MDD patients homozygous for the 5-HTTLPR S allele (Smits et al., 2004; Serretti et al., 2007), and better response and remission rates in association with the L allele (Porcelli et al., 2012). These findings have been particularly consistent in studies with Caucasian samples, and lack of consideration of sample ethnicity has been proposed by some (Crisafulli et al., 2011; McGuffin et al., 2011) as a means of explaining the negative results of a forth meta-analysis (Taylor et al., 2010).

There is growing evidence of an effect of the 5-HTTLPR on hippocampal volume, although there have been some negative findings (Hickie et al., 2007; Cole et al., 2011b). Recent studies argue against a main effect of the polymorphism on the volume of the hippocampus and instead suggest that the relationship between hippocampal volume reduction and the 5-HTTLPR S allele may be mediated by gender, and/or exposure to stress or depression. Price et al. (2013) found the S allele associated with smaller hippocampal volume and more depressive symptoms only in healthy male participants, the same relationship was absent in females. In contrast, Everaerd et al. (2012) reported decreased hippocampal volume among healthy female S allele carriers; while in males, the S allele was associated with smaller hippocampal volumes only if the participants also had a history of childhood adversity. The moderating role of stress is further supported by the findings of smaller hippocampal volumes associated with the S allele and higher waking cortisol levels in healthy older adults (O'Hara et al., 2007).

In major depressive disorder, several studies have reported smaller hippocampal volumes in patients homozygous for the 5-HTTLPR S allele relative to healthy controls with the same genotype (Taylor et al., 2005; Frodl et al., 2010; Eker et al., 2011). Taylor et al. (2005) reported this finding in elderly patients with an early age of onset (< 50 years), while an association between the L/L genotype and smaller hippocampal volumes was found in patients with late-onset geriatric depression, suggesting that age of onset may affect the 5-HTTLPR genotype-hippocampal volume relationship. Frodl et al. (2010) found evidence that patients with the S allele and a history of childhood neglect had smaller hippocampal volumes than patients with only one risk factor (genetic or environmental). Finally, Eker et al. (2011) reported a significant 5-HTTLPR genotype by

diagnosis interaction, with smaller hippocampal volumes in MDD patients homozygous for the S allele in both hemispheres.

1.3.3.2 Norepinephrine transporter polymorphism. The norepinephrine transporter (NET) is responsible for reuptake of NE into the presynaptic neuron. NET is the target of tricyclic antidepressants (including desipramine and imipramine), norepinephrine reuptake inhibitors (NRIs), and serotonin/norepinephrine reuptake inhibitors (SNRIs). The expression of *SLC6A2* (the gene that encodes the NET) is altered in patients with MDD (Klimek et al., 1997). A polymorphism has been discovered in the promoter region of the gene, NET -182T/C (rs2242446), and although its functional consequences are unknown, there have been some reports of an association between this SNP and depression and antidepressant response. NET -182T/C has been associated with MDD in several studies with Asian samples although identification of the risk allele has varied. Inoue et al. (2004) found C(-182) homozygotes had less susceptibility to develop MDD relative to T(-182) allele carriers. In contrast, Ryu et al. (2004) and Sun et al. (2008) reported that the C(-182) allele was more common among patients relative to controls. Further, no association was found between NET -182T/C and MDD in 2 other studies (Owen et al., 1999; Zill et al., 2002), and a recent meta-analysis failed to confirm association between the SNP and depression (Zhou et al., 2014). In terms of the effects of the polymorphism on treatment response in MDD, the T(-182) allele is associated with better response to milnacipram (an SNRI) compared to the C(-182) allele (Yoshida et al., 2004). Associations between NET polymorphisms and antidepressant responsiveness

have not been widely replicated (Min et al., 2009). No association between NET -182T/C and hippocampal volume has been reported in the literature.

1.3.3.3 Serotonin 1A receptor polymorphism. The serotonin 1A receptor (5-HT_{1A}) acts as an autoreceptor and its activation inhibits serotonin neuron firing and diminishes serotonin release (Dubovsky and Thomas, 1995; Kapur and Remington, 1996). Antidepressant compounds that work on 5-HT_{1A} desensitize raphe 5-HT_{1A} autoreceptors, which results in increased 5-HT neurotransmission. There are also 5-HT_{1A} heteroreceptors on post-synaptic neurons that mediate 5-HT actions on target neurons. 5-HT_{1A} -1019C/G (rs6295), a SNP in the promoter region of *HTR1A* (the gene encoding the 5-HT_{1A} receptor), has been found to be associated with altered expression and function of *HTR1A* (Lemonde et al., 2003; Albert and Lemonde, 2004). 5-HT_{1A} -1019C/G is associated with MDD and suicide (Lemonde et al., 2003; Le François et al., 2008). The G(-1019) allele fails to bind repressors Deaf1, Hes 1 and Hes 5, and leads to upregulation of 5-HT_{1A} receptor expression (Lemonde et al., 2003; Albert and Lemonde, 2004). Further, the G(-1019) allele results in an impaired repression of the 5-HT_{1A} receptor leading to elevated inhibition of basal raphe neuronal activity (Lemonde et al., 2003) and may increase the number of inhibitory 5-HT_{1A} autoreceptors (Porcelli et al., 2011). These effects may have implications for treatment response, as there is some evidence of an association between 5-HT_{1A} -1019C/G and antidepressant responsiveness in MDD patients. Caucasian patients who are G(-1019) allele carriers have been shown to have poorer response to SSRIs (Lemonde et al., 2004; Serretti et al., 2004; Parsey et al., 2006) and the 5-HT_{1A} agonist/5-HT_{2A} antagonist flibanserin (Lemonde et al., 2004). In contrast,

in Asian populations, better treatment response has been reported in G(-1019) homozygotes relative to C(-1019) allele carriers (Hong et al., 2006; Yu et al., 2006; Kato et al., 2009). Despite these reported associations, there have also been several studies that failed to find an effect of 5-HT_{1A} -1019C/G on antidepressant responsiveness (Lemonde et al., 2004; Peters et al., 2004; Serretti et al., 2004; Arias et al., 2005; Levin et al., 2007; Noro et al., 2010). In meta-analysis, the effects of 5-HT_{1A} -1019C/G on treatment response from all published studies were negative, as was a subanalysis of studies with Caucasian samples (Kato and Serretti, 2010). However, subanalysis in Asian populations showed 5-HT_{1A} -1019C/G to be a potential predictor of antidepressant response, with G(-1019) homozygotes showing better response relative to C(-1019) allele carriers (Kato and Serretti, 2010). No associations between 5-HT_{1A} -1019C/G and hippocampal volume have been reported.

1.3.3.4 Serotonin 2A receptor polymorphism. The 5-HT_{2A} -102T/C SNP (rs6313) has been shown to alter receptor expression with low expression of the C(-102) allele relative to the T(-102) allele (Polesskaya et al., 2006). 5-HT_{2A} -102T/C has been associated with MDD risk (Zhang et al., 1997; Kishi et al., 2010), with the C(-102) allele present in higher frequencies in MDD patients relative to bipolar patients and healthy controls (Zhang et al., 1997), and in patients with suicidal ideation (Du et al., 2000). Several studies have shown that the 5-HT_{2A} -102T/C SNP is associated with antidepressant responsiveness in patients. Poorer response has been reported in T(-703) homozygotes relative to C(-703) allele carriers (Minov et al., 2001, Cusin et al., 2002). Meta-analysis of the effects of 5-HT_{2A} -102T/C on treatment response from seven published studies was

nonsignificant (Kato and Serretti, 2010); however, in subanalysis of studies of 5-HT_{2A} -102T/C with Asian subjects, C(-102) homozygotes showed favourable SSRI response compared to T(-102) allele carriers (Kato and Serretti, 2010). The relationship between 5-HT_{2A} -102T/C and hippocampal volume has not been investigated.

1.3.3.5 Catechol-O-methyltransferase. Aside from the serotonin system, noradrenergic and dopaminergic systems also have a significant impact on the pathogenesis of major depressive disorder and are involved in the treatment of the condition through interaction with serotonergic pathways (Guiard et al., 2008). Catechol-O-methyltransferase (COMT) is a methylation enzyme that degrades catecholamines, particularly dopamine (DA) (Bertocci et al., 1991; Grossman et al., 1992). A functional polymorphism in the *COMT* gene (COMT Val158Met, rs4680) has been described in which a G > A transition at codon 158 results in a valine (Val) to methionine (Met) amino acid substitution (Lachman et al., 1996). The Val allele in this polymorphism is 4 times more active in metabolizing dopamine than the Met allele (Weinberger et al., 2001; Chen et al., 2004). As a result, the Met allele is associated with an increased concentration of DA, thought to have adverse consequences in MDD and to delay antidepressant response (Arias et al., 2006). Associations have been found between major depression and both the COMT Met allele (Ohara et al., 1998) and the Val allele (Massat et al., 2005). A meta-analysis also suggests association between COMT Val158Met and higher risk for suicidality (Kia-Keating et al., 2007).

There is some evidence of an effect of COMT Val158Met on antidepressant responsiveness. The Met allele has been associated with poorer and more delayed

response to citalopram (Arias et al., 2006) and mirtazapine (Szegedi et al., 2005); yet more recent studies have found poorer antidepressant response among Val/Val homozygotes (Baune et al., 2008; Tsai et al., 2009; Benedetti et al., 2010).

COMT is widely expressed in the hippocampus and prefrontal cortex (Matsumoto et al., 2003). Imaging studies have shown a relationship between the COMT Val158Met polymorphism and variation in hippocampal function, namely in emotional processing (Drabant et al., 2006; Heinz and Smolka, 2006) and episodic memory (Bertolino et al., 2006). To date there have also been several structural imaging studies investigating the relationship between COMT Val158Met and hippocampal volume. Among healthy individuals, there have been reports of decreased bilateral volume of the hippocampus in Val/Val homozygotes (Taylor et al., 2007; Cerasa et al., 2008), and Val-allele carriers (Honea et al., 2009). Further, Ehrlich et al. (2010) found increased hippocampal volume with each copy of the COMT-Met allele in a sample containing healthy individuals and patients with schizophrenia. Contrasting these findings, Wang et al. (2013) reported larger hippocampal volume associated with the Val allele in a sample of healthy Asian participants, suggesting population-specific genetic effects. Still other studies have failed to find any effect of COMT Val158Met on hippocampal structure (Zinkstok et al., 2006; Dutt et al., 2009).

Recent research suggests a lack of main effect of the COMT polymorphism but instead gene by gene and gene by environment interaction effects of the SNP on hippocampal volume. Radua et al. (2014), found a COMT Val158Met by 5-HTTLPR interaction effect where healthy participants who either carried the COMT Met allele and 5-HTTLPR S allele or were both COMT Val and 5-HTTLPR L homozygotes had smaller

hippocampal volumes relative to individuals with other combinations of alleles. Rabl et al. (2014), reported smaller hippocampal volume among healthy individuals with the COMT Met allele and exposure to life stress.

1.3.3.6 Brain-derived neurotrophic factor polymorphism. Brain-derived neurotrophic factor (BDNF) is an important neurotrophin that facilitates neuronal plasticity and promotes the growth and survival of neuronal populations (Huang and Reichardt, 2001). A SNP in the 5' prodomain region of the BDNF gene produces a valine to methionine substitution in codon 66. The BDNF Val66Met polymorphism (rs6265) interferes with the activity-dependent secretion of BDNF (Egan et al., 2003) and is implicated in increasing susceptibility for the development of MDD (Schumacher et al., 2005). Several studies have shown an association between BDNF Val66Met and major depressive disorder (Castrén, 2004). Meta-analyses suggest that while BDNF Val66Met genotype does not exert a major influence on the development of the disorder (Chen et al., 2008), the Met allele appears to moderate the relationship between stress and depression (Hosang et al., 2014). There is also evidence of an association between BDNF Val66Met and pharmacological response in patients. An early meta-analysis of 4 studies found more favourable response to treatment among Met allele carriers (Kato and Serretti, 2010). An updated meta-analysis (Niitsu et al., 2013) found better antidepressant response in patients with the heterozygous Val/Met genotype compared to those homozygous for either the Met or Val alleles.

The BDNF Val66Met polymorphism is of particular interest in the present study due to its apparent effect on hippocampal structure. Meta-analysis has confirmed bilateral

hippocampal reductions in healthy BDNF Val66Met Met allele carriers relative to Val/Val homozygotes (Hajek et al., 2012). Further studies not included in the aforementioned meta-analysis have reported similar findings including reduced hippocampal gray matter volume in healthy Met allele carriers with high trait depression (Joffe et al., 2009), and smaller total hippocampal volume in both healthy and bipolar disorder Met allele carriers (Chepenik et al., 2009). Smaller hippocampal volumes have also been shown in Met allele carriers from samples of patients with MDD (Frodl et al., 2007), and melancholic depression (Cardoner et al., 2013). Despite this evidence there have been additional published reports that failed to find associations between BDNF Val66Met and hippocampal volume (Benjamin et al 2010; Karnik et al., 2010). Similar to findings concerning the effects of the 5-HTTLPR S allele, the BDNF Met allele effects on hippocampal volume have been suggested to be more pronounced in individuals exposed to early life stress (Gatt et al., 2009; Carballedo et al., 2013).

1.4 Study rationale, research objectives and hypotheses

The preceding review presented extensive evidence of morphological changes in the brain in patients with major depressive disorder. These atrophic changes have been associated with clinical course of depression, with increased brain volume reductions reported in patients with longer illness durations, more depressive episodes, and poorer treatment responsiveness. Patients with treatment-resistant depression experience continued exposure to the stress-related mechanisms that may underlie the structural modifications seen in the disorder. Brain atrophy in depression is progressive with continuing illness but it may be reversible with antidepressant treatment. What remains

unclear is whether brain volume can be positively affected by pharmacological intervention even if patients fail to respond clinically to the treatment. The primary aim of this thesis was to explore longitudinal changes in brain structure in patients with treatment-resistant major depressive disorder according to their response to intensive pharmacotherapy.

The most consistent structural modification reported in depression is reduced volume of the hippocampus. Genetic factors are thought to underlie this atrophy. There is some evidence that candidate gene risk alleles associated with poorer antidepressant response in depression also confer greater risk of hippocampal volume reduction. A secondary aim of this thesis was to investigate the effects of monoaminergic-related gene variants on hippocampal volume in the patient sample and a matched healthy control group at baseline imaging.

1.4.1 Statement of research objectives

Research objective 1: To examine the roles of clinical responsiveness and antidepressant treatment in lessening whole-brain atrophy in treatment-resistant depression (Chapter 2).
Hypothesis: It was hypothesized that brain volume would be positively affected by pharmacological intervention only in patients who achieved sustained remission.

Research objective 2: To track longitudinal structural changes in patients within specific cortico-limbic regions of interest during treatment (Chapter 3).

Hypothesis: Patients who achieved sustained remission over follow-up were hypothesized to have less structural decline in the regions of interest relative to patients who failed to remit.

Research objective 3: To determine whether there was an association between certain monoamine-related gene polymorphisms and hippocampal volume, and if so, whether this association differed in patients with treatment-resistant depression and healthy individuals (Chapter 4).

Hypothesis: Candidate gene risk alleles were hypothesized to be associated with reductions in hippocampal volume.

1.5 Methodological approach

The data included in the 3 manuscripts that comprise this thesis all derive from the same prospective, longitudinal imaging dataset. A sample of patients with treatment-resistant major depressive disorder underwent structural magnetic resonance imaging at study initiation (baseline) and were then followed clinically and treated with intensive pharmacotherapy over an approximate 1-year follow-up period. Patients underwent a second (follow-up) MRI scan after either 6-months of sustained remission from depression or after 12-months of failure to remit. A group of matched, healthy individuals was scanned once to provide cross-sectional comparison data for the patients' baseline imaging scans. Patients and controls also provided blood samples for imaging genetics analyses. This section provides a brief description of the imaging approaches employed to analyze the dataset.

A primary goal of the thesis was to examine how brain structure changes over time in patients undergoing treatment for depression. The imaging data was first examined using whole-brain approaches (Chapter 2). Since atrophic changes in the brain are subtle and slow to develop, the methods used for its measurement must be accurate, sensitive, and reproducible (Wei et al., 2004; Durand-Debief et al., 2012). SIENA (Structural Image Evaluation using Normalization of Atrophy) provides a fully automated analysis of whole-brain volume change between 2 or more temporally separated MRI scans (Smith et al., 2001, 2002). SIENA was used to obtain a global estimate of the percentage brain volume change (PBVC) between patients' baseline and follow-up scans. Specifically, combined gray and white matter volume change over time was measured, with positive numbers representing increased brain volume, and negative numbers representing decreased brain volume. SIENA has been shown to provide accurate estimation of brain volume change over time in healthy individuals (de Bresser et al., 2011), and degenerative atrophy in Alzheimer's disease (Smith et al., 2007), multiple sclerosis (De Stefano et al., 2010), and Huntington's disease (Majid et al., 2011).

A limitation of SIENA is the inability to identify the affected brain tissue type (gray or white matter) and the specific locus of volumetric change. Voxel-based morphometry (VBM) (Ashburner and Friston, 2000) was used to analyze patients' baseline and follow-up scans to localize regions of volume change observed through SIENA analysis (Chapter 2). VBM is a technique that permits an unbiased, automated volumetric evaluation of the entire brain independent of distinct regions of interest (ROIs). This approach is advantageous as it may identify unexpected brain structures undergoing volumetric change that may not otherwise be investigated with a ROI

approach. However, as VBM involves surveying across all voxels of the brain for statistically significant differences, it requires relatively large effect sizes or large sample sizes to detect changes that survive correction for multiple comparisons (Whitwell, 2009). This can decrease the sensitivity of VBM to reveal volume changes in smaller brain structures (Konarski et al., 2007; Bora et al., 2012). Further, tissue segmentation with VBM is less accurate in certain brain regions, such as the hippocampus, due to its convoluted nature (Ekstrom et al., 2009; Bora et al., 2012). For these reasons, ROI-based analyses were also conducted on the imaging data.

Due to consistent reports of progressive hippocampal volumetric alterations in patients with depression, this was a critical structure of interest in this study and a ROI approach was used to measure the volume of the hippocampus of patients and controls at each imaging time point. This data was used to examine hippocampal volume changes in the patient sample during treatment (Chapter 3), and to investigate the effects of monoaminergic-related single nucleotide polymorphisms on baseline hippocampal volume in patients and controls (Chapter 4). Cortical reconstruction and volumetric segmentation of the cross-sectional and longitudinal imaging data was performed with the FreeSurfer image analysis suite. FreeSurfer contains a fully automated structural imaging stream used to derive volume-based measures of the subcortical brain structures including the hippocampus (Fischl et al., 2002). In order to measure changes in hippocampal volume in patients between baseline and follow-up scans, images were processed using the FreeSurfer longitudinal pipeline (Reuter et al., 2012). The accuracy of automated hippocampal segmentation by FreeSurfer has been shown to be comparable to manual tracing (Cherbuin et al., 2009; Morey et al., 2009; Sánchez-Benavides et al.,

2010; Doring et al., 2011). Moreover, due to its reproducibility over time, automated segmentation may be superior to manual tracing for longitudinal analyses (Cherbuin et al., 2009).

Additional cortical surface ROIs were selected for further study based on review of the literature and the results of the aforementioned VBM analyses that implicated cortico-limbic regions in treatment-mediated structural change over time. ROIs encompassing the rostral middle frontal gyrus (as a neuroanatomical representative of the dorsolateral prefrontal cortex), the anterior cingulate cortex, orbitofrontal cortex and inferior temporal gyrus were examined through cortical thickness analyses (Chapter 3). The thickness of the gray matter cortical ribbon decreases over time in normal aging and in various neurodegenerative diseases. Cortical thinning is often regionally specific and longitudinal measurement of cortical thickness can be used to track disease-related changes and the effects of treatment. The FreeSurfer surface-based imaging stream was used to derive measures of cortical thickness across the cortical mantle (Fischl and Dale, 2000). Cortical surface gyri were labeled using the automated FreeSurfer cortical parcellation procedure (Desikan et al., 2006). The resulting data can be analyzed using vertex-based analyses (where cortical thickness values are compared at each vertex across the entire cortical surface) or by calculating mean cortical thickness across specified ROIs. The latter approach was chosen for the analyses presented in this thesis, as the statistical limitations described for voxel-based analyses (see VBM above) also apply to vertex-based analyses.

