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Mood related insights : functional and structural MRI studies in depression and anxiety disorders

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Citation

Tol, M. J. van. (2011, May 26). *Mood related insights : functional and structural MRI studies in depression and anxiety disorders*. Retrieved from https://hdl.handle.net/1887/17672

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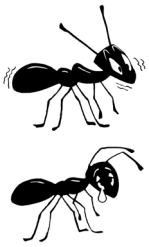

MOOD RELATED INSIGHTS

FUNCTIONAL AND STRUCTURAL MRI STUDIES
IN DEPRESSION AND ANXIETY DISORDERS

MARIE-JOSÉ VAN TOL

Marie-José van Tol
Mood Related Insights: functional and structural MRI studies in depression and anxiety disorders

cover design and illustrations: David Kops
Print: GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede
The Netherlands

The infrastructure for the NESDA study (www.nesda.nl) is greatly acknowledged. The NESDA infrastructure is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Quality of Healthcare (IQ healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute).

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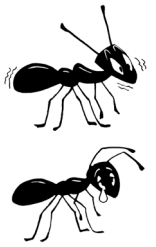

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FUNCTIONAL AND STRUCTURAL MRI STUDIES
IN DEPRESSION AND ANXIETY DISORDERS

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 26 mei 2011
klokke 13.45 uur

door

Marie-José van Tol
geboren te Amstelveen
op 25 December 1980

Promotiecommissie

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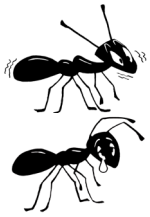


CHAPTER 1

GENERAL INTRODUCTION

In this thesis, the focus is on the brain. More specifically, the focus is on abnormalities in brain function and brain volume in patients with major depressive disorder (MDD) and anxiety disorders as measured using Magnetic Resonance Imaging (MRI). The main questions of this thesis are: 1) do MDD and anxiety disorders share brain abnormalities that can explain the shared symptoms and high comorbidity (i.e., concurrent manifestation)?; 2) should MDD with comorbid anxiety disorder be considered a different diagnostic category than MDD without comorbid anxiety and anxiety disorders without comorbid MDD when considering abnormalities in structure and function of regions associated with emotion perception and regulation? Furthermore, we will explore whether shared risk factors of both MDD and prevalent anxiety disorders are associated with brain abnormalities, abnormalities that may give insight in the aetiology of the disorders.

CLINICAL CHARACTERISTICS

MDD is among the most prevalent and disabling psychiatric disorders in adult age and is in the top-10 of diseases with the largest global disease burden (Blair et al., 2008; Lopez, Mathers, Ezzati, Jamison, & Murray, 2006). Symptoms of MDD include a persistent low or sad mood, a diminished capacity to experience pleasure, difficulties in eating and sleeping, and concentration problems. A diagnosis of MDD or any other mental disorder, except for posttraumatic stress disorder, is solely based on the presented symptoms. Aetiological or biological factors including a positive family history of depression and anxiety, genetics, adverse life events, and childhood trauma are not considered in the process of diagnostics. Nevertheless, these factors have been recognized to play an important role in the aetiology of MDD (Belmaker & Agam, 2008). Appendix 1 lists the diagnostic criteria of MDD according to the Diagnostic and Statistical Manual of mental disorders (DSM) version IV.

About 20-30% of the male population and 30-40% of the female population in western countries will experience at least one episode of MDD (Kruijshaar et al., 2005). At any point in time, four to ten percent of the population will suffer within one year from a major depressive episode that will typically last 24 weeks (Kruijshaar et al., 2005). Also, risk of recurrence is high, and most strongly predicted by illness severity and comorbidity of (social) anxiety disorders (Melartin et al., 2004). In the outpatient setting, approximately one-third will recover from MDD, whereas two-third of the MDD patients will experience at least one more episode of MDD within 5 years (Holma, Holma, Melartin, Rytsala, & Isometsa, 2008). MDD is associated with increased mortality rates, illness costs, and loss of productivity (Cassano & Fava, 2002).

Ten to 30% of those who suffer from MDD also meet criteria for an anxiety disorder, such as social anxiety disorder, panic disorder, and generalized anxiety disorder (Gorman, 1996; Gorman & Coplan, 1996; Ressler & Mayberg, 2007). Panic disorder is characterized by an excessive fear for panic attacks, social

anxiety disorder by extensive fear for social situations, and generalized anxiety disorder by excessive general worrying. Diagnostic criteria for these anxiety disorders are listed in detail in Appendix 1. Prevalence of anxiety disorders is also high, with a mean life-time prevalence of 28% (Ressler & Mayberg, 2007). The economic burden of anxiety disorders is comparable to that of MDD in terms of loss of productivity, quality of life decrement, and medical care utilization (Hoffman, Dukes, & Wittchen, 2008).