CHAPTER 2

Brain-Volume Increase with Sustained Remission in Patients with Treatment-Resistant Unipolar Depression

2.1 Overview

This manuscript describes the results of the whole-brain analyses of the longitudinal imaging data from the treatment-resistant patient sample. The main research questions addressed in this study were whether the brain volume reductions observed over time in depression could be stabilized with adequate pharmacological intervention even if patients fail to respond clinically to the treatment, and whether the atrophy could be reversed with sustained remission. Further, voxel-based morphometry was used to localize the observed gray and white matter volume changes to specific brain areas.

2.2 Statement of author contribution

The experimental design of this study was drafted by Pierre Blier with imaging analyses planned by Jennifer Phillips. Patients were diagnosed, clinically assessed and treated by Pierre Blier, Fahad Aldosary and Philippe Tremblay. Adaptation of staging score methodology and calculation of staging scores was performed by Lisa Batten with the assistance of Pierre Blier. Imaging data was analyzed by Jennifer Phillips. The article was written and the figures were produced by Jennifer Phillips. All authors critically reviewed and approved of the final manuscript. Further contributions by acknowledged collaborators included design of the MRI acquisition sequence by Andra Smith, and study coordination and patient recruitment by Chantal Hébert. This study was supported by a University of Ottawa Medical Research Fund grant awarded to Pierre Blier.

2.3 Title page

Brain-volume increase with sustained remission in patients with treatment-resistant unipolar depression

Jennifer L. Phillips[1,2], Lisa A. Batten[1], Fahad Aldosary[1], Philippe Tremblay[1], and Pierre Blier[1,2]

[1]Mood Disorders Research Unit, University of Ottawa Institute of Mental Health Research, Ottawa, Canada; [2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada

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MRI, Gatineau, Québec, Canada), for their technical assistance. The authors also thank Chantal Hébert, RN, and Maria da Silva (both of the University of Ottawa Institute of Mental Health Research, Ontario, Canada) for study coordination and administrative support, respectively. The acknowledged individuals report no financial or other potential conflicts of interest related to the subject of this article.

Potential Conflicts of Interest: PB has received research support or speakers honoraria from, or has served as a consultant to, AstraZeneca, Bristol-Meyers Squibb, Eli Lilly, Janssen, Labopharm, Lundbeck, Pfizer, Schering-Plough, Sepracor, Servier, Shire, Takeda, and Wyeth. JLP, LAB, FA, and PT report no competing interests.

2.4 Abstract

Objective: Previous magnetic resonance imaging (MRI) studies have demonstrated brain-volume reductions in unipolar major depressive disorder (MDD). It is not clear whether these atrophic changes can be stabilized with antidepressant treatment and/or reversed with remission. The objective of this study was to prospectively examine brain-volume changes in patients with treatment-resistant depression, comparing those who achieved sustained remission with those who did not remit.

Method: This prospective observational cohort study investigated the roles of clinical responsiveness and antidepressant treatment in lessening brain atrophy in depression. Data were collected between October 2004 and December 2008. Baseline MRI scans were obtained from 28 outpatients with treatment-resistant MDD (diagnosed according to DSM-IV criteria) who were recruited from the Mood Disorders Research Unit at the Royal Ottawa Mental Health Centre, Ottawa, Ontario, Canada. Twenty-seven patients underwent follow-up scanning after either 6 months of sustained remission (Montgomery-Åsberg Depression Rating Scale score $\leq$ 12) or 12 months of failure to remit. Longitudinal whole-brain and voxel-based gray and white matter volume changes were estimated.

Results: Twelve patients (mean age at baseline = 47.5 years) achieved sustained 6-month remission. In contrast to nonremitters (n = 15; mean age at baseline = 44.3 years), remitted patients demonstrated a significant mean increase in whole-brain volume during follow-up ($F_{1,27} = 9.51$, $P = .005$). Within-subject voxel-based morphometry analyses identified increased gray matter volume in remitters in the right orbitofrontal cortex ($t_{11} = 7.61$, $P = .006$) and the right inferior temporal gyrus ($t_{11} = 6.65$, $P = .004$). Nonremitters

showed decreased white matter volume in the left anterior limb of the internal capsule (t_{13} = 3.86, P = .04).

Conclusions: Given that remitters exhibited a mean increase in brain volume while nonremitters lost volume, pharmacotherapy in the absence of sustained remission is most likely insufficient to elicit brain-volume increase in MDD. The findings suggest that clinical remission rather than pharmacotherapy may be the key factor involved in driving volumetric recovery in treatment-resistant depression.

2.5 Introduction

Cross-sectional magnetic resonance imaging (MRI) studies have documented brain-volume reductions in patients with unipolar major depressive disorder (MDD) relative to healthy individuals. Such effects have been commonly localized to frontal, limbic and striatal regions (Koolschijn et al., 2009), areas implicated in the emotional, cognitive, metabolic and endocrine alterations seen in the disorder. Decreased trophic support in depression evidenced by stress-induced reductions in levels of brain-derived neurotrophic factor (Dwivedi et al., 2003), is thought to contribute to these volumetric alterations (aan het Rot et al., 2009).

Chronic antidepressant treatment has been shown to increase brain-derived neurotrophic factor expression (Nibuya et al., 1995; Russo-Neustadt et al., 2000), suggesting that certain pharmacotherapies may have neurotrophic effects (Sairanen et al., 2007). Since neurotrophic factors are important mediators of neuronal plasticity (Lu and Gottschalk, 2000), antidepressant-induced neurotrophin increase may restore plasticity and protect against volume loss. In fact, studies have shown evidence of volume recovery

with antidepressant treatment- for example, a 4.6% increase in hippocampal volume in patients with posttraumatic stress disorder treated with paroxetine (Bremner et al., 1995). The progressive nature of brain atrophy in depression (MacQueen et al., 2003) emphasizes the importance of capitalizing on the effects of pharmacotherapy on brain volume. Furthermore, findings of increased hippocampal volume loss with increasing duration of untreated depression (Sheline et al., 2003) highlight the importance of early treatment initiation. A longitudinal study found less volume decline in the anterior cingulate, hippocampus and prefrontal cortex of patients who remitted during 3-year follow-up relative to nonremitters (Frodl et al., 2008b). Cross-sectional studies have found more gray matter volume in the dorsolateral prefrontal cortex of remitted compared to unremitted patients (Li et al., 2010) and increased subgenual prefrontal cortex volume with antidepressant treatment only in patients who were in remission (Yucel et al., 2009). These findings suggest that structural modifications in MDD may also be associated with treatment response. However, it is not clear whether the observed reductions in brain atrophy in such studies can be attributed to the patients' treatment or the alleviation of the depression itself.

The purpose of this prospective observational cohort study was to investigate the roles of clinical responsiveness and antidepressant treatment in lessening brain atrophy in depression. Staging methods, developed to assess resistance to antidepressant treatment (Fava, 2003), were employed to provide novel information on the relationship between treatment resistance and volumetric alterations in MDD. We hypothesized that brain volume would be increased in patients who achieved sustained remission from depression and, secondly, that brain volume would be positively affected by pharmacological

intervention only if patients achieved remission with the treatment. These hypotheses were addressed in a prospective longitudinal imaging study of patients with treatment-resistant depression who were receiving intensive pharmacotherapy over a follow-up period of approximately 1 year. Patients with treatment-resistant depression were selected so as to obtain a balanced proportion of remitted and unremitted patients for comparison.

2.6 Method

2.6.1 Participants

Twenty-eight outpatients with treatment-resistant MDD were recruited from the Mood Disorders Research Unit at the Royal Ottawa Mental Health Centre, Ottawa, Ontario, Canada. Primary diagnosis of MDD was made by psychiatric consultation on the basis of DSM-IV criteria. Treatment resistance was defined as current episode duration of at least 6 months, failure to remit following treatment with at least 2 antidepressants at adequate dosage for at least 6 weeks each (determined through retrospective chart review of treatment response prior to enrollment), a 17-item Hamilton Depression Rating Scale ($HDRS_{17}$; Hamilton, 1960) score $\geq$ 18, and a Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg, 1979) score $\geq$ 22. Handedness was evaluated with the Edinburgh Handedness Inventory (Oldfield, 1971). Exclusion criteria included history of manic, hypomanic or mixed episode; diagnosis of posttraumatic stress disorder or any psychotic disorder, eating disorder, or substance-related disorder; presence of major medical illnesses; history of significant head trauma; exposure to oral or intravenous steroids; intelligence quotient (IQ) < 80; or any contraindications to MRI.

The Royal Ottawa Mental Health Centre Research Ethics Board approved the protocol. All participants provided written informed consent.

Data were collected between October 2004 and December 2008. Twenty-eight patients underwent clinical assessment and MRI scan at baseline. Twenty-seven patients were followed longitudinally (1 patient was lost to follow-up) and assessed by administration of the MADRS (chosen for its sensitivity to change) at baseline and each subsequent visit. During the study, patients underwent intensive pharmacotherapy under the care of study investigators with the goal of attaining remission. All patients were taking medication at enrollment and received treatment throughout follow-up. At patient visits (once every 2 weeks), if an approximately 20% symptom improvement was not detected (Szegedi et al., 2009), an increase in doses (if tolerated) or a medication change was implemented. Medication choices were based on the drugs' different mechanisms of action and potential synergistic effects on the serotonin, norepinephrine and dopamine systems (Blier, 2006). Follow-up scans were obtained after either a 6-month period of sustained remission (MADRS score ≤ 12 at each visit) or a 12-month period of failure to remit.

Severity of treatment resistance was measured through determination of staging scores calculated using treatment history. This method (modified from Fava, 2003) considers number of failed medication trials, optimization and intensity of dosages, and use of augmentation and combination strategies (see Supplementary Tables S2.2-S2.4). Each patient was assigned 2 numerical scores: (1) a retrospective staging score, reflecting treatment during the 5-year period preceding study enrollment (obtained through medical-chart and pharmacy-record review for the index episode) and (2) a prospective

staging score, reflecting treatment approaches used from baseline to study termination. Scores were calculated by assigning points as follows: 1 point for each antidepressant used for at least 6 weeks at an effective dosage, one-half point for treatment strategies given at or above the maximum recommended effective dosage, one-half point for medications added as augmentation or combination strategies regardless of dosage, and 3 points for each trial of electroconvulsive therapy. During follow-up, patients received individualized treatment with medications from the following drug classes: tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), norepinephrine reuptake inhibitors, dopamine agonists, atypical antipsychotics, and others (see Supplementary Tables S2.2-S2.4). No patients underwent electroconvulsive therapy during follow-up.

2.6.2 Brain imaging

T1-weighted magnetic resonance images were obtained at 1.5-T (Siemens Magnetom Symphony; Siemens AG, Erlangen, Germany) using the same acquisition protocol for all images (repetition time = 1500 milliseconds; echo time = 4.38 milliseconds; flip angle = 15°; matrix = 256 x 256 mm; slice thickness = 1 mm; voxel resolution = 1 x 1 x 1 mm). Baseline and follow-up images of most patients (n = 25) were obtained on the same scanner at St-Joseph MRI, Gatineau, Quebec, Canada. The baseline scans of 3 patients were obtained at the Ottawa Hospital, Ottawa, Ontario, Canada, with their follow-up scans acquired at St-Joseph MRI.

Images were converted to NIfTI-1.1 (Neuroimaging Informatics Technology Initiative) format using MRIConvert, version 2.0 (Lewis Center for Neuroimaging,

University of Oregon, Eugene, Oregon). Images were manually reoriented and centered on the anterior commissure using the Statistical Parametric Mapping (SPM) display routine (SPM5; Wellcome Department of Imaging Neuroscience, London, United Kingdom). Estimates of whole-brain volume were obtained using Structural Image Evaluation Using Normalization of Atrophy (SIENA), version 2.6 (Oxford Centre for Functional MRI of the Brain [FMRIB], Oxford, United Kingdom; Smith et al., 2001), part of the FMRIB Software Library (FSL), version 4.1.1 (FMRIB, Oxford, United Kingdom; Smith et al., 2004). For baseline scans, normalized brain volume (total brain volume normalized for head size) was estimated with SIENAX, version 2.6 (FMRIB, Oxford, United Kingdom; Smith et al., 2001), the cross-sectional extension of SIENA.

Longitudinal brain-volume change was estimated with SIENA, an automated technique that measures combined gray and white matter volume change. Patients' baseline and follow-up images were aligned to each other, resampled into the space halfway between the images, and segmented to find brain/non-brain edge points. Perpendicular edge displacement was estimated, and mean edge displacement was converted into an estimate of percentage brain-volume change (PBVC) between the 2 time points, with positive and negative numbers representing increased and decreased brain volume, respectively.

To localize regions of volume change, images were analyzed using voxel-based morphometry (Ashburner and Friston, 2000) in SPM5 running in MATLAB, version 7.3 (The MathWorks, Natick, Massachusetts). Voxel-based morphometry permits automated voxel-wise comparison of gray and white matter volumes. Skull-stripped images were segmented into tissue classes, and tissue segments were normalized using Diffeomorphic

Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007), a nonlinear iterative registration algorithm that creates and registers images to a population-specific template. Modulation was specified to ensure preservation of volumes. An affine transformation was applied to convert images to Montreal Neurological Institute space. Images were smoothed with an 8 mm Gaussian kernel.

2.6.3 Statistical analyses

The demographic and clinical variables and SIENAX-estimated baseline normalized brain volume of remitters and nonremitters were compared with 2-tailed independent-sample *t* tests or χ^2 tests using PASW Statistics, version 18.0 (SPSS Inc., Chicago, Illinois). Differences in PBVC were assessed with an analysis of covariance, adjusted for age and interscan interval. Pearson correlations were calculated to examine the relationships between PBVC and age and change in MADRS scores during follow-up. Stepwise regression analysis was performed to examine clinical predictors of PBVC, treating PBVC as a dependent variable and age, percentage of time in remission (calculated as [days in remission/interscan interval] x 100), and prospective staging scores as predictor variables. For all analyses, results were considered significant at $P < .05$.

For voxel-based morphometry analyses, the framework of the general linear model was employed to estimate within-group gray matter volume and white matter volume differences between baseline and follow-up with paired *t* tests (with scanner as a covariate). An absolute intensity threshold mask of 0.2 was employed in model specification. Contrasts were defined to examine gray matter volume and white matter

volume changes in remitter and nonremitter groups. Statistical results were first thresholded at an uncorrected voxel-level P value < .001, and, then, to account for non-uniform smoothness in the imaging data, an SPM non-stationarity correction toolbox (Worsley et al., 1999) was used to generate t statistic maps with cluster-size P values at .05 corrected for multiple comparisons. Coordinates were assigned to regions by automated labeling using the Harvard-Oxford cortical and subcortical atlases (Center for Morphometric Analysis, Charlestown, Massachusetts) and the Johns Hopkins University ICBM DTI-81 (International Consortium for Brain Mapping; diffusion tensor imaging) white matter atlas (Mori et al., 2005).

2.7 Results

2.7.1 Demographic data

During follow-up, 12 patients (44%) achieved sustained 6-month remission, while 15 patients (56%) failed to achieve sustained remission. Although remitted patients had lower MADRS scores at baseline relative to nonremitters ($t_{25} = 3.26$, $P = .003$), within-group analyses revealed significant decreases in the MADRS scores of both groups, with lower mean final MADRS scores in remitters (Figure 2.1). At baseline, the groups did not significantly differ on demographic variables, age at onset, number of previous episodes, $HDRS_{17}$ scores, or retrospective staging scores (Table 2.1), highlighting the homogeneity of the sample at enrollment. During follow-up, relative to nonremitters, remitted patients had significantly lower prospective staging scores, had a larger decrease in MADRS scores, spent more time in remission, and had shorter interscan intervals (see Table 2.1).

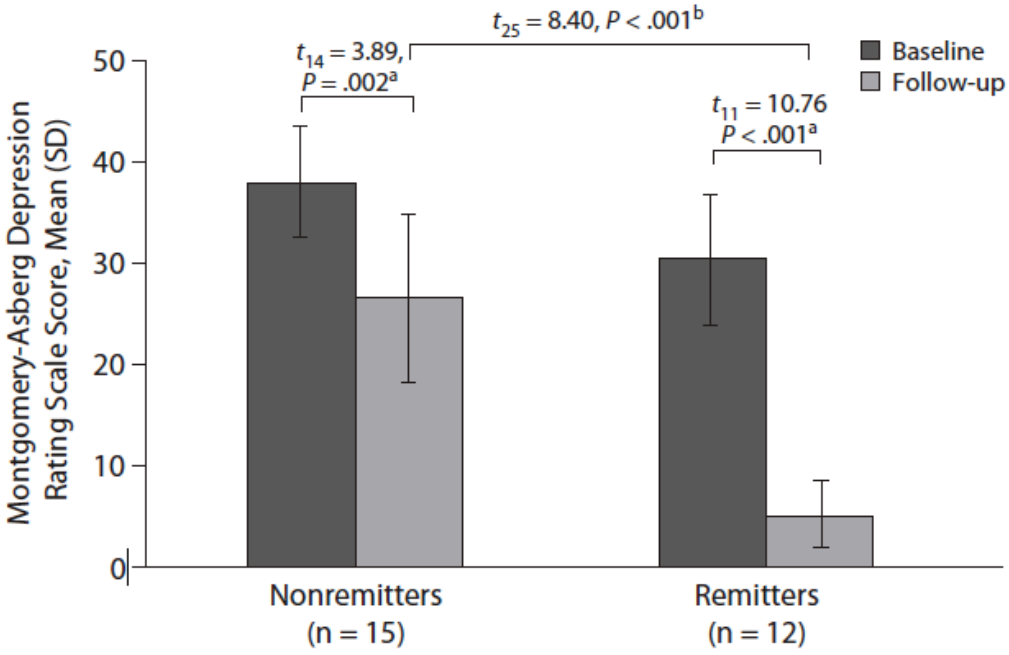

Figure 2.1. Change in depressive symptoms over follow-up (N = 27).

[a]Paired *t* test.

[b]Independent-samples *t* test.

Table 2.1. Demographic and clinical data of remitted and nonremitted patient groups.

Variable	Remitted Patients (N = 12), n	Nonremitted Patients (N = 15), n	Analysis		
			χ^2	*df*	*P*
Gender, male/female	5/7	4/11	0.68	1	.41
Handedness, right/left	9/3	14/1	1.78	1	.18
No. of depressive episodes, A/B/C[a]	4/3/5	7/3/5	0.49	1	.78
	Mean (SD)	Mean (SD)	*t*		
Age at baseline, y	47.5 (10.6)	44.3 (10.2)	0.80	25	.43
Age at follow-up, y	48.5 (10.7)	45.4 (10.3)	0.77	25	.45
Interval between scans, d	331 (107)	417 (38)	2.93	25	.007
Age at illness onset, y[b]	31.3 (14.2)	29.7 (14.0)	0.29	24	.78
Baseline $HDRS_{17}$ score	22.5 (4.2)	24.9 (4.7)	1.33	25	.20
Change in MADRS score[c]	−25.0 (8.1)	−11.1 (11.1)	3.63	25	.001
Retrospective staging score[d]	5.1 (2.7)	5.5 (3.1)	0.37	25	.72
Prospective staging score[e]	4.4 (2.1)	7 (2.4)	2.92	25	.007
Follow-up spent in remission, %[f]	62 (14)	7 (16)	9.49	25	<.001
Normalized brain volume, mL[g]	1,482 (65)	1,464 (68)	0.72	25	.48

[a]Number of episodes prior to study enrollment expressed as categories: A = 1-2 episodes, B = 3-4 episodes, C = 5+ episodes.

[b]Data not available for 1 subject.

[c]Change in MADRS score was calculated as [follow-up MADRS – baseline MADRS].

[d]Treatment history of the index episode from 5 years prior to study enrollment to baseline, calculated according to the modified Massachusetts General Hospital staging method for treatment resistance (Fava, 2003), with 1 point assigned for each drug used for the treatment of depression for at least 6 weeks at an effective dosage, plus one-half point for treatment strategies given at or above the maximum recommended dosage, plus one-half point for medications added as augmentation or combination strategies, plus 3 points for each adequate trail of electroconvulsive therapy.

[e]Treatment history from baseline to study termination, calculated as above.

[f]Percentage of time in remission was calculated as [(consecutive days in remission between baseline and final assessment/days between images) x 100].

[g]Baseline total brain-tissue volume (gray matter plus white matter), normalized for participant head size, was calculated with SIENAX, version 2.6 (Oxford Centre for Functional MRI of the Brain, Oxford, United Kingdom).

Abbreviations: $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale, MADRS = Montgomery-Åsberg Depression Rating Scale, MRI = magnetic resonance imaging.