Approximately 50% of persons who suffer from an anxiety disorder will suffer from at least one major depressive episode in their lives (Gorman, 1996), although comorbidity figures vary between 20% and 90%. The comorbid condition of depression and anxiety disorders has been associated with more severe psychopathology (Kessler, Chiu, Demler, & Walters, 2005; Roy-Byrne et al., 2000; Rush et al., 2005), increased disability (Gorman, 1996), and worse outcome compared with patients with only a diagnosis of MDD or anxiety disorders (Bruce et al., 2005; Gorman, 1996; Gorman & Coplan, 1996; Melartin et al., 2004; Roy-Byrne et al., 2000; Rush et al., 2005). Because of these differences, it has been suggested that MDD with comorbid anxiety disorders and MDD without comorbid anxiety disorders should be considered as different diagnostic groups (Penninx et al., 2010). At the same time, MDD and anxiety disorders might share a common neurobiological pathology. This is suggested because of their high comorbidity, but also because patients with MDD and anxiety disorders tend to respond to the same treatment strategies such as treatment with Selective Serotonin Reuptake Inhibitors (SSRIs)[1] and cognitive behavioral therapy[2] (Ressler & Mayberg, 2007). Furthermore, MDD and anxiety disorders show overlap in symptoms, such as restlessness, irritability, feelings of rejection, dysphoria, sleep and appetite disturbances, increased self-consciousness, and oversensitivity to criticism (Gorman, 1996). Both MDD and anxiety disorders are associated with increased vulnerability to psychosocial stress, as reflected by an abnormal cortisol response of the hypothalamus-pituitary-adrenal (HPA)-axis[3] (Veen et al., 2009; Vreeburg et al., 2009; Vreeburg et al., 2010), although findings have been inconsistent (Chida & Steptoe, 2009). Also, MDD and anxiety disorders are associated with common risk factors, including neuroticism, a positive family history of depression and anxiety, childhood trauma, and aversive life events (Spinhoven et al., 2010; Warner, Mufson, & Weissman, 1995; Weinstock & Whisman, 2006). These shared risk factors also suggest a shared aetiology in MDD and anxiety disorders.

DIAGNOSTIC CLASSIFICATION

The discussion whether MDD and anxiety disorders should be considered separate diagnostic categories with specific phenomenological and neurobiological characterizations, or should be considered different manifestations of the same disorder is not new. Since the late 1960's researchers attempted to find shared and unique phenomenological, treatment, course and outcome characteristics of MDD, anxiety disorders, and comorbid depression-

1 Since the 1950's, successful treatment of MDD with tricyclic medication led to the monoamine hypothesis of MDD and anxiety disorders. This hypothesis predicts that MDD and anxiety disorders are characterized by deficient monoaminergic transmission. Nowadays, selective serotonin reuptake inhibitors (SSRIs) and to a lesser extent selective noradrenalin reuptake inhibitors (SNRIs) are administered. These medications produce fewer side effects than the older tricyclic medication. SSRIs and SNRIs primarily act by preventing uptake of the monoamines by the pre-synaptic receptors, thereby promoting uptake of the agents by the post-synaptic receptors. However, up to 60% of patients may not achieve a sufficient response (Ressler & Mayberg, 2007).

2 Cognitive behavioral therapy is based on the hypothesis that MDD and anxiety disorders are in part the product of a maladaptive set of depressive or anxious cognitions. In cognitive-behavioral therapy, these cognitions are challenged and exchanged for more adaptive cognitions and behavior.

anxiety (Stavrakaki & Vargo, 1986). A problem in psychiatric classification is that psychiatric disorders are not discrete biomedical entities with clear phenotypic boundaries. Consequently, classification of psychiatric disorders is not stable and subject to ongoing discussion between scientist and clinicians. For instance, before 1994, the year that version IV of the DSM was published, symptomatology of panic disorder and generalized anxiety disorder was considered as one entity and referred to as anxiety neurosis. In contrast, generalized anxiety disorders is currently considered a separate disorder that is principally related to MDD owing to the large overlap in symptomatology.