2.7.2 Imaging analysis

Total normalized brain volume of remitted and nonremitted patients did not differ significantly at baseline (see Table 2.1). The SIENA analyses demonstrated positive mean PBVC in remitted patients (+0.30% [standard deviation = 0.70%]), while nonremitters showed negative mean PBVC (-0.35% [standard deviation = 1.08%]) over follow-up (Figure 2.2). In accordance with patients' mean normalized brain volume at baseline (see Table 2.1), these changes represent an approximate 4.5-mL brain-volume increase in remitters and an approximate 5.1-mL volume loss in nonremitters. Analysis of covariance adjusted for age and interscan interval revealed a significant main effect of outcome group (remission status) on PBVC ($F_{1,27} = 9.51$, $P = .005$), with age contributing significant variance ($F_{1,27} = 16.53$, $P < .001$). A post hoc *t* test revealed significant between-group differences in PBVC ($P = .005$, Bonferroni corrected). Further, Pearson correlation analyses demonstrated significant negative correlations between patients' baseline age and PBVC ($r = -0.52$, $P = .003$) and between change in MADRS scores and PBVC ($r = -0.37$, $P = .03$). These results indicate associations between aging and volume loss and between symptom improvement and volume increase.

Patients' PBVC was regressed on age, percentage of time in remission, and prospective staging scores using stepwise linear regression. The first variable, age, resulted in a significant increase in explained variance in PBVC ($\Delta R^2 = 0.27$, $F_{1,25} = 9.07$, $P = .006$), as did the second variable, percentage of time in remission ($\Delta R^2 = 0.12$, $F_{1,24} = 4.47$, $P = .04$). Together, age ($\beta = -0.53$, $t = -3.32$, $P = .003$) and percentage of time in remission ($\beta = 0.34$, $t = 2.12$, $P = .04$) were the best predictors of PBVC (adjusted $R^2 = 0.33$; $F_{2,24} = 7.40$, $P = .003$). Prospective staging score was not a significant predictor

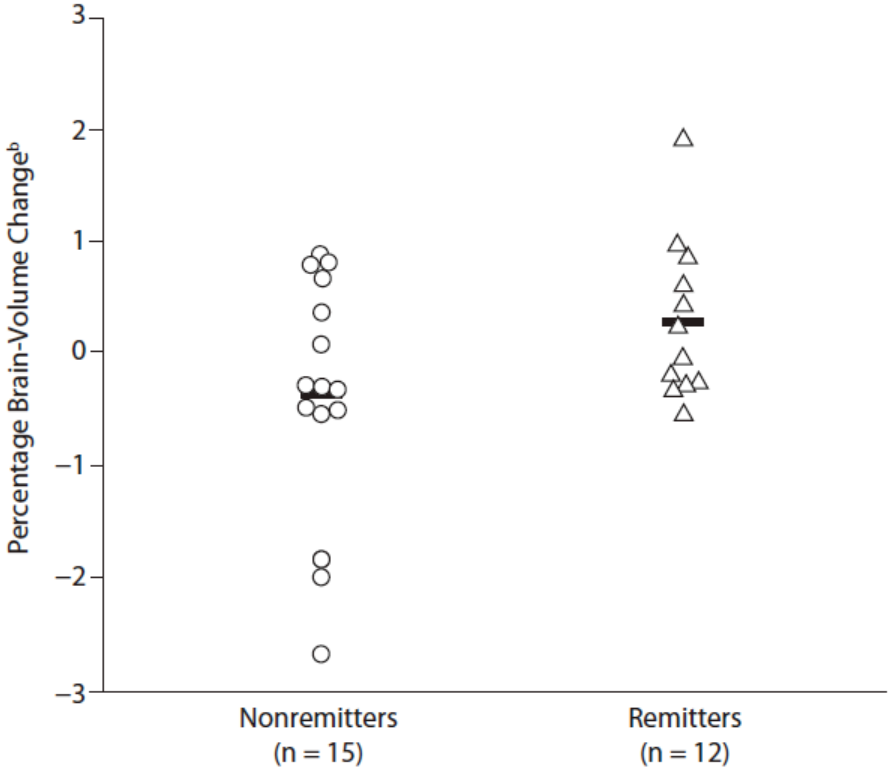

Figure 2.2. Percentage brain-volume change over follow-up (N = 27).[a]

[a]Each circle or triangle represents a patient; horizontal bars indicate group means.

[b]Analysis of covariance (adjusted for age and interscan interval); significant main effect of outcome group: $F_{1,27} = 9.51$, $P = .005$.

variable (β = -0.06, t = -0.26, P = .80).

In longitudinal voxel-based morphometry analyses, paired t tests in the remitted group revealed significant clusters of increased gray matter volume in the right orbitofrontal cortex and the right inferior temporal gyrus, while a cluster of decreased gray matter volume was found in the right superior parietal lobule (Figure 2.3). No significant changes in gray matter volume were detected in nonremitters.

Voxel-based morphometry analyses revealed no changes in white matter volume in remitters. Within nonremitters, while there were no significant regions of white matter volume increase, there was a significant cluster of decreased white matter volume detected in the left anterior limb of the internal capsule (Figure 2.4).

2.8 Discussion

The main finding of this study was that, although both remitted and nonremitted patients received intensive pharmacologic treatment and showed some degree of clinical improvement during the study, mean whole-brain volume increase was seen in the group of patients who achieved sustained remission, while mean brain-volume loss was evident in nonremitters. Forty-four percent of patients achieved sustained remission, a remarkable number considering the severity of treatment resistance demonstrated. Although the inclusionary definition of treatment resistance was consistent with traditional descriptions, most patients were significantly more resistant than these criteria suggest. Many patients had previously experienced failure of up to 5 antidepressant trials, 3 had experienced failure of electroconvulsive therapy, and most had experienced chronic depressive episodes since their illness onset. Of the remitted patients, all remitted while receiving SSRIs or SNRIs given in combination with other medications, while no patients

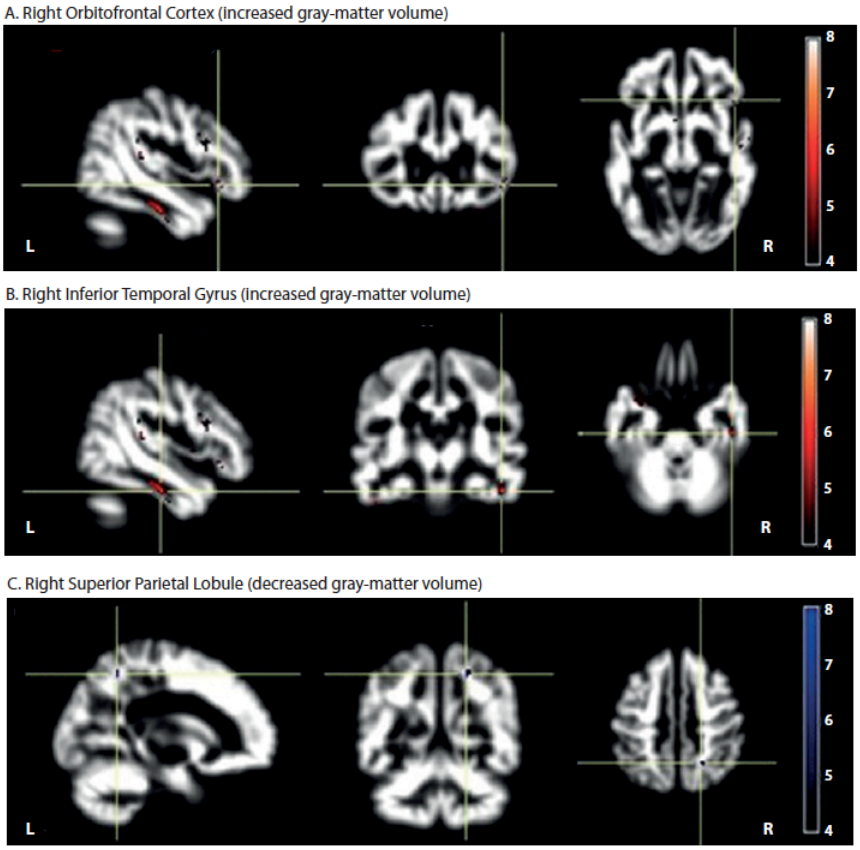

Figure 2.3. Regions of gray matter volume change over follow-up in remitted patients (N = 12). Clusters of increased gray matter volume from baseline to follow-up (in red) were detected in (A) the right orbitofrontal cortex (t_{11} = 7.61, P = .006; MNI coordinates: 50, 28, -6) and (B) the right inferior temporal gyrus (t_{11} = 6.65, P = .004; MNI coordinates: 50, -18, -26). Decreased gray matter volume (in blue) was detected in (C) the right superior parietal lobule (t_{11} = 6.28, P = .03; MNI coordinates: 22, -54, 54). Statistical parametric maps were thresholded at cluster-sized P value of .05, nonstationarity corrected for multiple comparisons and overlaid on the DARTEL-registered population-specific template. Crosshairs are centered on coordinates of local maxima. Abbreviations: DARTEL = Diffeomorphic Anatomic Registration Through Exponential Lie Algebra, L = left, MNI = Montreal Neurological Institute, R = right.

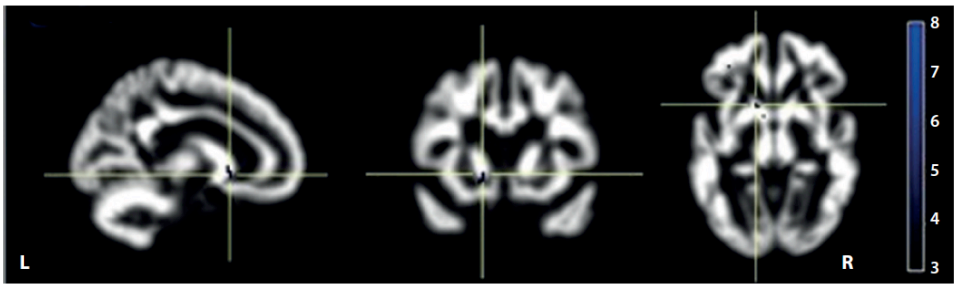

Figure 2.4. White matter volume decrease over follow-up in the left anterior limb of the internal capsule in nonremitted patients (N = 15). A cluster of decreased white matter volume from baseline to follow-up (in blue) was detected the left anterior limb of the internal capsule (t_{13} = 3.86, P = .04; MNI coordinates: -14, 16, -4). A statistical parametric map was thresholded at cluster-sized P value of .05, nonstationarity corrected for multiple comparisons and overlaid on the DARTEL-registered population-specific template. Crosshairs are centered on coordinates of local maxima. Abbreviations: DARTEL = Diffeomorphic Anatomic Registration Through Exponential Lie Algebra, L = left, MNI = Montreal Neurological Institute, R = right.

remitted while receiving SSRI treatment in monotherapy. Studies have shown combination therapy to be superior to SSRI monotherapy from treatment initiation (Blier et al., 2009, 2010), and augmentation and combination strategies are now commonly thought to represent an important means to enhance response among pharmacotherapy-resistant patients (Fava, 2009). The effects of such treatment strategies on brain volume, however, have yet to be determined. Previous studies have shown increases in whole-brain gray matter volume in psychiatric patients following short-term treatment with various medications. These findings include a 4% gray matter volume increase in bipolar patients following 4-week treatment with lithium (Moore et al., 2000; Sassi et al., 2002), and a 3% gray matter volume increase in schizophrenia patients treated with atypical antipsychotics (Garver et al., 2005). While antidepressants have been shown to increase brain volume regionally, in the hippocampus in posttraumatic stress disorder (Bremner et al., 1995) and in the anterior cingulate in MDD (Yucel et al., 2009), for example, to our knowledge, this study is the first to demonstrate whole-brain volume increase in unipolar MDD patients undergoing pharmacotherapy.

Volume changes were expected in the fronto-limbic areas implicated in depression, especially the hippocampus, anterior cingulate and prefrontal cortices (Frodl et al., 2008b; Koolschijn et al., 2009). In remitted patients, gray matter volume increases were localized to the right orbitofrontal cortex and inferior temporal gyrus. Studies have found decreased gray matter volume and abnormal glucose metabolism and cerebral blood flow in the orbitofrontal cortex in MDD patients relative to controls (Drevets, 2007). Conversely, nonremitted patients demonstrated white matter volume reduction in the left anterior limb of the internal capsule. Diffusion tensor imaging studies have shown

reduced fractional anisotropy (a measure of white matter integrity) in this region in MDD and have shown an association between decreased fractional anisotropy values and symptom severity (Zou et al., 2008). Further, diffusion tensor imaging-based tractography has revealed structural projections between the anterior limb of the internal capsule and several brain regions commonly implicated in depression and antidepressant response (Gutman et al., 2009).

Voxel-based morphometry revealed no hippocampal volume increases in remitters. Although a recent longitudinal study found modest increases in hippocampal volume in MDD patients following 3-year antidepressant treatment (Frodl et al., 2008a), no hippocampal modifications were observed at 1-year follow-up (Frodl et al., 2004). Hippocampal volume changes were not detected in another MDD sample following 7 months of SSRI treatment (Vythilingam et al., 2004). Antidepressant-mediated changes in hippocampal volume may not have been detected in this study because the changes may require a longer time period to become apparent.

This study is one of the first to examine morphological changes in a well-defined sample of patients with treatment-resistant MDD. Moreover, this study is among the first to investigate longitudinal brain-volume changes associated with remission status in depression. Despite these strengths, the study has certain limitations that warrant consideration. The first is the relatively small sample size. Although the longitudinal design of the study permitted powerful within-subject analyses, these results must be replicated in larger samples. Second, due to the naturalistic treatment approach used, it is not possible to perform in-depth analyses of the effects of any individual medication or combination/augmentation strategy on brain volume. Such analyses would require

longitudinal imaging studies combined with randomized medication trials. Third, region-of-interest approaches in structural imaging studies provide more power to detect changes compared to voxel-based morphometry. Given the subtlety of the magnitude of volume changes in this study, region-of-interest analyses may be required to detect changes in small structures such as the hippocampus. The use of voxel-based morphometry, however, provides an unbiased volumetric assessment across the entire brain and is thus valuable, as it may have identified regions that respond to treatment and/or remission that have not been previously reported and/or investigated.

Antidepressants, mood stabilizers, and atypical antipsychotics are each thought to contribute to brain-volume increase through their neurotrophic and neuroprotective effects (Hunsberger et al., 2009). While all patients were treated with drugs from pharmacologic classes shown to protect against brain-volume loss, as a group, nonremitters had significantly higher prospective staging scores than remitters (indicating exposure to more intensive treatment due to pharmacologic nonresponse). Despite this fact, nonremitters on average still showed evidence of brain atrophy, suggesting that pharmacotherapy in the absence of sustained remission might be insufficient to elicit brain-volume increase in MDD. This finding is supported by the results of the regression and correlation analyses. Overall, brain atrophy was shown to increase with increasing patient age, consistent with findings in healthy individuals (Enzinger et al., 2005). Further, while clinical improvement was associated with increased brain volume and time in remission was a significant predictor of volume change, treatment intensity was not a significant predictor variable. In other words, the amount of time a patient spent in remission was a better predictor of his or her brain-volume change than was the intensity

of pharmacotherapy he or she received. Collectively, these results suggest that, in treatment-resistant depression, remission rather than pharmacologic treatment is most likely the key factor involved in halting atrophy and driving volumetric recovery. This finding is an important one since the restoration of brain volume in patients with treatment-resistant depression may have positive implications for their future prognosis.

2.9 Clinical points

- Achieving sustained remission, not merely receiving medication treatment, may prevent brain-volume loss in depression.
- Using combinations of 2–3 medications is often necessary to achieve remission in depression, as in other medical illnesses.
- Remission of depression can be achieved using combinations of medications with different mechanisms of action.

2.10 Supplementary materials

Disclaimer: This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

Table S2.2. Staging score methodology.

Staging method to classify retrospective[a] and prospective[b] degree of treatment-resistance in major depressive disorder.

(1) For each adequate trial of an antidepressant medication (6 weeks at or above minimum recommended dose), 1 point was assigned per trial.

(2) For each optimization of dosage (treatment given at or above maximum recommended dose), an additional 0.5 points was assigned per trial.

(3) For each medication added to a primary antidepressant as an augmentation or combination strategy (treatment given for at least 6 weeks regardless of the dosage), an additional 0.5 points was assigned per trial.

(4) For each adequate trial of electroconvulsive therapy (at least nine sessions), an additional 3 points was assigned per trial.

[a]Retrospective staging scores reflect treatment approaches taken during the five-year period preceding study enrollment. No points were assigned in retrospective chart reviews if trial dosage, time frame, or strategy was unclear.

[b]Prospective staging scores were calculated from baseline visit to first remission visit. No points were assigned if drugs were discontinued due to side-effects or non-compliance. Medication changes made during sustained remission were not included in calculations.

Table S2.3. Minimum and maximum doses of antidepressant and other medications used in the calculation of staging scores for treatment resistance.

Drug Class **Generic Name**	**Minimum Recommended Dose (mg/day)**	**Maximum Recommended Dose (mg/day)**
Tricyclic Antidepressants		
clomipramine	150	250
amoxapine	150	250
amitriptyline	150	250
maprotiline	100	250
desipramine	150	250
nortriptyline	75	125
doxepin	150	250
trimipramine	150	250
imipramine	150	250
protriptyline	30	60
Monoamine Oxidase Inhibitors (MAOIs)		
isocarboxazid	30	60
phenelzine	45	90
tranylcypromine	30	60
moclobemide	300	900
Selective Serotonin Reuptake Inhibitors (SSRIs)		
fluvoxamine	50	300
paroxetine	20	60
fluoxetine	10	60
sertraline	50	150
citalopram	20	60
escitalopram	10	30
Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs)		
venlafaxine	125	250
duloxetine	60	100
Norepinephrine Reuptake Inhibitor		
atomoxetine	40	100

Table S2.3 (cont.)

Drug Class **Generic Name**	**Minimum Recommended Dose (mg/day)**	**Maximum Recommended Dose (mg/day)**
Other Antidepressants		
trazodone	300	600
nefazodone	300	600
bupropion	300	450
mirtazapine	15	45
pinodolol	7.5	15
topiramate	300	600
Atypical Antipsychotics		
quetiapine	50	600
clozapine	25	100
olanzapine	5	20
ziprasidone	40	100
aripiprazole	5	30
paliperidone	1.5	9
risperidone	0.25	4
Other Medications		
lithium	600	1200
triiodothyronine	0.025	0.05
buspirone	20	60
Dopamine agonists		
pramipexole	1	5
bromocriptine	2.5	5

Table S2.4. Treatment approaches used by patients at baseline and follow-up imaging and remission status at follow-up.