It has also been suggested that MDD and anxiety disorders should be characterized along one continuum from anxiety to depression (Stavrakaki & Vargo, 1986). Such an approach could explain the overlapping symptomatology and the high 'turn-over' rate, that is the 'replacement' of a primary diagnosis of an anxiety disorder with a primary diagnosis of MDD over the course of the disease. Models including more than one continuum have been proposed as well (Clark & Watson, 1991; Goldberg, 2000; Wardenaar et al., 2010a; Wardenaar et al., 2010b), including dimensions of general distress, physiological or anxious arousal, anhedonia/lack of positive affect, mood and cognition/negative affect, and sleep.

Such dimensional approaches have the advantage of avoiding problems associated with a categorical classification, especially when studying biological factors associated with MDD and anxiety disorders. Describing patients along dimensions of depression and anxiety symptomatology avoids artificial comorbidity that may arise owing to the large overlap in symptoms. Next, no strict dichotomy between healthy and ill is needed, and subsyndromal levels of depressed and/or anxious symptoms are not systematically overlooked. Finally, the use of a categorical disorder classification system to describe patients leads to high levels of heterogeneity within diagnostic classes: e.g. in MDD, patients receive the same diagnosis even if they only overlap on one of nine symptoms.

For the forthcoming version of the DSM, version V, it has been proposed to include a dimensional characterization of mood and anxiety symptoms next to the categorical classification for the diagnosis of mood and anxiety disorders. Such an approach may fill the gap between clinicians and scientist in an attempt to further identify and acknowledge the shared and unique phenomenological and neurobiological characteristics of various psychiatric disorders. Importantly, taking into account aetiological factors (e.g., childhood trauma, negative life events, family history), psychological factors (e.g., personality factors, dysfunctional cognitive schemas), and laboratory findings (e.g. neuroimaging findings, genetics, stress hormone measurements) could have implications for the psychiatric nomenclature (Rounsaville et al., 2002). Considering information on aetiology, symptomatology, and neurobiology could help answer the question of how to regard MDD and prevalent anxiety disorders: consider the symptomatology as manifestations of the same disorder, classify the disorders as distinct diagnostic groups, or characterize

3 The HPA-axis is the body's primary stress-response and feed-back system. Cortisol, a glucocorticoid, is the primary human stress hormone that is synthesized in the adrenal glands. Cortisol is the end product of a cascade initiated by hormones released by the hypothalamus. The stress hypothesis of MDD and anxiety disorders states that a disrupted homeostasis of the human stress system is at the base of the disorder.

patients along axes within a multi-dimensional space. However, as of yet, the common and disorder specific neurobiological characteristics of MDD and prevalent anxiety disorders have not been studied extensively. Also, support for the suggestion that MDD with and without comorbid anxiety disorders should be considered as different entities, with respect to their neurobiological profile, is limited. Moreover, the neurobiological impact of risk factors of depression and anxiety disorders has been hardly studied. Importantly, neurobiological insights could contribute to the discussion of how to regard MDD, anxiety disorders, and the comorbid condition of depression-anxiety. Accurate characterization of the disorders is important because the way patients are diagnosed has implications for both treatment and prognosis.

NEUROIMAGING

One approach in studying the neurobiological underpinnings of MDD and anxiety disorders is to focus on brain morphology and brain functioning. Technological developments in the past 30 years have made it possible to study the brain in a non-invasive way in vivo. Neuroimaging techniques such as Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) have contributed enormously to the knowledge of brain structure and function in healthy individuals and in psychiatric patients. Using PET, a radioactive tracer is injected in the bloodstream. Frequently used tracers are 18F-labeled glucose (Fluordeoxyglucose [FDG]) and 15O-labeled water[4]. Subsequently, distribution of the radioactively labeled glucose or water is imaged, giving an estimate of energy consumption (glucose metabolism) or perfusion across the brain. MRI does not require the administration of a radioactive tracer. Using functional (f)MRI, scans are acquired that are sensitive to changes in the ratio of oxygen bound to haemoglobin molecules in venous blood. This is called the Blood Oxygenation Level Dependency (BOLD) effect, and is reflective of neuronal activity (Logothetis, 2002). Using both 15O-PET and BOLD-fMRI, perfusion or activity in reaction to certain stimuli or responses are usually compared with a chosen baseline, and therefore provide relative measurements. In the last 10 years, researchers became increasingly interested in the low frequency fluctuations of the BOLD signal. These low frequency fluctuations are used to study synchronicity of time courses across the brain, providing an estimate of the functional connectivity of separate brain regions. Major advantages of fMRI are that scans are acquired without exposure to radiation, and that both the spatial and temporal resolution is better than for PET. MRI can also be used for structural imaging of brain volume (gray matter, white matter, and cerebro-spinal fluids), diffusion, and white matter tracts.

4 Other nuclear imaging applications of PET for biochemical imaging exist but will not be discussed in this thesis.

Neuroimaging in MDD

Up to 2005, brain activation has been studied in MDD predominantly with PET during the 'resting state', that is when patients are not engaged in a task. From 1998 on, researchers started using fMRI to study brain activity in MDD using

various emotional and cognitive tasks. Most consistent metabolism/perfusion (PET) and BOLD (fMRI) abnormalities have been observed in the cingulate gyrus, dorsolateral prefrontal cortex, orbitofrontal cortex, insula, hippocampus, and putamen, although little overlap is found between different activation studies (Fitzgerald, Laird, Maller, & Daskalakis, 2008). Emotional processes have been most frequently studied in MDD, such as emotional face viewing, emotional autobiographical script processing and emotional picture viewing. Such emotional paradigms have been used to test the hypothesis of abnormal limbic activation to disorder relevant information. Abnormal sensitivity of limbic areas might explain the heightened responsiveness to stress and negative emotions associated with MDD.

Cognitive theories of MDD predict that MDD is the product of automatic maladaptive thoughts and cognitions (i.e., negative views of the self, the world, and the future) and associated selective processing of negative information (Beck, 1976). When encountering negative events, only a limited pallet of stereotyped ideas is available, further biasing the view of self, the world, and the future in a negative way. Such biased information processing towards negative information is refered to as the mood-congruent bias, whereas biased information processing away from positive information has been refered to as the mood-incongruent bias (Beck, 1976; Bower, 1981; Bradley, Mogg, & Williams, 1995). These mood-congruent and mood-incongruent processing biases may affect memory formation and thereby further affect the course of the disorder in a negative way (Elliott, Rubinsztein, Sahakian, & Dolan, 2002). Also, cognitive abnormalities in depression have been related to deficits in effortful processing (Elliott, 1998; Hartlage, Alloy, Vazquez, & Dykman, 1993). Some researchers have proposed that stressful conditions, including depression and anxiety reduces cognitive capacity, resulting in diminished available attentional resources (Hasher & Zacks, 1979). Others proposed that cognitive capacity in MDD is unaffected, but that attentional resources in a depressed state are allocated to depression-relevant thoughts and information. This 'narrowed attention' is proposed to be the result of the availability of more relevant 'nodes' to link negative information to or by the incapacity to inhibit negative information flow (see Hartlage et al., 1993 for an overview). As a result, less attentional resources are available for (external) task-relevant processes.

In support of these theories, researchers observed abnormal activity of (para) limbic regions during 'mood-congruent' and 'mood-incongruent' information processing (Epstein et al., 2006; Fossati, Ergis, & Allilaire, 2002; Keedwell, Andrew, Williams, Brammer, & Phillips, 2005b; Sheline et al., 2001; Siegle, Thompson, Carter, Steinhauer, & Thase, 2007). Next, cognitive processes such as selective and sustained attention and executive control that are often reported to be abnormal in MDD (Fossati et al., 2002) have received attention in neuroimaging studies. Executive tasks are considered effortful, and therefore thought to be affected in MDD (Elliott, 1998). Importantly, these executive processes are thought to rely on cortical prefrontal cortex (PFC) regions, such

as the ventrolateral PFC, dorsolateral PFC, and anterior cingulate cortex (ACC) that are also implicated in mood regulation. Therefore researchers administered these cognitive tasks to test the functional integrity of these regions during non-emotional processes. Abnormal executive performance and associated abnormal activity of dorsolateral PFC and ACC regions have been observed (Fossati et al., 2002) and support the effortful processing hypothesis of depression. However, results have been inconsistent.

MRI has also been used to study brain morphometry in MDD. Structural abnormalities (gray matter volume reductions) in MDD have been most consistently found in the ACC and orbitofrontal cortex, and moderate volumetric reductions have been observed in the hippocampus, putamen and caudate nucleus (Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009). These volumetric abnormalities have been explained in light of the abnormal stress and emotion regulation associated with MDD.

Overall, brain imaging studies in MDD often lack an adequate sample size. Also, results may have been confounded by the inclusion of MDD patients with a variety of comorbid psychiatric disorders including anxiety disorders, posttraumatic stress disorder, obsessive compulsive disorder, and attention deficit hyperactivity disorder. Also, use of antidepressant medication may have confounded the results. Antidepressive drugs such as benzodiazepines, tricyclic antidepressants (TCAs), and antipsychotics could influence the cerebral blood flow (Matthew et al., 1995; Ngan, Lane, Ruth, & Liddle, 2002) and thereby the BOLD signal. In conclusion, imaging studies in MDD patients with adequate sample sizes that are able to control for confounding factors including psychiatric comorbidity and medication use are rare.