Patient	Baseline Treatment, Dosage (mg/day)	Follow-Up Treatment, Dosage (mg/day)	Status
1	citalopram 10, venlafaxine 225, quetiapine 50, trazodone 100	fluoxetine 40, quetiapine 300, trazodone 150	NR
2	venlafaxine 300, quetiapine 50, dextroamphetamine 20	tranylcypromine 20, atomoxetine 60	NR
3	venlafaxine 150	venlafaxine 150, bupropion 300	NR
4	escitalopram 10, atomoxetine 60, trazodone 100	bupropion 450, atomoxetine 60, trazodone 75	R
5	doxepin 300, flurazepam 30	venlafaxine 225, mirtazapine 30	NR
6	paroxetine 50	tranylcypromine 20, quetiapine 300	NR
7	fluoxetine 40, bupropion 300	fluoxetine 40, atomoxetine 25, trazodone 150	R
8	venlafaxine 300	moclobemide 900, bupropion 300	NR
9	venlafaxine 150, risperidone 0.5	venlafaxine 300, mirtazapine 45, pindolol 15, lamotrigine 100	R
10	bupropion 150	venlafaxine 300, bupropion 450	R
11	escitalopram 15, bupropion 150	escitalopram 30, buspirone 30, lithium 900, quetiapine 25	NR
12	paroxetine 50, trazodone 100	escitalopram 40, risperidone 2, lamotrigine 175	NR
13	venlafaxine 150, bupropion 150, trazodone 100	escitalopram 15, bupropion 150, mirtazapine 30	R
14	venlafaxine 300, amitriptyline 50, zopiclone 10	venlafaxine 300, amitriptyline 50, risperidone 2	NR
15	fluoxetine 40, clomipramine 50	escitalopram 10, bupropion 150, trazodone 100	R
16	venlafaxine 225, bupropion 300, risperidone 1	venlafaxine 300, nortriptyline 50, paliperidone 6	NR
17	moclobemide 750	escitalopram 40, pramipexole 3, bupropion 300	NR
18	paroxetine 20, bupropion 100, trazodone 50	paroxetine 20, bupropion 100, risperidone 1	R
19	paroxetine 20, amitriptyline 20, trazodone 150	paroxetine 40, pindolol 10, trazodone 150	R
20	paroxetine 60, mirtazapine 45, olanzapine 10, lithium 900	escitalopram 40, mirtazapine 60, buspirone 30, zopiclone 7.5	R
21	venlafaxine 225	venlafaxine 225, pindolol 10, trazodone 100	R
22	venlafaxine 225, olanzapine 10, trimipramine 100, temazepam 60, clonazepam 1, eltroxin 0.1	venlafaxine 150, ziprasidone 40, trimipramine 200, diazepam 20, levothyroxine 0.075	NR
23	citalopram 40, bupropion 150	escitalopram 20, atomoxetine 80, pramipexole 4, quetiapine 50	NR
24	venlafaxine 300, bupropion 150	venlafaxine 375	NR
25	escitalopram 20, bupropion 150, topiramate 100, temazepam 15	escitalopram 40, ziprasidone 40, pindolol 5, trazodone 100	NR
26	venlafaxine 187.5	venlafaxine 225, bupropion 450, trazodone 200	R
27	paroxetine 40, bupropion 150, trazodone 50	paroxetine 40, atomoxetine 40, modafinil 100, trazodone 150	R

Abbreviations: NR = nonremitter; R = remitter

CHAPTER 3

A Prospective, Longitudinal Study of the Effect of Remission on Cortical Thickness and Hippocampal Volume in Patients with Treatment-Resistant Depression

3.1 Overview

The first manuscript in the thesis described whole-brain volume increase in patients who achieved sustained remission and brain volume loss in nonremitters over follow-up. The current paper employed a region of interest approach to further examine the patients' longitudinal changes in brain structure over the follow-up period. Cortical thickness and brain volume were analyzed in specific cortico-limbic brain areas. The objectives of this paper were to determine whether the patient sample differed from a matched healthy control group at baseline imaging, whether patients' baseline brain structure at treatment initiation could predict their clinical response over follow-up, and how patients' remission status affected their volume or cortical thickness over time.

3.2 Statement of author contribution

The experimental design of this study was drafted by Pierre Blier with imaging analyses planned by Jennifer Phillips. Patients were diagnosed, clinically assessed and treated by Pierre Blier, Fahad Aldosary, and Philippe Tremblay. Healthy control recruitment and screening was performed by Lisa Batten. Imaging data was analyzed by Jennifer Phillips. The article was written and the figures were produced by Jennifer Phillips. All authors critically reviewed and approved of the final manuscript. Further contributions by acknowledged collaborators included design of the MRI acquisition sequence by Andra Smith, and study coordination and patient recruitment by Chantal Hébert. This study was supported by a University of Ottawa Medical Research Fund grant awarded to Pierre Blier.

3.3 Title page

A prospective, longitudinal study of the effect of remission on cortical thickness and hippocampal volume in patients with treatment-resistant depression

Jennifer L. Phillips[1,2], Lisa A. Batten[1], Fahad Aldosary[1], Philippe Tremblay[1], and Pierre Blier[1,2]

[1]Mood Disorders Research Unit, University of Ottawa Institute of Mental Health Research, Ottawa, Canada; [2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada

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Conflict of Interest: PB received grant funding and/or honoraria for lectures and/or participation in advisory boards for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Euthymics, Forest, Janssen, Lundbeck, Merck, Otsuka, Pfizer, Pierre Fabre, Servier, Shire, Takeda, and Valeant. PT has served as a consultant to Lundbeck. All other authors declare no conflicts of interest.

3.4 Abstract

Background: Magnetic resonance imaging studies have provided evidence of structural modifications in cortical-limbic regions in major depressive disorder (MDD). To date however, few studies have tracked structural changes in patients during treatment. This prospective, longitudinal imaging study investigated associations between brain structure and clinical responsiveness in a sample of patients with treatment-resistant MDD over an approximate one-year follow-up period.

Methods: FreeSurfer software was used to extract volume or cortical thickness values from 5 regions of interest (the hippocampus, rostral middle frontal gyrus, orbitofrontal cortex, anterior cingulate cortex and inferior temporal gyrus) in patients (n = 26) and matched healthy controls (n = 28). Analyses were conducted to compare volume and cortical thickness in patients and controls at baseline imaging; to determine whether patients' brain structure at treatment initiation was associated with response over follow-up; and to compare longitudinal changes in volume and cortical thickness in patients who achieved sustained 6-month remission (Montgomery-Åsberg Depression Rating Scale Score $\leq$ 12) with nonremitters.

Results: Patients and controls showed no structural differences at baseline. Among patients, thicker right anterior cingulate cortex at baseline was associated with greater symptom improvement over follow-up. Remitters and nonremitters showed subtle changes in volume and thickness over time in opposing directions, with increased hippocampal volume and cortical thickness in the rostral middle frontal gyrus and orbitofrontal cortex in remitters, and decreased volume or thickness in these regions in nonremitters.

Conclusions: The results suggest that longitudinal structural trajectories may differ in MDD patients according to their clinical response to treatment.

3.5 Introduction

Mounting evidence suggests that major depressive disorder (MDD) is associated with abnormalities in cortical and subcortical brain structures and their connective circuits. Volume-based magnetic resonance imaging (MRI) studies have primarily localized brain volume differences in patients with depression relative to healthy individuals to frontal and limbic brain regions. Specifically, meta-analyses of cross-sectional imaging studies have most commonly reported volume reductions in the prefrontal, orbitofrontal, and cingulate cortices, the hippocampus, and striatum in patients with MDD (Koolschijn et al., 2009; Arnone et al., 2012). Multimodal imaging has provided further evidence of dysfunction of the cortical-limbic circuit in depression with findings of altered anatomical connectivity in this network by diffusion tensor tractography (Fang et al., 2012) and abnormal effective connectivity by positron emission tomography (PET) measures of brain glucose metabolism (Seminowicz et al., 2004). Cytoarchitectural abnormalities in the cerebral cortex in MDD have also been widely reported in postmortem studies. Specifically, reduced neuronal and glial cell densities, neuronal size and cortical thickness have been found in the dorsolateral prefrontal, orbitofrontal and anterior cingulate cortices of patients with depression (Ongür et al., 1998; Rajkowska et al., 1999; Cotter et al., 2001, 2002). Collectively, this evidence implicates the cortical-limbic regions in the structural modifications observed in depressed patients.

Studies have also revealed a relationship between these regional structural abnormalities and a number of clinical variables in depression. Meta-analyses have shown that longer illness duration in patients is associated with greater gray matter (GM) volume decrease in the rostral anterior cingulate cortex and dorsomedial frontal cortex (Bora et al., 2012), and smaller hippocampal volume (McKinnon et al., 2009). Hippocampal volume is also associated with total number of depressive episodes (Videbech and Ravnkilde, 2004). Evidence of the relationship between structural brain changes and illness burden suggests that these modifications may be state-related rather than (or in addition to) trait-related markers, and thus may be reversible upon treatment or remission. In fact, meta-regression analysis of cross-sectional studies has shown larger hippocampal volume among remitted patients compared to currently depressed patients (Kempton et al., 2011).

Longitudinal structural imaging studies may determine whether atrophic changes are amenable to treatment but to date few such studies have been conducted in depression. Initial longitudinal research has revealed a depression-specific pattern of brain volume decline consistent with the areas identified as affected in cross-sectional studies (Frodl et al., 2008b). Moreover, these same regions appear to respond over time to antidepressant treatment and/or remission. Frodl and colleagues (2008b) found less GM volume decline in the hippocampus, prefrontal and anterior cingulate cortices in patients who achieved remission over 3-year follow-up compared to nonremitters. In a previous study, we found increased whole brain volume, and increased GM volume in the orbitofrontal cortex and inferior temporal gyrus in patients who achieved 6-month sustained remission (Phillips et al., 2012). These studies provide evidence that the extent

of brain atrophy in depression may be lessened or reversed in patients who achieve remission.

Finally, since treatment responders and nonresponders seemingly follow different structural trajectories over time, it is possible that structural differences present in individuals at treatment initiation (for example, smaller brain volume or thinner cortex) may predispose patients to poorer treatment response or vulnerability for relapse during treatment. Previous studies have shown smaller volume of the hippocampus (Frodl et al., 2004; MacQueen et al., 2008; Hoogenboom et al., 2013), and rostral middle frontal gyrus (Hoogenboom et al., 2013), and thinner posterior cingulate gyrus (Järnum et al., 2011) in pretreatment scans of patients who failed to achieve remission over follow-up compared to remitters.

In the present study, we prospectively examined cortical thickness and volume changes in specific cortical-limbic regions of interest (ROIs) in the same sample of patients with treatment-resistant depression previously examined by our group using whole-brain imaging techniques (Phillips et al., 2012). ROIs were selected a priori based on previous evidence of structural differences in these areas in MDD patients and comparison subjects, and potential associations between morphometric characteristics in these regions and treatment response or remission. The objectives were to determine whether the patients demonstrated cortical thickness or volume differences relative to healthy matched controls at baseline imaging; whether patients' brain structure at treatment initiation was associated with their clinical response over follow-up; and to compare longitudinal changes in cortical thickness and volume in patients who achieved sustained remission with nonremitters.

3.6 Methods

3.6.1 Participants

Twenty-eight outpatients with treatment-resistant depression (aged 18-65 years) were recruited from the Mood Disorders Research Unit at the Royal Ottawa Mental Health Centre, Ottawa, Ontario, Canada. This patient sample was previously reported by Phillips et al. (2012). Diagnosis of MDD was determined by psychiatric consultation on the basis of DSM-IV criteria (American Psychiatric Association, 1994). Treatment-resistance was defined as current episode illness duration of at least six months, failure to achieve remission after treatment with at least two antidepressants at adequate dosage for at least six weeks each, and presence of depressive symptoms corresponding to a Hamilton Rating Scale for Depression ($HDRS_{17}$; Hamilton, 1960) score ≥ 18 and a Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg, 1979) score ≥ 22. Exclusionary criteria for patients included diagnosis of post-traumatic stress disorder, any psychotic disorder, anorexia nervosa, or a history of manic, hypomanic or mixed episode. Twenty-nine age, gender and handedness-matched healthy control participants were recruited through community advertisement. Controls were free of psychiatric disorders confirmed through administration of the Scheduled Clinical Interview for DSM-IV-Nonpatient Edition (SCID-NP; First et al., 2002), and reported no history of mood or anxiety disorders among their first-degree relatives. Exclusion criteria for all participants were presence of major medical illnesses, neurological disorders, history of head injury with loss of consciousness, diagnosis of substance abuse or dependence, exposure to oral or intravenous steroids, IQ < 80, or contraindications to MRI. Handedness was evaluated with the Edinburgh Handedness Inventory (Oldfield,

1971).

All participants underwent baseline MR imaging at study inclusion (controls were examined with MRI only once at baseline). Patients underwent a second follow-up MRI scan after either a 6-month period of sustained remission (defined as MADRS score ≤ 12 at each visit) or after a 12-month period of failure to remit. All patients were receiving antidepressant treatment at time of image acquisition. Patients were followed longitudinally and treated with intensive pharmacotherapy under the care of study investigators with the goal of attaining remission. At study visits (once every two weeks), patients were assessed by administration of the MADRS, if an approximate 20% symptom improvement was not detected (Szegedi et al., 2009), an increase in doses (if tolerated), a change in medication, or an augmentation strategy was implemented. Medication choices were based on their different mechanisms of action and potential synergies on the serotonin, norepinephrine and dopamine systems (Blier, 2006). During follow-up, patients received individualized treatment with medications from the following classes: tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, selective norepinephrine reuptake inhibitors, dopamine agonists, atypical antipsychotics, and other medications including bupropion, mirtazapine, pindolol, trazodone, and lithium (see Phillips et al., 2012). No patients received electroconvulsive therapy during follow-up. The research protocol was approved by the Research Ethics Board of the Royal Ottawa Mental Health Centre. After complete description of the study to subjects, informed written consent was obtained.

3.6.2 Image acquisition and processing

T1-weighted magnetic resonance images were obtained at 1.5-T (Seimens Magnetom Symphony Systems, Siemens, Erlangen, Germany) using the same magnetization-prepared rapid gradient echo (MPRAGE) acquisition protocol (with TE =4.38 ms, TR =1500 ms, flip angle =15˚, field of view =250 mm, matrix =256x256, slice thickness =1 mm). Most baseline scans and all follow-up scans were obtained on the same scanner at St-Joseph MRI, Gatineau, Quebec, Canada. The baseline scans of 3 patients were obtained at the Ottawa Hospital, Ottawa, Ontario, Canada. MRI scans were reviewed by a licensed radiologist to rule out clinically significant neuroanatomical abnormalities.

Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite (version 4.5, http://surfer.nmr.mgh.harvard.edu) to derive measures of cortical thickness (Fischl and Dale, 2000), subcortical volume (Fischl et al., 2002), and total intracranial volume (Buckner et al., 2004). The technical details of these processing pipelines have been previously described in detail (Dale et al., 1999; Fischl et al., 1999). The cortical reconstructions for each participant were visually inspected for inaccuracies in segmentation and manually corrected as necessary by a single rater (J.L.P.) blind to subject identity, diagnostic group and time point. To measure changes in cortical thickness and volume in patients between baseline and follow-up scans, images were processed using the FreeSurfer longitudinal pipeline (Reuter et al., 2012).

3.6.3 Regions of interest

For this study, data was analyzed from 4 cortical and 1 subcortical region of

interest (ROI; Figure 3.1). Cortical surface gyri were labeled using the automated FreeSurfer cortical parcellation procedure (Desikan et al., 2006). Based on previous studies (Desikan et al., 2006; Kikinis et al., 2010), the rostral middle frontal gyrus was chosen as the gyral-based neuroanatomical representative of the dorsolateral prefrontal cortex (DLPFC, a functionally-defined area). The rostral middle frontal gyrus includes Brodmann area (BA) 46, the core component of the DLPFC (Rajkowska and Goldman-Rakic, 1995). For 2 of the cortical ROIs, prior to data extraction various cortical parcellations were combined to create customized ROIs. The anterior cingulate cortex (ACC) ROI included the rostral and caudal ACC parcellation labels. The medial and lateral orbitofrontal labels were combined to create the orbitofrontal cortex ROI. The inferior temporal gyrus parcellation label was used with no modifications. Mean cortical thickness values (calculated as the mean distance between the pial and gray/white matter surfaces across the specified region) were extracted from these 4 ROIs for each hemisphere. Volume was extracted from a single subcortical ROI, the hippocampus, defined using the FreeSurfer automated subcortical segmentation procedure (Fischl et al., 2002).

3.6.4 Statistical analysis

Statistical analyses were conducted using PASW Statistics version 18.0 (SPSS Inc., Chicago, Illinois). Demographic and clinical variables of patient and control groups, and remitter and nonremitter groups were compared with two-tailed independent samples *t* tests (for continuous variables) or chi square tests (for dichotomous variables). Within resultant patient groups (remitters and nonremitters), baseline and follow-up MADRS

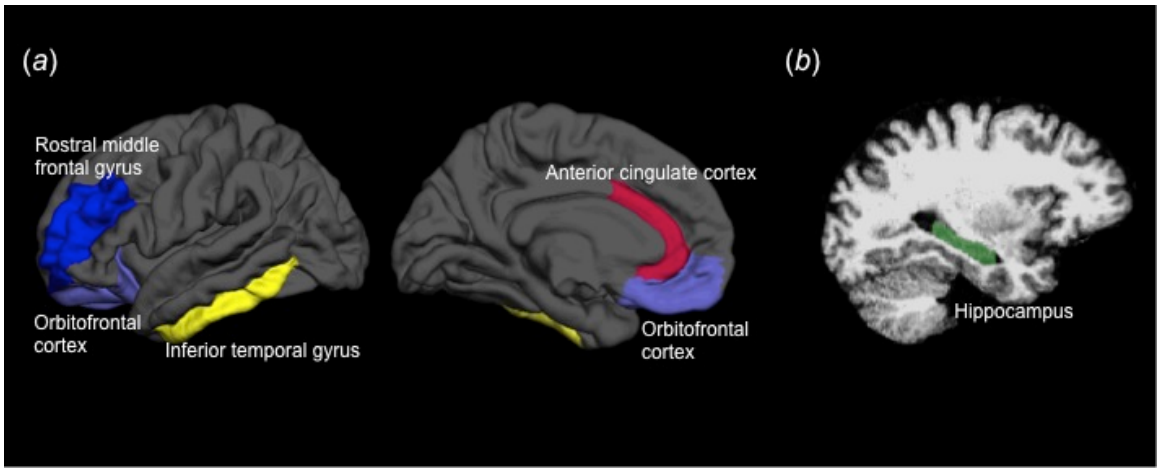

Figure 3.1. Regions of interest (ROIs) examined in this study. (*a*) Cortical surface-based ROIs rendered on the lateral (left) and medial (center) pial surface representations of the left hemisphere on an average brain. (*b*) The single subcortical ROI, the hippocampus, overlaid on a sagittal slice from a single participant (right).

scores were compared with paired *t* tests.

All neuroanatomical measures were examined for normality using the Shapiro-Wilk test. Cross-sectional analyses of volumetric and thickness data from each ROI were conducted to compare patient and control groups at baseline imaging. Baseline left and right hippocampal volumes were investigated using multivariate analysis of covariance (MANCOVA) with diagnosis (patient or control) as the independent variable, and total intracranial volume (TIV) and baseline scanner as covariates. Baseline mean cortical thickness values for each cortical ROI (rostral middle frontal gyrus, ACC, orbitofrontal cortex, inferior temporal gyrus) were examined by individual MANCOVAs adjusted for baseline scanner. The cortical thickness analyses were not adjusted for TIV since it does not affect the thickness of the cortical mantle (Buckner et al., 2004).

Among patients, exploratory analyses were conducted to test for correlations between baseline volume or mean cortical thickness measures in each ROI and change in MADRS scores over follow-up using Pearson correlation statistics.

Longitudinal analyses were conducted to compare volume and cortical thickness changes in patients who remitted over follow-up and nonremitters. Repeated measures ANCOVAs were conducted for each ROI with volume or mean cortical thickness as the dependent variable, hemisphere (left or right) and time (baseline or follow-up) as the within-subject variables, and remission status (nonremitter or remitter) as the between-subject variable, with interscan interval (number of days between scans), baseline scanner, and TIV (for volume analyses only) as covariates. The main effects for remission status and time, and the remission status x time interaction are reported for each ROI. Simple effects were obtained by deconstructing significant interactions.

A two-tailed P value < 0.05 was considered statistically significant for all comparisons unless otherwise noted. For cortical thickness analyses, main effects and interactions were considered significant after application of a Bonferroni correction for multiple comparisons with a significance level of $P < 0.01$ ($P < 0.05/4$ number of comparisons (cortical ROIs) in each hemisphere). For exploratory correlation analyses, results were reported at uncorrected P values ($P < 0.05$).

3.7 Results

3.7.1 Demographic and clinical characteristics

Following MR image acquisition, 3 participants were excluded from the study, one patient due to poor quality MRI data, one patient due to lack of follow-up data, and one control subject due to evidence of brain tumour. The final sample for this study consisted of 54 participants, including 26 patients with treatment-resistant MDD examined at baseline and follow-up, and 28 matched healthy controls examined at baseline only. Patient and control groups did not differ significantly on demographic variables (age, gender or handedness), or baseline total intracranial volume (TIV; Table 3.1).

Mean (± S.D.) duration of follow-up for patients was 379 (±88) days. During follow-up, 12 patients achieved sustained 6-month remission, while 14 patients failed to achieve sustained remission. Comparison of remitter and nonremitter groups (Table 3.1) revealed no differences in demographic variables, age at onset, or number of previous depressive episodes, highlighting the homogeneity of the patient sample. Remitters had significantly lower MADRS scores at baseline and follow-up imaging relative to

Table 3.1. Demographic, clinical and volumetric characteristics of study participants ($N = 54$).