Neuroimaging in anxiety disorders

The literature on brain function and structure in the highly prevalent anxiety disorders panic disorder, social anxiety disorder, and generalized anxiety disorder is limited. The less prevalent anxiety disorders posttraumatic stress disorder and obsessive-compulsive disorder have been most extensively studied. As mentioned earlier, a diagnosis of posttraumatic stress disorders is made only in the presence of a traumatic experience in the (near) past. Other anxiety disorders are diagnosed solely based on presented symptomatology without including aetiological factors in the diagnostic process. Obsessive-compulsive disorder on the other hand has been characterized by a different neurobiological profile compared with other anxiety disorders (Radua, van den Heuvel, Surguladze, & Mataix-Cols, 2010; Rounsaville et al., 2002; van den Heuvel et al., 2005b). Also, for the new version of the DSM, obsessive compulsive disorder has been proposed for possible reclassification in another or separate diagnostic category. Therefore, these anxiety disorders and their associated neurobiological abnormalities may not be representative of the cluster of anxiety disorders at large.

The focus of activation studies in anxiety disorders has traditionally been on

threat perception and fear-conditioning, and hardly any studies focused on executive and attentional processes. Overall, generalized anxiety disorder has received very limited attention in the field of neuroimaging. In social anxiety disorder, emotional paradigms most consistently demonstrated increased responsiveness of the amygdala and the insula (Etkin & Wager, 2007). In anxiety disorders, studies investigating dorsal involvement in emotional processes are limited in number. In one paper normal attentional control and related prefrontal activation was reported during non-emotional task in patients with panic disorder, but compromised functioning was observed under emotional distraction (van den Heuvel et al., 2005b). Overall, few studies have studied whether effortful processing is compromised in anxiety disorders as well.

The focus of most structural imaging studies in anxiety disorders has been on panic disorder, and researchers reported decreased amygdalar, insular, dorsal ACC volume (Asami et al., 2008; Massana et al., 2003; Uchida et al., 2008). A recent meta-analysis showed that reduced dorsal ACC and putaminal volume are the most consistently observed abnormalities in panic disorder (Radua et al., 2010), although considered together with posttraumatic stress disorder. Similar to the research in MDD, most studies in anxiety disorders lack sufficient sample size and adequate controlling for comorbidity and medication use.

NEUROANATOMICAL MODELS OF MOOD AND ANXIETY DISORDERS

Based on the variety of brain regions associated with abnormal structure or function, MDD and anxiety disorders are not considered disorders of a single brain region; Instead, MDD and anxiety disorders are considered the result of complex deficient interactions between highly integrated networks of limbic-cortical regions (Seminowicz et al., 2004). Several neuroanatomical models have been proposed in an attempt to explain the variety of symptoms observed in MDD including depressed mood, anhedonia, somato-vegetative dysregulation, and concentration problems. These models propose that symptoms are based on abnormal functioning of both (para)limbic and neocortical brain regions. (Para)limbic regions are primarily associated with perception of emotional information and the generation of a primary emotional response, whereas the neo-cortical regions are mainly associated with cognitive and emotional control. (Para)limbic, or 'ventral' [5], regions include amongst others the amygdala, insula, ventral striatum, thalamus, and subgenual ACC. Dorsolateral PFC, dorsal ACC, and dorsomedial PFC are considered 'dorsal' regulatory neocortical areas, in the context of emotion regulation models.

5 'Ventral' or 'inferior' refers to the lower portion of the brain. 'Dorsal' or 'superior' refers to the top portion of the brain.