	Group; mean (±S.D.)[a]					
Characteristics	Controls ($n = 28$)	Patients ($n = 26$)	P value[b]	Nonremitters ($n = 14$)	Remitters ($n = 12$)	P value[c]
Age, years	45.7 (10.6)	46.0 (10.4)	0.91	44.7 (10.5)	47.5 (10.6)	0.50
Gender, *n* male/female	10/18	8/18	0.70	3/11	5/7	0.40
Handedness, *n* right/left[d]	25/3	22/4	0.61	13/1	9/3	0.31
Baseline TIV, mm^3	1529301 (126250)	1526607 (163255)	0.95	1511361 (196469)	1544395 (119641)	0.62
Age at illness onset, years[e]	---	30.3 (14.1)	---	29.5 (14.5)	31.2 (13.5)	0.76
No. depressive episodes, A/B/C[f]	---	10/6/10	---	6/3/5	4/3/5	0.88
Baseline MADRS score	---	34.6 (7.0)	---	38.2 (5.2)	30.3 (6.4)	0.002
Follow-up MADRS score	---	16.7 (12.7)	---	26.6 (8.6)	5.3 (3.3)	<0.001
Change in MADRS score	---	-17.9 (11.8)	---	-11.7 (11.3)	-25.0 (8.0)	<0.001
Interscan interval[g]	---	379.4 (88.3)	---	421.1 (36.6)	330.8 (106.5)	0.007

[a]Unless otherwise indicated.
[b]Controls versus patients, Independent samples t test or X^2 test.
[c]Nonremitters versus remitters, Independent samples t test or X^2 test.
[d]Handedness was measured using Edinburgh Handedness Inventory (Hamilton, 1960).
[e]Data missing for 1 participant.
[f]Number of episodes prior to study enrollment expressed as categories: A = 1-2 episodes, B = 3-4 episodes, C = 5+ episodes.
[g]Number of days between scans.
Abbreviations: MADRS = Montgomery-Åsberg Depression Rating Scale, SD = standard deviation, TIV = total intracranial volume.

nonremitters. However, within-group paired *t* tests revealed significant decreases in MADRS scores over follow-up in both groups (remitters $t_{11} = 10.76$, $P < 0.001$; nonremitters $t_{13} = 3.89$, $P = 0.002$). Remitters also had shorter interscan intervals (days between MRI scans) compared to nonremitters (this variable was included as a covariate for all longitudinal analyses).

3.7.2 Cross-sectional imaging results

Morphometric data were normally distributed. Baseline hippocampal volumes and mean cortical thickness values for each ROI are presented in Table 3.2. Cross-sectional comparisons of patients and controls at baseline revealed no between-group differences in hippocampal volume or mean cortical thickness in the rostral middle frontal gyrus, ACC, orbitofrontal cortex, or inferior temporal gyrus (for each ROI no significant main effect of diagnosis).

Among patients, exploratory analyses were conducted to test for correlations between baseline volume or thickness measures and change in symptoms over follow-up. There was a significant negative correlation between patients' mean right anterior cingulate cortical thickness and change in MADRS score over follow-up ($r = -0.42$, $P = 0.035$; Figure 3.2). This indicates that thicker right ACC at baseline was associated with greater symptom improvement (larger decrease in MADRS scores). No significant correlations were observed between change in MADRS and baseline volume or mean cortical thickness in the other ROIs.

Table 3.2. Cross-sectional comparisons of volumetric and thickness data of study participants at baseline imaging ($N = 54$).

Region of Interest		Mean (±S.E.) Patients ($n = 26$)	Controls ($n = 28$)	F	df	P value[a]
		Volume (mm^3)				
Hippocampus	LH	4307.0 (67.3)	4344.6 (64.8)	0.16	1,50	0.70
	RH	4363.4 (63.1)	4394.7 (60.7)	0.12	1,50	0.73
		Mean cortical thickness (mm)				
Rostral middle frontal gyrus	LH	2.452 (0.03)	2.482 (0.03)	0.59	1,51	0.45
	RH	2.436 (0.02)	2.474 (0.02)	1.17	1,51	0.28
Anterior cingulate cortex	LH	2.781 (0.04)	2.878 (0.04)	2.86	1,51	0.10
	RH	2.704 (0.04)	2.766 (0.03)	1.51	1,51	0.22
Orbitofrontal cortex	LH	2.648 (0.03)	2.675 (0.03)	0.44	1,51	0.51
	RH	2.613 (0.03)	2.615 (0.03)	0.001	1,51	0.97
Inferior temporal gyrus	LH	2.884 (0.03)	2.948 (0.03)	1.96	1,51	0.17
	RH	2.891 (0.03)	2.950 (0.03)	2.04	1,51	0.16

[a]For each anatomical region of interest, individual multivariate analyses of covariance were conducted with volume or mean cortical thickness as the dependent variable, diagnosis (patient or control) as the independent variable and baseline scanner and total intracranial volume(for volumetric analyses only) as covariates.

Abbreviations: LH = left hemisphere, RH = right hemisphere, SE = standard error.

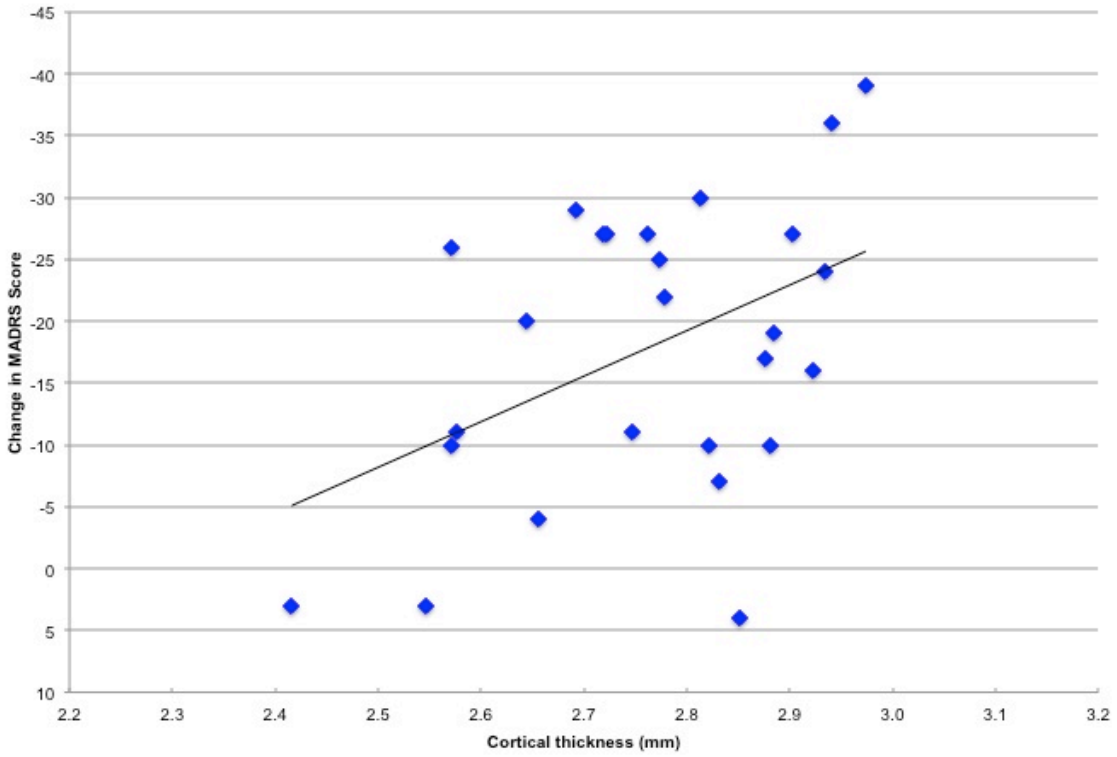

Figure 3.2. Correlation between patients' baseline mean cortical thickness of the right anterior cingulate cortex and their change in MADRS score over follow-up (where decrease in MADRS score indicates clinical improvement). Abbreviations: MADRS, Montgomery-Åsberg Depression Rating Scale.

3.7.3 Longitudinal imaging results

For the longitudinal imaging analyses, volume and cortical thickness changes over follow-up in the ROIs were examined in patients with repeated measures ANCOVAs (Table 3.3). Averaged across both time points, volume and thickness measures did not differ in remitted and nonremitted patient groups (there was no significant main effect of remission status for any ROI). Similarly, the changes in volume or thickness over follow-up were not statistically significant when considering the entire patient sample (both remitters and nonremitters; no significant main effect of time for any ROI survived correction for multiple comparisons [$P < 0.01$]).

There were however significant remission status x time interaction effects for hippocampal volume and rostral middle frontal gyrus and orbitofrontal cortical thickness. These significant interaction terms indicate that the mean paired differences in volume or cortical thickness from baseline to follow-up in remitters differed significantly from nonremitters (structural change over time differed according to patients' remission status). Graphical representation of patients' imaging measures at baseline and follow-up for each ROI for which the interaction effect was detected demonstrate morphological changes in opposing directions with a consistent pattern of increased volume or cortical thickness in remitters and decreased volume or thickness in nonremitters (Figure 3.3). Posthoc simple effects analyses revealed that the slight changes in volume or thickness seen in each group over follow-up were themselves not statistically significant (no significant main effect of time within either group [remitters or nonremitters] for any ROI showing a significant remission status x time interaction).

Table 3.3. Longitudinal analyses of volumetric and thickness data of patients over follow-up (n = 26)[a].

	Main effect of remission status			Main effect of time			Time x remission status interaction		
Morphometric Variable	*F*	df	*P value*	*F*	df	*P value*	*F*	df	*P value*
Volume									
Hippocampus	0.12	1,21	0.737	3.76	1,21	0.066	8.15	1,21	0.009
Mean Cortical Thickness									
Rostral middle frontal gyrus	0.54	1,22	0.470	3.98	1,22	0.059	10.07	1,22	0.004
Anterior cingulate cortex	0.29	1,22	0.593	0.83	1,22	0.373	3.01	1,22	0.097
Orbitofrontal cortex	0.60	1,22	0.447	6.01	1,22	0.023	9.31	1,22	0.006
Inferior temporal gyrus	0.09	1,22	0.762	0.05	1,22	0.835	5.57	1,22	0.028

[a]For each anatomical region of interest, individual repeated measures analyses of covariance were conducted with interscan interval, baseline scanner and total intracranial volume (for volumetric analyses only) as covariates.

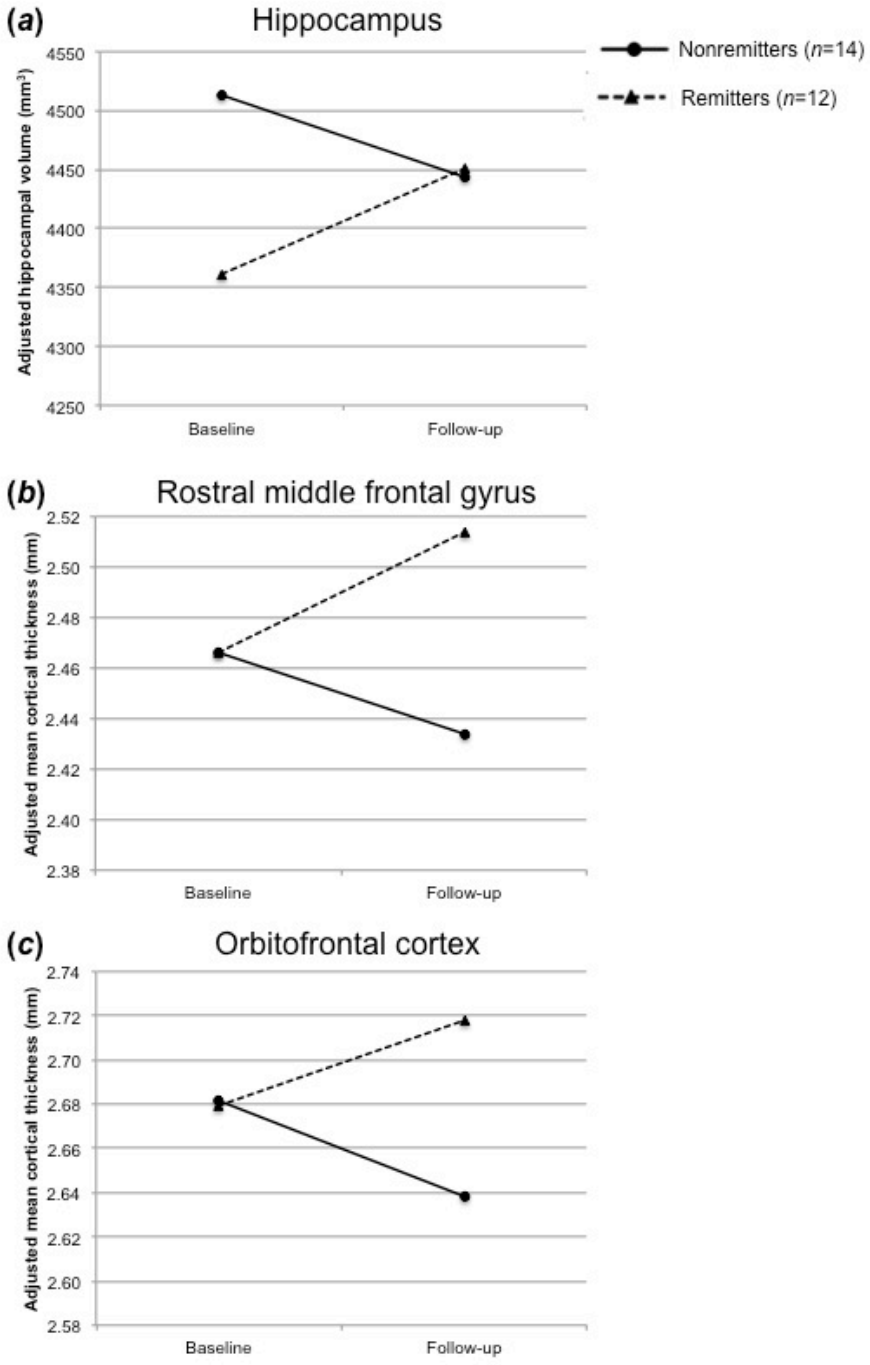

Figure 3.3. Graphical representation of the significant remission status x time interaction effects on volume of the hippocampus (*a*), and mean cortical thickness of the rostral middle frontal gyrus (*b*) and orbitofrontal cortex (*c*).

3.8 Discussion

There were three main findings in this study, 1) patients did not differ from control participants in volume or cortical thickness in any of the specified ROIs at baseline imaging, 2) among patients, thicker right anterior cingulate cortex at baseline was associated with greater symptom improvement over follow-up, and 3) the direction of longitudinal hippocampus volume and rostral middle frontal gyrus and orbitofrontal cortical thickness changes in patients differed according to their remission status, with increasing volume or thickness in remitters over time and decreasing volume or thickness in nonremitters.

In contrast to meta-analyses demonstrating smaller hippocampal volume among MDD patients relative to controls (Campbell et al., 2004; Videbech and Ravnkilde, 2004; Koolschijn et al., 2009; Arnone et al., 2012), the cross-sectional comparison of patients and controls at baseline imaging revealed no differences in hippocampal volume. There were also no between-group differences in mean cortical thickness in the surface ROIs examined in this study. In recent years, several groups have found reduced cortical thickness in patients in prefrontal regions, including the orbitofrontal cortex (Järnum et al., 2011; Tu et al., 2012; Grieve et al., 2013; Na et al., 2014), DLPFC (van Tol et al., 2014), and rostral middle frontal gyrus (Tu et al., 2012; Na et al., 2014). However, all these studies used vertex-based analyses (where cortical thickness values are compared at each vertex across the entire cortical surface) whereas this study employed a ROI approach and examined mean cortical thickness values. Reported clusters of decreased cortical thickness in vertex-based analyses may be very regionally specific and it is

possible that such differences may not be detectable when cortical thickness values are averaged across regions.

The exploratory correlation analyses demonstrated an association between patients' cortical thickness in the right ACC at treatment initiation and their change in MADRS scores over follow-up, with thicker ACC at baseline predicting greater symptom improvement. Numerous functional imaging modalities (electroencephalogram, functional MRI, and PET) have demonstrated the importance of pretreatment rostral ACC activity in the prediction of pharmacological response in MDD patients (reviewed in Pizzagalli, 2011). Regarding structural imaging, there is some evidence for the predictive potential of the structural correlates of the ACC for antidepressant treatment response. Previous studies have shown prediction of clinical response to pharmacotherapy among patients in association with increased cortical depth of the subgenual ACC (Coryell et al., 2005), greater GM volume in the right rostral ACC (Costafreda et al., 2009), and diffusion tensor imaging measures of white matter connectivity between the subgenual ACC and limbic regions (Korgaonkar et al., 2014). Within the other ROIs, there were no further correlations identified between any neuroanatomical measure and change in clinical symptoms. Thus, the effect appears specific to the ACC although as the analyses were not corrected for multiple comparisons, these results should be interpreted with caution.

In our previous report of whole-brain volume change in this patient sample, sustained remitters showed overall brain volume increase while nonremitters lost brain volume over follow-up (Phillips et al., 2012). In the longitudinal analyses, similar results were seen in several ROIs; with remitted and nonremitted patients demonstrating subtle

increases and decreases respectively in hippocampal volume, and cortical thickness in the rostral middle frontal gyrus and orbitofrontal cortex. Posthoc analyses revealed that the magnitude of the changes in volume and cortical thickness observed over time within each group were themselves not statistically significant. Rather, it was the finding that the structural changes occurred in opposing directions according to patients' remission status over follow-up that was significant.

The volume and thickness changes were small which may indicate that a longer follow-up period is necessary to elicit marked structural changes and to determine whether the trajectories of morphometric change observed in remitters and nonremitters continue as time progresses. Frodl and colleagues investigated hippocampal volume changes in a cohort of MDD patients after 1- and 3-year follow-up periods. At 1-year, they found no changes in hippocampal volume (Frodl et al. 2004), yet at 3-year follow-up, they found a modest increase in hippocampal volume in patients who had received consistent antidepressant treatment over the entire study period (Frodl et al., 2008a). In the present study, all patients received intensive pharmacotherapy over follow-up yet only remitters showed increasing volume or thickness over time, suggesting that remission itself may be driving structural recovery rather than antidepressant treatment. This distinction may be important for the hippocampus as the only other studies to document hippocampal volume increases over time in MDD patients failed to include either nonremitted patients (Ahdidan et al., 2011), or treatment non-responders (Schermuly et al., 2011). Nevertheless, although we found increasing orbitofrontal cortical thickness only in remitters (with cortical thinning in nonremitters), Järnum et al. (2011) found increased cortical thickness in the orbitofrontal cortices over 6-month

follow-up in their full patient sample which included both remitted and nonremitted patients. This finding supports an effect of antidepressant treatment on cortical thickness changes in the orbitofrontal cortex. However, the consistent pattern of decreasing volume or cortical thickness in nonremitters in this study suggests that for certain brain regions or morphometric measures, antidepressant-mediated structural recovery may only occur if patients respond clinically to the treatment.

Several questions persist regarding the relationship between structural changes and treatment response in depression. It is not known whether normalization of atrophic changes are necessary for patient response to antidepressants, or whether response to treatment, and thus, alleviation of continuing depressive symptoms contribute to the structural recovery seen in remitted patients. Furthermore, while there are a number of potential mechanisms that may contribute to volume or cortical thickness increases observed with MRI in antidepressant-treated remitted patients (including regeneration of neurons, increase in glial cell numbers, synaptogenesis, larger neuropil volume, more blood vessels, or less apoptosis); any discussion of these possibilities remains speculative and further research is necessary to address these questions.

The design of this study permitted the investigation of longitudinal volume and cortical thickness changes in patients according to their response to treatment. It elicited different information than would be obtained by cross-sectional comparison of remitted patients with currently ill patients, or comparison of how patients' brain structure changes over time relative to a healthy control group without consideration of their clinical outcome. Despite these strengths, this study has certain limitations that merit comment. There was no follow-up imaging of control participants, which precludes the possibility

of studying normal progressive volume and cortical thickness changes in healthy individuals over the time period investigated. Further, as all patients were treated at baseline imaging and received pharmacotherapy throughout the follow-up period, the longitudinal effects of untreated depression on brain structure (separate from any pharmacological effects) could not be studied. Similarly, due to the naturalistic treatment approach used, the individual effects of any particular medications or augmentation strategies on volume or thickness changes could not be analyzed. Although the latter is less of a concern for this particular study as the goal was to investigate the effects of overall clinical outcome (remission status) on longitudinal structural change. Finally, the study contained a relatively small sample size, although the patient group was very homogenous and well characterized clinically.