In a prominent neuroanatomical model of limbic-cortical dysregulation developed by Helen Mayberg in 1997 and updated in 2004 (Seminowicz et al.), increased activation of ventral paralimbic regions and decreased activation of dorsal neocortical regions are proposed as prominent features of MDD, responsible for sad mood. The Mayberg model emphasizes the importance of a third 'emotion-cognition integration component', including the rostral ACC,

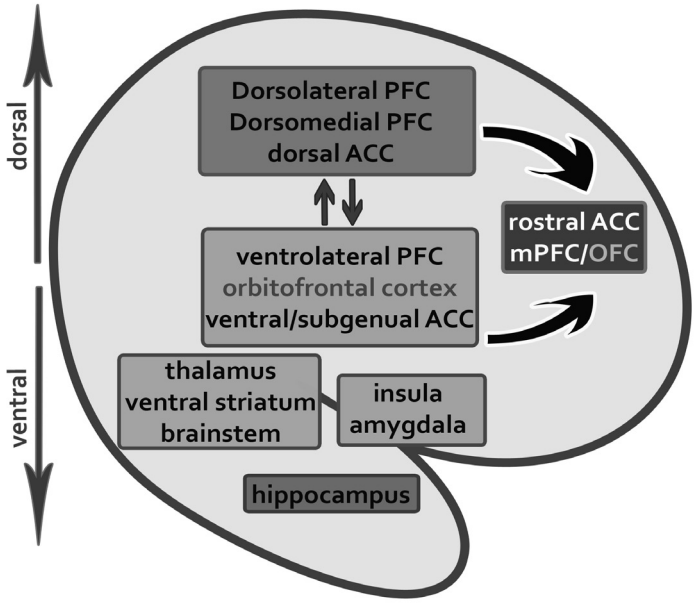


FIGURE 1:

Graphical representation of the combined models of Mayberg (1997; Seminowicz et al., 2004) and Phillips (Phillips, Drevets, Rauch, & Lane, 2003b) displayed within the contours of the brain, as viewed from aside. In this model the orbitofrontal cortex (OFC), is depicted in gray, as it serves a different role in the two models. Finally, the hippocampus is also depicted in gray, as in both models, this structure is considered to serve a dorsal regulatory role, despite its ventral location.
In Box I, an overview of functions of several ventral and dorsal regions and their relation with the psychopathology in MDD and anxiety disorders is presented.

medial PFC, and orbitofrontal cortex. In his model, the rostral component serves as a modulatory hub between ventral 'autonomic integration' regions (e.g., sub- genual ACC, thalamus) and dorsal 'sensory-cognitive integration' regions (e.g., dorsolateral PFC, hippocampus) and is implicated in normal mood regulation.

A second neuroanatomical model, developed by Mary Phillips and colleagues (2003b) proposes that direct deficient interactions between 'ventral' ((para) limbic) and 'dorsal' (neocortical) regions could result in the symptomatology of a range of psychiatric disorders such as schizophrenia, bipolar disorder, and MDD. This Phillips model predicts that excessive activity of ventral and decreased activation of dorsal structures gives rise to the experience of inappropriate threat and a decreased range of emotional states. Figure 1 depicts the models of Mayberg and Phillips combined for the purpose of this thesis.

Although frequently associated with salience detection (See box I), and therefore an evident region of interest, the amygdala is not included in every model of mood regulation in MDD and in the most consistent functional or structural abnormalities reported to date in MDD (Seminowicz et al., 2004). In the study of anxiety disorders, disrupted interaction between amygdalar nuclei and dorsal PFC structures has been proposed to underlie the symptomatology (Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010; Etkin, Prater, Schatzberg, Menon, & Greicius, 2009), giving a more central role for the amygdala in the pathophysiology of anxiety disorders than in the models of MDD. The model

of Mayberg (1997) is primarily proposed for MDD, whereas the model of Phillips (2003b) is considered a more general framework for psychiatric disorders, such as posttraumatic stress disorder and schizophrenia. To what extent these models are applicable to thefunctional and structural neuropathology of prevalent anxiety disorders and to comorbid depression-anxiety is barely studied.

AIM OF THE PRESENT STUDY

Most studies performed to date focused either on MDD or on anxiety disorders and failed to systematically exclude comorbid psychiatric disorders. Also, studies performed to date failed to test for shared and unique characteristics in brain function and structure of MDD and anxiety disorders. Importantly, no study explicitly studied the comorbid condition of MDD and anxiety disorders and compared the neurophysiological profile of this comorbid condition with 'singular' (i.e. without comorbidity) MDD and anxiety. Also, owing to the small numbers of participants included in most studies, comorbidity of MDD on the correlates of anxiety, and vice versa could not be controlled for. This limited power (small sample size) constitutes a further problem, as it has been shown that reliable and replicable task effects in fMRI studies are only obtained in samples of over approximately 15 persons (Thirion et al., 2007). Small sample sizes lead to unreliable results and do not allow one to control for important confounders, such as use of antidepressive medication, illness severity, and psychiatric comorbidity. The inconsistencies in imaging results reported so far could be the result of such factors.