In summary, our findings suggest that cortical thickness of the right ACC may be associated with treatment-resistant MDD patients' change in depressive symptoms over follow-up. Further, the results indicate that while the patient sample did not differ from healthy controls on baseline volume or cortical thickness in the ROIs examined, over time patients did demonstrate subtle structural changes, the direction of which varied according to their remission status over follow-up. Remitted patients showed a pattern of increasing volume or thickness over follow-up in several ROIs, while nonremitters demonstrated decreasing volume and cortical thinning over time. Although the longitudinal changes themselves were small in magnitude, the opposing direction of change seen in the two patient groups is an interesting finding that warrants further study.

CHAPTER 4

Impact of Monoamine-Related Gene Polymorphisms on Hippocampal Volume in Treatment-Resistant Depression

4.1 Overview

The second manuscript in this thesis included a comparison of the morphometric characteristics of the patient sample and a matched healthy control group at baseline imaging. Results revealed no differences in hippocampal volume in patients relative to controls. In the present paper, a cross-sectional approach was used to further examine the effects of depression and genetic variation on the volume of the hippocampus in study participants. The specific objectives were to determine whether there was an association between certain genes in the monoaminergic system and baseline hippocampal volume, and, if so, whether the association differed in patients compared to controls.

4.2 Statement of author contribution

The experimental design of this study was drafted by Pierre Blier. Patients were diagnosed and clinically assessed by Pierre Blier, Fahad Aldosary, and Philippe Tremblay. Healthy control recruitment and screening was performed by Lisa Batten. Investigated gene polymorphisms were selected by Pierre Blier and Jennifer Phillips. Genetic analyses were conducted by Lisheng Du. Imaging and statistical analyses were performed by Jennifer Phillips. The article was written by Jennifer Phillips. All authors critically reviewed and approved of the final manuscript. Further contributions by acknowledged collaborators included design of the MRI acquisition sequence by Andra Smith, and study coordination, patient recruitment, and blood draws by Chantal Hébert. This study was supported by a University of Ottawa Medical Research Fund grant awarded to Pierre Blier.

4.3 Title page

Impact of monoamine-related gene polymorphisms on hippocampal volume in treatment-resistant depression

Jennifer L. Phillips[1,2], Lisa A. Batten[1], Philippe Tremblay[1], Fahad Aldosary[1], Lisheng Du[1], and Pierre Blier[1,2]

[1]University of Ottawa Institute of Mental Health Research, Ottawa, Canada

[2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada

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4.4 Abstract

Objective: In major depressive disorder (MDD), single nucleotide polymorphisms (SNPs) in monoaminergic genes may impact disease susceptibility, treatment response, and brain volume. The objective of this study was to examine the effect of such polymorphisms on hippocampal volume in patients with treatment-resistant MDD and healthy controls. Candidate gene risk alleles were hypothesized to be associated with reductions in hippocampal volume.

Methods: Twenty-six outpatients with treatment-resistant MDD and 27 matched healthy controls underwent magnetic resonance imaging (MRI) and genotyping for 6 SNPs in monoaminergic genes (serotonin transporter [*SLC6A4*], norepinephrine transporter [*SLC6A2*], serotonin 1A and 2A receptors [*HTR1A* and *HTR2A*], catechol-O-methyltransferase [*COMT*], and brain-derived neurotrophic factor [*BDNF*]). Hippocampal volume was estimated using an automated segmentation algorithm (FreeSurfer).

Results: Hippocampal volume did not differ between patients and controls. Within the entire study sample irrespective of diagnosis, C allele-carriers for both the NET -182T/C [rs2242446] and 5-HT_{1A} -1019C/G [rs6295] polymorphisms had smaller hippocampal volumes relative to other genotypes. For the 5-HTTLPR (rs25531) polymorphism, there was a significant diagnosis by genotype interaction effect on hippocampal volume. Among patients only, homozygosity for the 5-HTTLPR short (S) allele was associated with smaller hippocampal volume. There was no association between the *5HT2A*, *COMT* and *BDNF* SNPs and hippocampal volume.

Conclusion: The results indicate that the volume of the hippocampus may be influenced by serotonin- and norepinephrine-related gene polymorphisms. The NET and 5-HT_{1A} polymorphisms appear to have similar effects on hippocampal volume in patients and controls while the 5-HTTLPR polymorphism differentially affects hippocampal volume in the presence of depression.

4.5 Significant outcomes

- Single nucleotide polymorphisms in the norepinephrine transporter and serotonin 1A receptor genes were associated with hippocampal volume in patients with major depressive disorder and controls.
- Homozygosity for the short (S) allele of the serotonin transporter polymorphism (5-HTTLPR) was associated with smaller hippocampal volume in patients with major depressive disorder, suggesting an effect of the risk allele only in the presence of depression.

4.6 Limitations

- Given the small sample size the results should be interpreted with caution.
- Genetic effects on hippocampal volume are likely the result of the interaction of multiple genes and the study did not investigate gene-gene interaction effects.

4.7 Introduction

A number of studies have recently been conducted to investigate the pharmacogenetics of antidepressant response; namely, how an individual's genetic makeup affects how he or she responds to antidepressant drugs. Of particular interest for major depressive disorder (MDD) are single nucleotide polymorphisms (SNPs) in genes involved in the synthesis, transport, signal transduction and degradation of monoamines as the monoaminergic systems are the primary targets of all currently available classes of antidepressants. Genetic variation within the monoaminergic system has been shown to be associated with susceptibility to the development of mood disorders, alterations in patients' response to treatment (Kato and Serretti, 2010) and various imaging phenotypes (Scharinger et al., 2011).

Most depressed patients will fail to achieve complete remission upon a first trial of antidepressant treatment and are left with no response or significant residual symptoms. The potential consequences of continuing depressive symptoms include decreased recovery rates as the length of depressive episodes increase and greater likelihood of future depressive episodes (McIntyre et al., 2014). Given the lengthy duration required to evaluate the effects of an antidepressant trial, there is a need to identify drugs with a higher probability of success to avoid further treatment delay in resistant patients. At least some of the variation in pharmacological outcome in MDD is thought to have a genetic basis (Kato and Serretti, 2010); thus, a treatment-resistant sample may be rich in risk alleles for candidate genes involved in antidepressant response.

Monoamine-related gene polymorphisms have also been associated with various

characteristics measured using neuroimaging (Scharinger et al., 2011). A recent large magnetic resonance imaging (MRI) study of healthy individuals revealed an inverse association between hippocampal volume and severity of self-reported depressive symptoms (Brown et al., 2014). Moreover, meta-analyses of cross-sectional MRI studies have reported reduced volume of the hippocampus among MDD patients relative to controls (Campbell et al., 2004; Videbech and Ravnkilde, 2004; Koolschijn et al., 2009; McKinnon et al., 2009; Arnone et al., 2012). Recent findings suggest that genetic factors may modulate stress-related changes in hippocampal volume in depression (Frodl et al., 2008c). Furthermore, hippocampal volume is associated with various clinical factors related to treatment course, including patients' age of disease onset (Lloyd et al., 2004), number of previous depressive episodes and duration of illness (McKinnon et al., 2009), treatment responsiveness (Frodl et al., 2004), speed of treatment response (Sheline et al., 2012), and remission status (Arnone et al., 2013).

The present study investigated the relationship between certain monoamine-related gene variants and hippocampal volume in patients with treatment-resistant depression and healthy controls. Genes were selected based on their involvement in the mechanism of action of antidepressant drugs and previous evidence of their potential association with treatment response in MDD. Among these were genes encoding monoamine transporters: *SLC6A4* (serotonin transporter [5-HTT]), *SLC6A2* (norepinephrine transporter [NET]); serotonin receptors: *HTR1A* (5-HT_{1A}), and *HTR2A* (5-HT_{2A}); a monoamine metabolic enzyme: *COMT* (catechol-O-methyltransferase); and *BDNF* (brain-derived neurotrophic factor).

4.7.1 Aims of the study

The aims of this study were to examine the prevalence of monoamine-related polymorphisms in a sample of patients with treatment-resistant depression relative to healthy controls, and to compare gene effects on hippocampal volumes in patient and control groups. Candidate gene risk alleles were hypothesized to be associated with reductions in hippocampal volume.

4.8 Materials and methods

4.8.1 Participants

Twenty-eight outpatients with treatment-resistant depression (aged 18-65 years) were recruited from the Mood Disorders Research Unit at the Royal Ottawa Mental Health Centre, Ottawa, Ontario, Canada. This patient sample was previously reported by Phillips et al. (2012). Diagnosis of MDD was established by psychiatric consultation on the basis of DSM-IV criteria (American Psychiatric Association, 1994). Classification of treatment-resistance was based on current episode illness duration of at least six months, failure to achieve remission after treatment with at least two antidepressants of different classes at adequate dosage for at least six weeks each, and presence of depressive symptoms corresponding to a Hamilton Rating Scale for Depression ($HDRS_{17}$; Hamilton, 1960) score $\geq$ 18 and a Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg, 1979) score $\geq$ 22. Diagnosis of post-traumatic stress disorder, any psychotic disorder, anorexia nervosa, or a history of manic, hypomanic or mixed episode were exclusionary criteria for patients. All patients were receiving antidepressant treatment at time of MR image acquisition.

Twenty-nine age, gender, and handedness-matched healthy controls were recruited from the community through advertisement. Controls were free of psychiatric disorders confirmed through administration of the Scheduled Clinical Interview for DSM-IV-Nonpatient Edition (SCID-NP; First et al., 2002), and reported no history of mood or anxiety disorders among their first-degree relatives. Exclusion criteria for all participants were presence of major medical illnesses, neurological disorders, history of head injury with loss of consciousness, diagnosis of substance abuse or dependence, exposure to oral or intravenous steroids, IQ < 80, and contraindications to MRI. Handedness was evaluated with the Edinburgh Handedness Inventory (Oldfield, 1971). Participants underwent MR imaging and blood draw for genetic testing at study inclusion. The research protocol was approved by the Research Ethics Board of the Royal Ottawa Mental Health Centre. After complete description of the study to subjects, informed written consent was obtained.

4.8.2 Image acquisition, processing and analysis

T1-weighted magnetic resonance images were obtained on a 1.5 T scanner (Seimens Magnetom Symphony Systems, Siemens, Erlangen, Germany) using the same magnetization-prepared rapid gradient echo (MPRAGE) acquisition protocol: repetition time 1500 ms, echo time 4.38 ms, flip angle 15˚, field of view 250 mm, matrix 256 x 256, slice thickness 1 mm. The scans of all controls (n = 27) and most patients (n = 23) were obtained on the same scanner at St-Joseph MRI, Gatineau, Quebec, Canada, while the scans of 3 patients were obtained at the Ottawa Hospital, Ottawa, Ontario, Canada. MRI

scans were reviewed by a licensed radiologist to rule out clinically significant neuroanatomical abnormalities.

Images were processed and analyzed with the FreeSurfer image analysis suite, version 4.5 (http://surfer.nmr.mgh.harvard.edu; Dale et al., 1999; Fischl et al., 1999) to automatically generate volume estimates for subcortical regions (Fischl et al., 2002). The cortical reconstructions for each participant were visually inspected for inaccuracies in segmentation and manually corrected if necessary by a single rater blind to subject identity, diagnostic group and time point. Automated hippocampal segmentation by FreeSurfer has been shown to be comparable to manual tracing (Cherbuin et al., 2009; Morey et al., 2009; Sánchez-Benavides et al., 2010; Doring et al., 2011). Estimates of total intracranial volume were obtained from FreeSurfer (Buckner et al., 2004).

4.8.3 Genotyping

DNA was isolated from whole blood for polymerase chain reaction analyses using standard phenol extraction methods. DNA was genotyped for 6 polymorphic variants selected based on previous evidence of potential associations between the candidate gene and MDD, and the specific SNP and treatment response in MDD patients (Table 4.1; Serretti et al., 2007; Kato and Serretti, 2010). For the 5-HTTLPR (serotonin transporter-linked polymorphic region) polymorphism, participants were classified as homozygous for the long allele (L/L genotype) or short allele (S/S genotype), or heterozygous (L/S genotype). Of the two functional variants of the L allele (L_A and L_G; Nakamura et al., 2000), the L_G allele expresses serotonin at levels comparable to the S allele (Hu et al., 2006), thus L_G alleles were reclassified as S alleles. For the remaining polymorphisms,

Table 4.1. Selected candidate gene polymorphisms.

Gene	Polymorphism	dbSNP ID
Serotonin transporter - *SLC6A4*	5-HTTLPR	rs25531
Norepinephrine transporter - *SLC6A2*	NET -182T/C	rs2242446
Serotonin 1A receptor - *HTR1A*	5-HT_{1A} -1019C/G	rs6295
Serotonin 2A receptor - *HTR2A*	5-HT_{2A} -102T/C	rs6313
Catechol-O-methyltransferase - *COMT*	COMT Val158Met	rs4680
Brain-derived neurotrophic factor - *BDNF*	BDNF Val66Met	rs6265

individuals were classified as homozygous for the major allele, heterozygous, or homozygous for the minor allele. *BDNF* (brain-derived neurotrophic factor) Val/Met and Met/Met genotypes were collapsed under a single heading (Met-carrier) due to the scarcity of Met/Met homozygotes in the sample.

4.8.4 Statistical analysis

Comparison of demographic variables (age, gender, handedness) and total intracranial volume (TIV) of patient and control groups were examined by independent samples *t* tests (for continuous variables) or χ^2 tests (for dichotomous variables). Distribution differences of genotype frequencies between patients and controls were examined by χ^2 test. The χ^2 test was used to assess for Hardy-Weinberg equilibrium.

Left and right hippocampal volumes of patients and controls were compared through multivariate analysis adjusted for TIV and scanner. The genotype variant effects on hippocampal volume in patients and controls were investigated using individual analyses of covariance (ANCOVA) for each individual genetic polymorphism, with left and right hippocampal volume as the dependent variables, diagnosis (patient or control), and genotype (homozygous for the major allele, heterozygous, or homozygous for the minor allele) as independent variables, and TIV and scanner as covariates. Post-hoc *t* tests, Bonferroni-corrected for multiple comparisons, were used to compare hippocampal volume among resultant diagnostic or genotype groups. In the case of significant diagnosis by genotype interaction effects, separate ANCOVAs were conducted for patient and control groups with the variables and covariates as described above. All

statistical analyses were conducted using PASW Statistics, version 18.0 (SPSS Inc, Chicago, Illinois). A P value < 0.05 was considered significant for all comparisons.

4.9 Results

Complete imaging and genetic data were available for 53 study participants (26 patients and 27 controls). Two participants were excluded following MR image acquisition, 1 patient due to poor quality MRI data, and 1 control subject due to evidence of brain tumour. Additionally, 2 subjects (1 patient and 1 control) were excluded for failure to provide blood samples. Patient and control groups did not differ significantly on age, gender or handedness (Table 4.2).

Genotype frequencies did not differ between patient and control groups (Table 4.3). All polymorphisms were in Hardy-Weinberg equilibrium ($P > 0.05$).

Patients and controls did not differ in total intracranial volume (Table 4.2). Multivariate analysis adjusted for TIV and scanner revealed no significant main effect of diagnosis on hippocampal volume in the left ($F_{1,52} = 0.27$, $P = 0.60$) or right hemisphere ($F_{1,52} = 0.16$, $P = 0.69$). This indicates that patient and control groups had similar hippocampal volumes (Table 4.2).

In the entire study sample, multivariate ANCOVA revealed no significant main effect of 5-HTTLPR genotype on left or right hippocampal volumes (Table 4.4), indicating similar hippocampal volumes among individuals with 5-HTTLPR L/L, L/S and S/S genotypes. There were, however, significant diagnosis by 5-HTTLPR genotype interactions for the left and right hippocampus (Table 4.4), indicating that 5-HTTLPR genotype had differing effects on hippocampal volume in the patient and control groups.

Table 4.2. Demographic, clinical and volumetric characteristics of study participants.

	Group; mean (±SD)[a]		
Characteristics	Patients (n = 26)	Controls (n = 27)	P value[b]
Age, years	46.0 (10.4)	45.4 (10.7)	0.83
Gender, n male:female	8:18	9:18	0.84
Handedness, n right:left[c]	22:4	24:3	0.65
Age at illness onset, years	30.3 (13.8)		
MADRS score	34.6 (7.0)		
No. depressive episodes, A/B/C[d]	10/6/10		
Total intracranial volume, mm^3	1526600 (163300)	1536100 (123200)	0.81
Left hippocampal volume, mm^3	4305 (458)	4373 (356)	0.60
Right hippocampal volume, mm^3	4369 (377)	4406 (383)	0.69

[a]Unless otherwise indicated.

[b]Independent samples t test or X^2 test.

[c]Handedness was measured using the Edinburgh Handedness Inventory (Hamilton, 1960).

[d]Number of episodes prior to study enrollment expressed as categories: A = 1-2 episodes, B = 3-4 episodes, C = 5+ episodes

Abbreviations: MADRS = Montgomery-Åsberg Depression Rating Scale (Montgomery and Åsberg, 1979), SD = standard deviation.

Table 4.3. Genotype distributions of monoamine-related gene polymorphisms in patients and controls

SNP; group	Genotype, *n* (%)			*P* value[a]
5-HTTLPR	L/L	L/S	S/S	
All participants	14 (26.4)	22 (41.5)	17 (32.1)	
Patients	9 (34.6)	9 (34.6)	8 (30.8)	0.39
Controls	5 (18.5)	13 (48.2)	9 (33.3)	
NET -182T/C	T/T	T/C	C/C	
All participants	34 (64.1)	19 (35.9)	0 (0.0)	
Patients	15 (57.7)	11 (42.3)	0 (0.0)	0.34
Controls	19 (70.4)	8 (29.6)	0 (0.0)	
5-HT_{1A} -1019C/G	C/C	C/G	G/G	
All participants	15 (28.3)	27 (50.9)	11 (20.8)	
Patients	8 (30.8)	13 (50.0)	5 (19.2)	0.92
Controls	7 (25.9)	14 (51.9)	6 (22.2)	
5-HT_{2A} -102T/C	T/T	T/C	C/C	
All participants	17 (32.1)	23 (43.4)	13 (24.5)	
Patients	7 (26.9)	10 (38.5)	9 (34.6)	0.24
Controls	10 (37.0)	13 (48.1)	4 (14.9)	
COMT Val158Met	Val/Val	Val/Met	Met/Met	
All participants	16 (30.2)	24 (45.3)	13 (24.5)	
Patients	8 (30.8)	10 (38.4)	8 (30.8)	0.51
Controls	8 (29.6)	14 (51.9)	5 (18.5)	
BDNF Val66Met	Val/Val	Val/Met	Met/Met	
All participants	40 (75.5)	12 (22.6)	1 (1.9)	
Patients	21 (80.8)	5 (19.2)	0 (0.0)	0.38
Controls	19 (70.4)	7 (25.9)	1 (3.7)	

[a]Compared with the control group by X^2 test.

Abbreviations: 5-HT_{1A} = serotonin 1A receptor, 5-HT_{2A} = serotonin 2A receptor, 5-HTTLPR = serotonin transporter-linked polymorphic region, BDNF = brain-derived neurotrophic factor, COMT = catechol-O-methyltransferase, NET = norepinephrine transporter, SNP = single nucleotide polymorphism.

Table 4.4. Investigations of genotype variant effects on hippocampal volume in patients and controls.[a]

		Main effect of diagnosis			Main effect of genotype			Diagnosis x genotype interaction		
SNP		*F*	*df*	*P* value	*F*	*df*	*P* value	*F*	*df*	*P* value
5-HTTLPR	LH	1.03	1,52	0.32	0.60	2,52	0.56	6.35	2,52	0.004
	RH	1.06	1,52	0.31	1.12	2,52	0.34	4.65	2,52	0.015
NET -182T/C	LH	0.07	1,52	0.79	4.41	1,52	0.04	0.01	1,52	0.92
	RH	0.02	1,52	0.90	3.89	1,52	0.055	0.95	1,52	0.34
5-HT_{1A} -1019C/G	LH	0.01	1,52	0.94	1.76	2,52	0.19	1.01	2,52	0.35
	RH	0.02	1,52	0.89	5.88	2,52	0.005	1.00	2,52	0.38
5-HT_{2A} -102T/C	LH	0.43	1,52	0.52	0.52	2,52	0.60	0.05	2,52	0.95
	RH	0.53	1,52	0.47	0.69	2,52	0.51	0.52	2,52	0.60
COMT Val158Met	LH	0.12	1,52	0.73	0.55	2,52	0.58	0.04	2,52	0.96
	RH	0.06	1,52	0.82	0.36	2,52	0.70	0.02	2,52	0.98
BDNF Val66Met	LH	0.11	1,52	0.74	0.03	1,52	0.86	0.06	1,52	0.81
	RH	0.19	1,52	0.66	0.01	1,52	0.94	0.04	1,52	0.84

[a]Individual analyses of covariance for each genetic polymorphism, with left and right hippocampal volume as dependent variables, diagnosis, and genotype as independent variables, and total intracranial volume and scanner as covariates.