In addition to including predominantly medicated patients, the inclusion of severely ill, hospitalized patients in previous studies may have confounded results. Hospitalization may be a very stressful experience itself, which often involves change of medication or administration of a sedative drug. Including patients who suffer from concentration problems related to the negative side effects of hospitalization or use of psychotropic acting drugs may result in activation abnormalities. Subsequently, these abnormalities might be falsely attributed to the psychopathology. Also, cognitive dysfunction may be an important predictor for hospitalization (Jaeger, Berns, Uzelac, & vis-Conway, 2006). Therefore, inpatients may be characterized by a distinct symptom- or functional impairment profile than outpatients.

Furthermore, few studies have investigated whether functional and structural abnormalities that are observed in the 'active' depressed or anxious state continue into the remitted phase. PET studies have demonstrated abnormal orbitofrontal, ACC and dorsolateral PFC perfusion in the remitted state in MDD in a mood provocation paradigm, indicating a functional vulnerability that could explain the heightened sensitivity to relapse into another MDD episode. Recent evidence exist that those who reach full remission after six weeks of SSRI treatment, differ in both brain morphology and cognitive functioning from patients who do not reach remission (Li et al., 2010). However, whether

such 'trait' phenomenon are observed during cognitive and emotional tasks in MDD patients, or are observed in patients with remitted anxiety disorders, is barely studied.

In anxiety disorders, studies primarily focused on the signaling function of ventral regions in response to negative or threat eliciting stimuli. Dorsal PFC involvement during cognitive tasks has hardly been studied in panic disorder, and studies on PFC involvement in social anxiety disorder and generalized anxiety disorder are virtually absent. Studying fMRI correlates of cognitive tasks that depend on intact functioning of dorsal PFC regions such as the dorsolateral PFC, dorsal ACC and inferior frontal gyrus is important because of the role these regions play in (models of) emotional regulation.

The studies presented in the current thesis aimed to overcome such limitations by studying both cognitive and emotional processes in a large sample of outpatients with MDD and anxiety disorders (panic disorder, social anxiety disorder, generalized anxiety disorder), and healthy controls. Participants were recruited from the Netherlands Study of Depression and Anxiety (NESDA; described below), a large-scale, observational study in the outpatient setting. This allowed for the inclusion of large numbers of participants and to study MDD and anxiety in a 'real-life' setting. By including a large sample of patients with both MDD and anxiety disorders, the shared and unique correlates in MDD and anxiety disorders could be studied. At the same time, this set-up allowed to explicitly test for the effect of comorbidity of depression and anxiety, use of antidepressant medication, and illness severity.

THE NETHERLANDS STUDY OF DEPRESSION AND ANXIETY

The Netherlands Study of Depression and Anxiety (NESDA) is a multi-center, longitudinal, observational cohort study, that aims to (1) gain insight in the long-term prognosis of depression and anxiety disorders in terms of course and public health consequences, (2) study depression and anxiety in concert, and (3) integrate biological and psychosocial paradigms of mood and anxiety disorders. NESDA has been designed to be representative of those with depressive and anxiety disorders in different health care settings and stages of developmental history. Therefore, the sample is stratified for setting (community, primary care and specialized mental health) and set up to include a range of psychiatric disorders. NESDA has a focus on MDD, dysthymia, social anxiety disorder, panic disorder, and generalized anxiety disorder. Persons diagnosed with a primary clinical diagnosis of a psychiatric disorder not subject of NESDA that is likely to affect course trajectory, such as posttraumatic stress disorder, personality disorders, and obsessive-compulsive disorder, were not included in NESDA.

Amongst the psychosocial and biological measures included in NESDA are: Demographic interview, current and life-time psychopathology interview, personal history interview (trauma, life events), health consequences interview (medication use, loss of productivity, disability), perceived need for care questionnaires, current depressive and anxiety state questionnaires,

personality measures, worrying,- depressive and anxious cognitions measures, health behavior interview (sports, smoking, alcohol, drugs use, sleep), saliva sampling for cortisol measures, measures of heart-rhythm variability, and genetic measures. For a complete description of the instruments included in NESDA, see Penninx et al. (2008). Most important for this thesis is the inclusion of functional and structural MRI in NESDA.

NESDA NEUROIMAGING STUDY

A subsample (n=301; 200 female, age range: 18-57) of the total NESDA sample (N=2981) (See Figure 2 for a flow chart and a graphical representation of the MRI sample), was invited to participate in the NESDA neuroimaging study between October 2004 and April 2007. The NESDA neuroimaging study is a longitudinal study that was designed to study cognitive and emotional processes, as well as brain structure in MDD and anxiety disorders. In this thesis 'anxiety disorders' refers to panic disorder, social anxiety disorder and generalized anxiety disorder, unless otherwise specified.