Abbreviations: Abbreviations: 5-HT_{1A} = serotonin 1A receptor, 5-HT_{2A} = serotonin 2A receptor, 5-HTTLPR = serotonin transporter-linked polymorphic region, BDNF = brain-derived neurotrophic factor, COMT = catechol-O-methyltransferase, LH = left hemisphere, NET = norepinephrine transporter, RH = right hemisphere, SNP = single nucleotide polymorphism.

Analyses of covariance conducted separately on patients and controls indicated significant main effects of 5-HTTLPR genotype on hippocampal volumes in both groups. In controls, there was a significant main effect of 5-HTTLPR genotype on right hippocampal volume ($F_{2,26} = 5.38$, $P = 0.01$), with Bonferroni-corrected post-hoc *t* tests revealing significantly larger right hippocampal volume among controls with 2 copies of the 5-HTTLPR S allele relative to those with only one copy (5-HTTLPR L/S genotype; $P = 0.01$; Figure 4.1A). The effect of the 5-HTTLPR genotype on left hippocampal volumes in the control group did not reach statistical significance ($F_{2,26} = 2.67$, $P = 0.09$). In patients, there was a significant main effect of 5-HTTLPR genotype on left hippocampal volume ($F_{2,25} = 3.55$, $P = 0.04$), with Bonferroni-corrected post-hoc *t* tests revealing significantly smaller left hippocampal volume among patients with the 5-HTTLPR S/S genotype relative to 5-HTTLPR L/S genotype ($P = 0.04$; Figure 4.1B), while the effect of the 5-HTTLPR genotype on right hippocampal volumes was not significant ($F_{2,25} = 1.17$, $P = 0.33$).

Multivariate ANCOVA revealed a significant main effect of NET -182T/C genotype on left hippocampal volumes in study participants and a near significant effect on the right hippocampus (Table 4.4). Post-hoc Bonferroni-corrected *t* tests revealed significantly larger left hippocampal volume among NET -182T/C T/T homozygotes relative to T/C heterozygotes ($P = 0.04$; Figure 4.2).

There was also a significant main effect of 5-HT_{1A} -1019C/G genotype on right hippocampal volumes in study participants (Table 4.4). Post-hoc Bonferroni-corrected *t* tests revealed significantly larger right hippocampal volume among individuals with the 5-HT_{1A} -1019C/G G/G genotype relative to C/C homozygotes ($P = 0.005$) and C/G

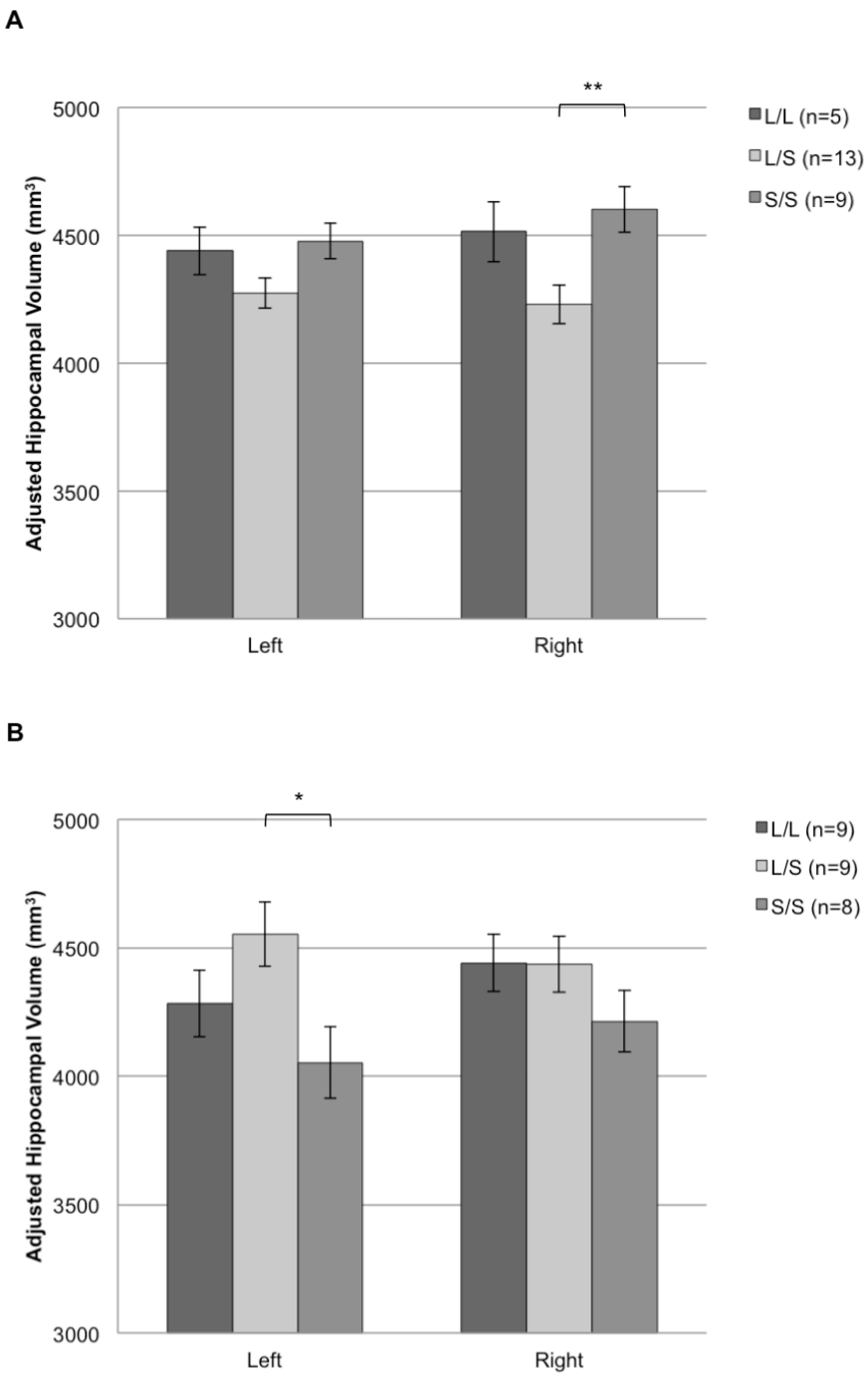

Figure 4.1. Total intracranial volume-adjusted left and right hippocampal volumes by 5-HTTLPR genotype in (A) controls (n = 27), and (B) patients (n = 26). The data are expressed as mean and standard error of the mean. *P < 0.05; **P < 0.01.

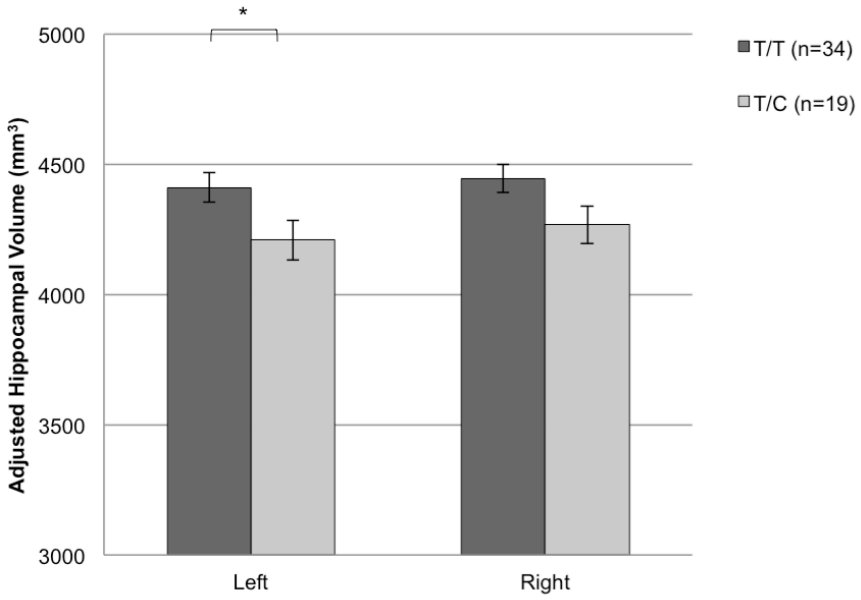

Figure 4.2. Total intracranial volume-adjusted left and right hippocampal volumes by NET -182T/C genotype in study participants (n = 53). The data are expressed as mean and standard error of the mean. *$P < 0.05$.

heterozygotes ($P = 0.03$; Figure 4.3).

Individual ANCOVAs for 5-HT_{2A} -102T/C, COMT Val158Met, and BDNF Val66Met revealed no significant main effects or diagnosis by genotype interaction effects on hippocampal volume (Table 4.4).

4.10 Discussion

Two main findings emerged from this pilot study: first, there was no identified effect of illness on hippocampal volumes; and second, three of the monoaminergic SNPs were associated with hippocampal volume. There was a relationship between homozygosity for the 5-HTTLPR S allele and reduced hippocampal volume among patients, and in the entire study sample, the NET -182T/C and 5-HT_{1A} -1019G/C SNPs were associated with hippocampal volume.

The 5-HTTLPR S allele is associated with reduced transcriptional activity of the 5-HTT promoter and diminished serotonin activity (Lesch et al., 1996). Several lines of evidence suggest that the short variant of 5-HTTLPR confers vulnerability to the development of depression especially in the presence of stressful life events (Karg et al., 2011). A meta-analysis has reported poorer and more delayed response to selective serotonin reuptake inhibitors among MDD patients homozygous for the 5-HTTLPR S allele (Serretti et al., 2007). In the current study, homozygosity for the S allele was associated with reduced hippocampal volumes in patients but not in controls. While some studies have failed to find an association between 5-HTTLPR and hippocampal volume (Hickie et al., 2007; Cole et al., 2011b), several other studies have found interactions between this SNP and other clinical and physiological factors which affect hippocampal

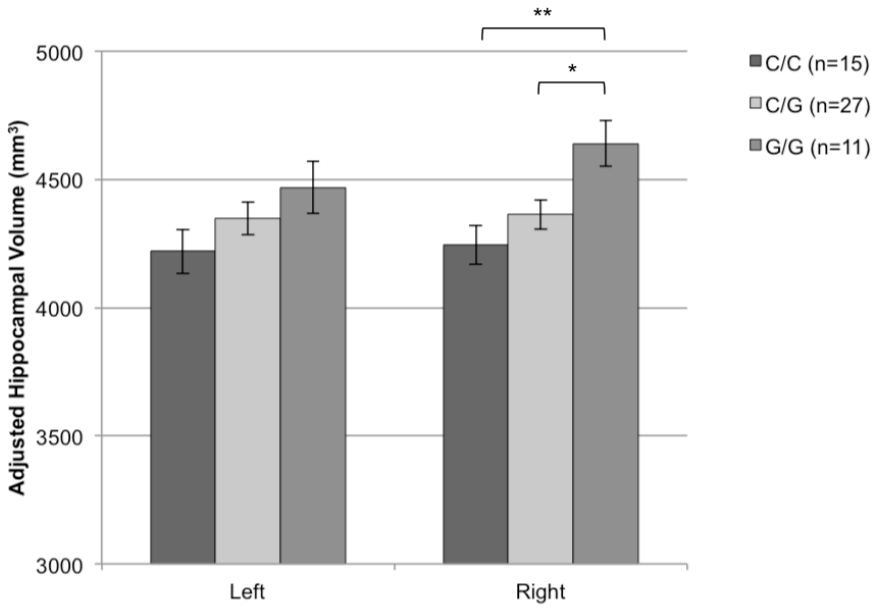

Figure 4.3. Total intracranial volume-adjusted left and right hippocampal volumes by 5-HT_{1A} -1019C/G genotype in study participants (n = 53). The data are expressed as mean and standard error of the mean. *$P < 0.05$; **$P < 0.01$.

volume. For example, S/S homozygosity has been reported to be associated with reduced hippocampal volume in elderly MDD patients with an early (≤ 50 years) age of onset (Taylor et al., 2005); in the present study, the patient sample had mean age of onset of 30 years (Table 4.2). Such individuals would be expected to have experienced multiple depressive episodes over a longer duration, which may correlate with greater exposure to elevated glucocorticoid levels (Sapolsky, 2000a) thought to contribute to hippocampal volume reductions (Tata and Anderson, 2010). This theory is consistent with the findings of other studies that have documented associations between the 5-HTTLPR S allele and reduced hippocampal volume with higher waking cortisol levels (O'Hara et al., 2007), the presence of childhood stress (Frodl et al., 2010; Everaerd et al., 2012), and the diagnosis of depression (Eker et al., 2011). Thus the effects of the 5-HTTLPR S allele on hippocampal volume may be moderated by a gene-by-environment interaction, in which the polymorphism alone is insufficient to affect hippocampal volume in the absence of an environmental stressor such as a depressive episode.

NET -182T/C had an effect on hippocampal volume in the full study sample. The NET -182T/C polymorphism is in the promoter region of the gene that encodes the norepinephrine transporter (Zill et al., 2002), considered a candidate gene for major depression. However, its functional consequences remain unknown and a recent meta-analysis failed to confirm an association between the polymorphism and MDD (Zhou et al., 2014). Like the 5-HTT, the NET is a major target of certain antidepressants including tricyclics, norepinephrine reuptake inhibitors and serotonin/norepinephrine reuptake inhibitors (SNRIs). The C(-182) allele has been associated with poorer response to milnacipran (an SNRI) compared to the T(-182) allele among MDD patients (Yoshida et

al., 2004); however, associations between NET polymorphisms and antidepressant responsiveness have not been widely replicated (Kato and Serretti, 2010). In the present study, C(-182) allele-carriers had smaller hippocampal volumes relative to T/T homozygotes. Associations between this polymorphism and hippocampal volume have not been previously reported in the literature.

Similar to NET -182T/C, the 5-HT_{1A} -1019C/G polymorphism had an effect on hippocampal volume irrespective of diagnosis. 5-HT_{1A} -1019C/G has been shown to alter receptor expression, with the G(-1019) allele in the *HTR1A* promoter region failing to bind repressors Deaf1, Hes1 and Hes5, leading to upregulation of presynaptic 5-HT_{1A} autoreceptor expression (Lemonde et al., 2003; Albert and Lemonde, 2004). The G(-1019) allele has been associated with MDD and suicide (Lemonde et al., 2003; Le François et al., 2008), and among MDD patients, reduced treatment response to serotonergic antidepressants (Kato and Serretti, 2010), increased amygdala reactivity (Dannlowski et al., 2007), and in patients with comorbid MDD and borderline personality disorder, reduced amygdala volume (Zetzsche et al., 2008). There is a high concentration of 5-HT_{1A} receptors in the dentate gyrus and it is via these receptors that serotonergic antidepressants are thought to influence adult neurogenesis (Radley and Jacobs, 2002). Since the G(-1019) allele is associated with fewer postsynaptic 5-HT_{1A} receptors (Czesak et al., 2006), one would expect a reduction in hippocampal neurogenesis in association with the G allele (Le François et al., 2008), which could have consequences for hippocampal volume. In the present study, however, G/G homozygotes had larger hippocampal volumes than individuals with one or two copies of the C allele. Given that a relatively small proportion of the total volume of the hippocampus is represented by the

dentate gyrus, and that the magnitude of adult neurogenesis in the human hippocampus is probably too low to account for volume changes within the hippocampus itself (Czéh and Lucassen, 2007; Spalding et al., 2013), alterations to neurogenesis alone would be unlikely to explain the volume effects of 5-HT_{1A} -1019C/G.

Findings of NET -182T/C and 5-HT_{1A} -1019C/G effects on hippocampal volume in both patients and controls independent of a diagnosis of MDD, suggest an association with development. Serotonin plays a role in the regulation of brain development and acts as a trophic factor as well as a neurotransmitter (for review see Gaspar et al., 2003). 5-HT_{1A} receptor activation affects early postnatal dendritic maturation in the hippocampus (Gross et al., 2002), in particular affecting the length and number of dendritic spines in hippocampal neurons (Yan et al., 1997). The effects of these particular polymorphisms on hippocampal development are unknown but it is possible that they may affect neurodevelopmental processes that have consequences for hippocampal volume in adults.

A limitation of the present study is the relatively small sample size. While the focus of this paper was to investigate the effects of various monoamine-related SNPs on hippocampal volume, the candidate genes selected generally have only modest associations with MDD diagnosis and treatment response and therefore much larger sample sizes are required to replicate association studies. Further, the genetic effects on hippocampal volume are likely the result of the interaction of multiple genes in addition to environmental influences and the study was underpowered to investigate gene-gene interaction effects on hippocampal volume. For these reasons, the findings of this study should be considered preliminary and further research is necessary.

The present data indicate that hippocampal volume may be influenced by serotonin- and norepinephrine-related gene polymorphisms. The NET and 5-HT_{1A} polymorphisms appear to exert their effects on hippocampal volume similarly in patients and controls; while the 5-HTTLPR polymorphism differentially affects hippocampal volume in the presence of depression. Given the putative role of both the hippocampus and of monoamine-related candidate genes in depression and antidepressant response, it will be beneficial to elucidate how these various factors interact in order to potentially identify valid markers of depression and predictors of treatment response.

CHAPTER 5

General Discussion

5.1 Summary of findings

The primary aim of this work was to prospectively track changes in brain structure in patients with treatment-resistant major depression while they underwent intensive pharmacotherapy with the goal of attaining remission. The main findings of the research presented in this thesis are summarized below.

Forty-four percent of the 27 patients who completed the study achieved sustained 6-month remission. Longitudinal imaging analyses revealed that these remitted patients had a mean increase in whole-brain volume over the follow-up period, while nonremitted patients lost brain volume despite receiving more treatment strategies. Voxel-based morphometry (VBM) analyses were conducted to localize the observed volume changes. During the study period, remitters demonstrated increased gray matter volume in the orbitofrontal cortex and the inferior temporal gyrus, and nonremitters had white matter volume loss in the anterior limb of the internal capsule.

Changes in volume and cortical thickness in patients were also tracked within specific cortico-limbic ROIs. Consistent with the whole-brain analyses, different structural trajectories were observed for remitted and nonremitted patients within several ROIs. The direction of structural change over follow-up differed in patients according to their clinical response to treatment, with findings of increasing hippocampal volume and cortical thickness in the rostral middle frontal gyrus and orbitofrontal cortex in remitters, and decreasing volume and cortical thinning in these regions in nonremitters. Although no differences in ACC cortical thickness change were detected in remitter and nonremitter groups, baseline thickness of the ACC was predictive of patients' symptom change over follow-up.

Volume and cortical thickness measurements of patients within the cortico-limbic ROIs were also compared to those of healthy matched control participants at baseline imaging. While no between-group differences were observed, hippocampal volume differences between patients and controls emerged when genetic variation was considered. Smaller hippocampal volume was associated with homozygosity for the 5-HTTLPR S allele only in the patient group. In contrast, the NET -182T/C and 5-HT_{1A} -1019C/G polymorphisms were associated with hippocampal volume irrespective of diagnosis. The implications of these findings are discussed in more detail in the following sections.

5.2 Structural correlates of treatment-resistant depression

Studies have shown that brain volume decreases over time in healthy adults and that this atrophy accelerates with increasing age. Researchers employing SIENA (the same imaging methodology used in the current study to measure percentage brain-volume change [PBVC] over time) have reported mean annual brain-volume change amounts of -0.23% in a sample of 199 healthy study participants (Takao et al., 2012), and -0.40% in 201 neurologically asymptomatic elderly individuals (Enzinger et al., 2005). In the first paper presented in this thesis (Phillips et al., 2012), nonremitted depressed patients had a PBVC of -0.35% over follow-up (mean follow-up period for nonremitters was 417 days). This corresponds to an approximate annual brain volume change of -0.31% in nonremitters, which is comparatively higher than what might be expected based on the Takao et al. (2012) cohort. This speculation is intriguing as the Takao et al. (2012) sample had a considerably older mean age (56 years) relative to that of the

nonremitters (44 years). This suggests that the nonremitted patients experienced more brain volume loss over follow-up than would be expected for their age, however their atrophy does not appear to be as severe as that experienced by neurologically-healthy elderly individuals (Enzinger et al., 2005). The present study lacks direct longitudinal comparison data with healthy controls; however, the absence of whole-brain atrophy in the patients in stable remission suggests that the atrophic changes in nonremitters can be accounted for by their continuing depression. There have been no other published reports of longitudinal whole-brain volume change in depression. Frodl et al. (2008b) reported a depression-specific pattern of regional gray matter volume decline (more volume loss over time) in patients with depression relative to healthy individuals, and more volume decline in nonremitted patients compared to patients in stable remission. While the overall degree of atrophy observed in the nonremitters in the present study was subtle, whole-brain volume increase in the remitter group suggests that the longitudinal volume changes are state-related.

As hypothesized, structural changes were observed over time in several cortico-limbic regions previously implicated in major depression. Consistent with the whole-brain findings, the ROI analyses revealed brain volume and cortical thickness changes over follow-up in opposing directions in the remitted and nonremitted patient groups. VBM and surface-based cortical thickness are complementary measures. VBM provides a mixed measure of GM volume that includes cortical surface area and cortical thickness (Mechelli et al., 2005; Hutton et al., 2009; Palaniyappan and Liddle, 2012). Over follow-up, there were findings of increased GM volume and increasing mean cortical thickness in the orbitofrontal cortex in remitted patients with both whole-brain VBM and ROI

cortical thickness analyses. Järnum et al. (2011) reported an increase in orbitofrontal cortical thickness over 6-month follow-up in antidepressant-treated MDD patients. The authors hypothesized that this increase was pharmacologically driven as their sample included both remitted and currently ill patients. In contrast, in the current work, orbitofrontal volume and cortical thickness increase was only observed in patients in stable remission (cortical thinning was observed in nonremitters), suggesting that the effects observed in the orbitofrontal cortex in this sample of treatment-resistant patients was driven by treatment response. More research is needed to confirm this assertion.

Remitted patients also showed increased GM volume in the inferior temporal gyrus with VBM. Cortical thickness analyses in this region revealed the same pattern of structural change observed in the other ROIs (increasing thickness over time in remitters, thinning in nonremitters) at trend level (results did not survive correction for multiple comparison). The inferior temporal gyrus is not often identified as a region of interest for major depression but there is some evidence for structural abnormalities in this region in patients. Diffusion tensor imaging has shown abnormalities in white matter connectivity including decreased fractional anisotropy (FA) in the superior longitudinal fasciculi in the inferior temporal cortex in women with unipolar MDD relative to healthy women (Versace et al., 2010). Frodl et al. (2008b) identified the inferior temporal gyrus as one of several areas showing greater GM volume decline over 3-year follow-up in MDD patients compared to healthy controls. The present work is the first to report increased volume in this region in remitted patients during treatment. Further research of the extent and consequences of longitudinal change in the inferior temporal gyrus in depression is warranted.

VBM analyses identified GM changes over follow-up in patients only in the two regions discussed above. As hypothesized however, in ROI analyses, opposing structural trajectories were identified for remitted and nonremitted patients in the rostral middle frontal gyrus and the hippocampus (with increasing cortical thickness or volume in remitters and decreasing thickness or volume in nonremitters). There have been previous reports of longitudinal hippocampal volume increase in antidepressant-treated patients. However, while one study reported volume increase only in patients treated throughout the entire follow-up period (Frodl et al., 2008a), other studies have included samples of patients who were either all treatment responsive (Schermuly et al., 2011) or all in remission at follow-up scanning (Ahdidan et al., 2011), preventing the distinction between the effects of treatment and the effects of response or remission. In the present work, the consistent pattern of volume or cortical thickness increase in remitted patients suggests that structural recovery only occurs in patients with stable remission from depression.

Contrary to the other ROIs, remitted and nonremitted patients did not differ in ACC cortical thickness change over follow-up. However, thicker anterior cingulate cortex at treatment initiation predicted greater symptom improvement in patients over follow-up. These findings are consistent with previous studies that showed the potential of ACC cortical depth (Coryell et al., 2005), GM volume (Costafreda et al., 2009), and white matter integrity (Korgaonkar et al., 2014) measures to predict treatment response. It remains unclear whether structural change in this region and others leads to treatment response or whether treatment response follows structural recovery. The answers may

depend on the still unknown mechanisms that underlie the structural modifications observed in patients during the course of their illness and treatment.

5.3 Proposed mechanisms underlying structural atrophy and recovery

The data presented in this thesis support continuing brain atrophy in nonremitted depressed patients and limited structural recovery in patients who achieved sustained 6-month remission. The brain regions primarily implicated in the longitudinal changes were the prefrontal cortex and the hippocampus. The molecular mechanisms underlying the structural modifications in depression have not yet been elucidated. The following section outlines the major theories that have been proposed to account for structural atrophy and recovery observed in depression.

As reviewed in the general introduction, structural atrophy in depression is thought to be associated with chronic stress and in particular, the damage that follows prolonged glucocorticoid exposure (Sapolsky, 2000a; McEwen, 2005). Patients with major depression commonly exhibit hyperactivity of the HPA axis which can lead to increased amounts of the circulating glucocorticoid hormone cortisol (Naughton et al., 2014). Juruena et al. (2006) reported twice the salivary cortisol levels in a sample of patients with treatment-resistant depression compared to healthy controls. Animal studies have revealed that high levels of glucocorticoids are associated with neuron loss, atrophy of neuronal processes (McEwen and Seeman, 1999; Sapolsky, 2000b), and decreased adult neurogenesis (David et al., 2009). These cellular consequences may be implicated in the atrophic changes reported in depression (Sapolsky, 2000a; McEwen, 2005) and are most likely to affect the brain regions that are targets of stress hormones.

While the hippocampus has the highest concentration of glucocorticoid receptors in the brain (McEwen, 2005), the prefrontal cortex is also an important target of glucocorticoids (McEwen, 2007). In healthy adult males, higher salivary cortisol levels have been associated with reduced cortical thickness in the rostral middle frontal gyrus and medial orbitofrontal cortex (Kremen et al., 2010) (two regions where nonremitted patients showed cortical thinning over follow-up in the present study). Similarly, progressive increase in salivary cortisol levels over 5 years was shown to predict the degree of hippocampal atrophy in healthy elderly adults (Lupien et al., 1998). Despite this finding, studies that have investigated the relationship between cortisol levels and hippocampal atrophy in patients with depression have failed to report significant associations (O'Brien et al., 2004; Colla et al., 2007; Köhler et al., 2010). Research has shown that rates of hypercortisolemia in MDD patients vary according to the population sampled (Maes et al., 1994). While most studies report hypersecretion of cortisol in about 50% of MDD patients, up to 80% of patients with severe depression have elevated cortisol levels (Pariante and Millar, 2001; Pariante, 2003; Anacker et al., 2011). Therefore the hypothesis of glucocorticoid-mediated atrophy may still be relevant for the treatment-resistant sample examined in this thesis. Frodl et al. (2012) reported evidence of an association between reduced expression of glucocorticoid-inducible genes (genes that affect activation of the glucocorticoid system) and smaller hippocampal volume in MDD patients, lending further support for an association between the glucocorticoid system and brain atrophy in depression beyond that assessed with the measurement of salivary cortisol.

Findings of progressing atrophic changes with increasing duration of depression (Lorenzetti et al., 2009; McKinnon et al., 2009; Bora et al., 2012) support the glucocorticoid cascade hypothesis (Sapolsky et al., 1986). This hypothesis proposes that excessive glucocorticoid exposure leads to hippocampal damage which reduces cortisol-mediated feedback inhibition by the hippocampus, and results in further cortisol secretion, creating a feed-forward cascade of hippocampal damage. While there are an abundance of animal studies supporting this hypothesis, more translational research from animal to human studies is required (Frodl and O'Keane, 2013). Recent research has moved beyond this hypothesis seeking to further describe the molecular mechanisms underlying the associations between stress and brain atrophy in depression. In a recent study, Ota et al. (2014) reported an association between neuronal atrophy in the rat prefrontal cortex and increased expression of the protein REDD1 (regulated in development and DNA damage responses-1) under chronic stress conditions. Further, they found increased levels of REDD1 in postmortem prefrontal cortex sections from human subjects with MDD compared to controls, suggesting a potential mediator of stress-related structural changes observed in that region (Ota et al., 2014). Kang et al. (2012) reported lower expression of synapse-related genes in the dorsolateral prefrontal cortex of subjects with MDD associated with synapse loss in postmortem samples. While these examples illustrate potential causal mechanisms of atrophic change in depression, the exact nature of the atrophy remains unknown.

There is increasing evidence that antidepressant treatment may halt or reverse the brain volume reduction observed in patients with depression. Antidepressants ameliorate HPA axis hyperactivity and may protect against some of its consequences including

neuronal cell death and reduced neurogenesis (Anacker et al., 2011). Medications from various pharmacological classes including antidepressants, mood stabilizers, and atypical antipsychotics, are thought to generally contribute to morphological recovery through their neurotrophic and neuroprotective effects (Hunsberger et al., 2009). Several specific mechanisms have been proposed that could contribute to increased brain volume among antidepressant-treated patients, these include regeneration of neurons, increase in glial cell numbers, larger neuropil volume, more blood vessels, or less apoptosis (Czéh and Luccassen, 2007).

Animal models of stress-induced depression are associated with loss of neurons and atrophy of dendrites in the hippocampus, changes which can be reversed with antidepressants (reviewed in McEwen, 1999). Animal studies have shown that chronic antidepressant treatment increases adult neurogenesis (Malberg et al., 2000; Dranovsky and Hen, 2006) and that hippocampal neurogenesis is necessary for mediating the response to antidepressant treatment (Santarelli et al., 2003; Perera et al., 2011), suggesting a mechanism that could drive volume recovery in this area. However, in subjects with MDD, evidence of antidepressant-induced increases in neural progenitor cells is limited to the dentate gyrus (Boldrini et al., 2009), which constitutes only 6% of the total volume of the human hippocampus (Joelving et al., 2006). The magnitude of adult neurogenesis in humans is probably too low to account for the volume changes observed within the hippocampus in patients with depression (Czéh and Luccassen, 2007; Spalding et al., 2013). Furthermore, with reference to the present study, since the addition of new cells is isolated to such a small portion of the brain, neurogenesis is unlikely to

account for the whole-brain volume changes or prefrontal cortical thickness changes observed in the patients in stable remission.

Recent research has demonstrated an association between neurogenesis and angiogenesis in the adult human dentate gyrus. Specifically, a correlation was reported between increased neural progenitor cell number and capillary volume observed with antidepressant treatment and larger dentate gyrus volume (Boldrini et al., 2012). Antidepressant treatments increase vascular endothelial growth factor expression and induce the proliferation of vascular endothelial cells in the hippocampus (Nowacka and Obuchowicz, 2012). What remains unclear is whether angiogenesis occurs elsewhere in the cortex in response to treatment and, thus, whether it could contribute to the increased brain volume or increasing cortical thickness observed in remitted patients over follow-up.

Brain volume increases observed in patients receiving pharmacotherapy could also be accounted for by changes in glia. Glial cells provide metabolic support for neurons, and loss of glia could contribute to the atrophic changes and neuron loss caused by stress and depression. Stress and antidepressant treatment can modify gliogenesis, glial morphology, and glial cell numbers (Alonso, 2000; Czéh et al., 2006; Wennstrom et al., 2006). Research has shown histopathological evidence of reductions in glial cell numbers in subjects with MDD in the prefrontal, orbitofrontal and cingulate cortices (Rajkowska et al., 1999; Cotter et al., 2001, 2002). Although such changes have not been reported in the human hippocampus (Lucassen et al., 2001; Müller et al., 2001), in tree shrews there is evidence of stress-related reductions in the somal volume of astrocytes in the hippocampus that can be blocked by SSRI treatment (Czéh et al., 2006). Moreover, a

recent study revealed increasing dentate gyrus granule cell and glial cell numbers with age in antidepressant-treated subjects with MDD (Cobb et al., 2013) which may reflect the proliferating effects of antidepressant treatment.

Finally, a potential explanation for the opposing structural trajectories observed in remitted and nonremitted patients in the present study may come from the effects of antidepressant treatment on synaptogenesis. A recent postmortem study reported a decreased number of synapses in the prefrontal cortex of depressed subjects (Kang et al., 2012). Preclinical studies show that antidepressants can block or reverse decreased synaptic connections caused by chronic stress exposure (Magariños and McEwen, 1995; Bessa et al., 2009). Such changes could, in patients, possibly result in the reconnection of key cortico-limbic circuits (Duman, 2014). Recently, Li et al. (2011) reported rapidly sprouting cortical pyramidal neurons following acute ketamine infusion in the rat. Ketamine produces rapid antidepressant effects in patients with treatment-resistant depression (Berman et al., 2000; Zarate et al., 2006). Ketamine has faster effects on synaptic density relative to typical antidepressants which suggests the importance of synaptic alterations on treatment response in patients (Duman and Aghajanian, 2012). This may provide a theory as to why structural recovery was seen only in patients who achieved sustained remission in the present work despite all patients receiving intensive pharmacotherapy throughout the follow-up period.

The preceding discussion of the potential cellular bases that may underlie volume changes in patients over the course of their illness and treatment is entirely speculative as none of the purported mechanisms were measured directly in the research described herein. Likely, no single mechanism can fully account for the brain volume loss observed

over time in patients with continuing depression, nor for the structural recovery evident following antidepressant treatment or remission. Likely these longitudinal changes are driven by some combination of factors acting on different and perhaps complementary mechanisms which remain to be elucidated. Of relevance to the current work, perhaps the most interesting unanswered question is why, if volumetric increases are driven by pharmacological treatment (as the literature suggests), was volume increase only evident in remitted patients when all patients received intensive pharmacotherapy throughout the follow-up period? The results suggest that, in treatment-resistant depression, remission rather than antidepressant treatment is the key factor involved in halting atrophy and driving volumetric recovery. In fact, antidepressant-mediated structural modifications may themselves be necessary to elicit response to treatment among patients. Further research is needed to address these questions.

5.4 Genetic influences on hippocampal volume in depression

Major depression is a clinically heterogeneous disorder which may explain much of the inconsistency reported in cross-sectional structural imaging studies that have compared MDD patients with matched healthy controls. The most consistent structural finding in patients with depression is reduced volume of the hippocampus (Campbell et al., 2004; Videbech and Ravnkilde, 2004; Koolschijn et al., 2009; McKinnon et al., 2009; Arnone et al., 2012). Hippocampal volume reduction relative to controls is not detected in all samples of depressed patients however, including the treatment-resistant sample in the present study. Although these patients had many of the clinical characteristics commonly associated with hippocampal atrophy, such as multiple previous depressive episodes,

longer illness durations and poor treatment responsiveness (McKinnon et al., 2009; Kempton et al., 2011; Hsieh et al., 2002), their baseline hippocampal volumes did not differ from those of matched healthy individuals. Such findings emphasize the complex relationship between genes and environmental factors that contribute to variation in hippocampal volume (Rabl et al., 2014). Heritability studies have reported that genes have a significant influence on hippocampal development and volume (Bartley et al., 1997; Lyons et al., 2001; Schatzberg, 2002). Research also suggests genetic modulation of stress-related hippocampal volume changes (Frodl et al., 2008c). Such findings may indicate a need to consider the effects of genetic variation when conducting cross-sectional between-group comparisons of hippocampal volume.

As reported in the third paper included in this thesis, the NET -182T/C and 5-HT_{1A} -1019C/G polymorphisms showed similar effects on hippocampal volume in MDD patients and controls, signifying a likely association between these SNPs and brain development. In contrast, homozygosity for the 5-HTTLPR risk allele (the S allele) was associated with smaller hippocampal volume only in patients. This indicates a gene by environment interaction, whereby diagnosis or history of depression is necessary for the effects of 5-HTTLPR on hippocampal volume to become apparent. These results are consistent with previous reports of associations between the 5-HTTLPR S allele, smaller hippocampal volume, and cortisol levels, stress exposure, or diagnosis of depression (O'Hara et al., 2007; Frodl et al., 2010; Eker et al., 2011; Everaerd et al., 2012). The specificity of the effects of the risk allele on hippocampal volume to the patient population suggests that the SNP may also be of importance when considering longitudinal changes in hippocampal volume over the course of illness (although this was

not investigated in the present study). As previously reviewed, there is considerable evidence of progressing hippocampal volume changes over time during the course of illness in depression. There is also convincing evidence of an association between the 5-HTTLPR S allele and stress susceptibility. For example, a recent meta-analysis of 11 studies reported an association between homozygous carriers of the 5-HTTLPR S allele and increased cortisol reactivity to stressors (Miller et al., 2013). As reviewed in the previous section, HPA-axis hyperactivity in MDD patients is thought to be a potential mechanism leading to hippocampal atrophy in depression (Sapolsky, 2000a; McEwen, 2005).

Failure to consider additional gene by environment interaction effects may also explain the negative findings in the comparisons between patients and controls at baseline imaging in the present work. For example, research suggests smaller hippocampal volumes associated with the interacting effects of exposure to childhood stress and both the 5-HTTLPR (Frodl et al., 2010) and BDNF Val66Met polymorphisms (Carballedo et al., 2013; Frodl et al., 2014). It may also be important to investigate the effects of genes on particular subregions of the hippocampus individually, as has been done in recent imaging studies. For example, Rabl et al. (2014) identified the hippocampal subfield most affected by the 5-HTTLPR S allele as the subiculum, a region critical for mediating HPA axis inhibition (Herman et al., 1995), and susceptible to changes in neuroplasticity in response to stress (MacDougall and Howland, 2013). Identifying affected hippocampal subfields may help to clarify hypotheses regarding the relationships between genetic polymorphisms and neurodevelopmental processes or stress-related neuroplastic changes in the hippocampus.

5.5 Strengths, limitations and future directions

The research presented in this thesis provides one of the first descriptions of the longitudinal effects of antidepressant treatment and remission on brain structure in a clinically well-defined, homogeneous sample of patients with major depressive disorder. While other studies have shown that effective treatment may reduce depression-related brain atrophy in patients, no other studies have specifically investigated the potential effects of pharmacotherapy on brain volume change in treatment nonresponsive patients, or isolated the effects associated with sustained remission. Clinical nonresponsiveness in a significant number of patients was necessary in order to investigate whether reductions in brain atrophy or observations of structural recovery during the study period could be attributed to the treatment received by patients or the alleviation of their depressive symptoms. Treatment-resistant patients were selected in order to obtain a balanced proportion of remitted and nonremitted patients for comparison. This represents an important patient population given the prevalence and morbidity of treatment-resistant depression. Another strength of the study was its prospective, longitudinal design.

The primary limitation of the work described herein is the small sample size, which reduced the types of imaging and genetic analyses that could be conducted. Replication with larger samples would permit investigation of the effects of genetic variation on longitudinal changes in brain structure and the examination of gene-gene interaction effects on structural variables. Further, lack of follow-up imaging in healthy controls prevented the direct comparison of patients' morphometric changes over follow-up with normal brain structure change observed over the same time period in healthy individuals. A longer follow-up period in future studies may also prove beneficial in

order to determine whether the observed pattern of opposing structural trajectory over time in remitted and nonremitted patients continues during the course of their illness or sustained remission. Finally, the naturalistic treatment approach used in the study limits the investigation of the effects of any particular medication or combination/augmentation strategy on brain structure changes in patients over follow-up. Imaging in single-agent medication trials may be necessary to determine which drugs have the most beneficial effects on brain structure. Future studies may benefit from the measurement of patient cortisol levels (to investigate the correlation between longitudinal brain atrophy and cortisol hypersecretion in currently ill patients), and the measurement of childhood adversity and/or other chronic stressors thought to be associated with structural changes in patients with major depression.

5.6 Conclusion

All the patients included in this study received intensive treatment with medications shown to be beneficial for brain volume, and on average both remitted and nonremitted patient groups showed some degree of clinical improvement during treatment. Despite this, only remitters showed evidence of volume and cortical thickness increase over time while nonremitted patients demonstrated continuing whole-brain atrophy, decreasing hippocampal volume and prefrontal cortical thinning over follow-up. These results suggest that structural recovery in treatment-resistant depression is driven by remission rather than pharmacotherapy. Further, genetic determinants of antidepressant response were shown to affect hippocampal volume in the presence of depression. Research indicates that repeated depressive episodes lead to lasting structural

changes in the brain that may increase relapse potential and further risk of treatment-resistance. In fact, the progressive nature of brain volume loss in nonremitted patients suggests that treatment response may itself be an important determinant of atrophic changes. These findings emphasize the importance of treating patients to clinical remission as the restoration of brain structure within cortico-limbic networks in patients with treatment-resistant depression might lead to a better prognosis for the future.

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