Aims of the NESDA neuroimaging study at large, that include aims beyond the scope of this thesis, were to: (I) Study shared and unique neurophysiological correlates of MDD and anxiety disorders in an outpatient sample, related to basic cognitive processes, emotional processes, 'resting state', and variations in brain structure; (II) study the comorbid condition of MDD and anxiety disorders and make a comparison with both MDD and anxiety disorders on measures named in (I); (III) describe patients with MDD and anxiety disorders with respect

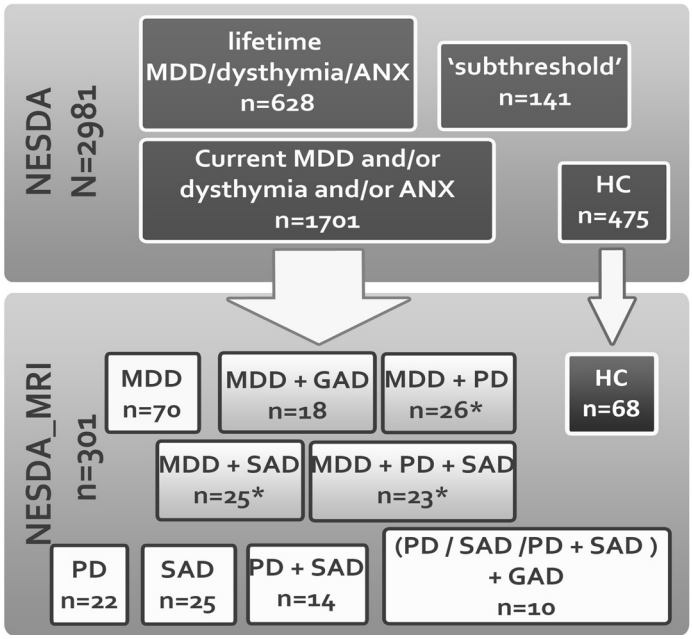


FIGURE 2:

NESDA flow-chart. * indicates that the subsample also includes patients with comorbid GAD. Current diagnosis refers to a half-year diagnosis. All MRI participants were selected from the NESDA 'current diagnosis' sample and the HC sample. In this figure, patients with a current diagnosis are not included in the 'life-time' sample. Approximately one-third of the included patients used anti-depressant medication at the time of scanning.

to their course, to identify neurophysiological correlates that are associated with outcome (recovery, recurrence, development of comorbidity; (IV) describe neurophysiological correlates of risk factors of MDD and anxiety disorders, including genetic variations; (V) test whether a dimensional model of psychopathology for MDD and anxiety disorders (to be developed in NESDA) would describe the functional and structural pathophysiology more accurate than a categorical classification system (DSM-IV classes). MRI measurements were planned at T0 (baseline measurement), T1 (follow up after two years), T2 (four or six years after the baseline measurement). Several tasks were administered to all participants during functional MRI: A non-emotional visuo-spatial planning task (Tower of London; ToL), an emotional word encoding and recognition task, and an emotional face viewing task. In addition, structural imaging for measurement of regional brain volume and white matter integrity measurements and functional imaging for 'resting state' fMRI was applied to study the brain's intrinsic organization when participants were not engaged in a task. Data acquired during execution of the Tower of London visuospatial planning task and the word encoding task will be discussed in this thesis, in addition to the structural MRI data.

OUTLINE OF THE THESIS

The main objective of this thesis is to describe the common and unique functional and structural MRI correlates of MDD and anxiety disorders, while explicitly testing for the effects of their comorbidity. The thesis is structured into two sections: in SECTION I, the functional and structural MRI correlates of a current diagnosis of MDD and/or anxiety disorders are investigated, while the effects of antidepressant medication use and illness severity are being studied as well. In SECTION II, the focus is on factors that are considered risk factors for developing mood and anxiety disorders.

SECTION I

In **Chapter 2**, common and unique neuroanatomical correlates of MDD and anxiety disorders are studied with an optimized voxel based morphometry (VBM) approach, a method that allows detecting regional volumetric differences across the whole brain. Aim of this study is to investigate whether emotional disturbances in MDD and anxiety could at least in part be explained by volumetric variations in regions related to emotional processing.

In **Chapter 3**, common and unique neurophysiological correlates of encoding and recognizing emotional words in MDD and anxiety disorders are investigated. Aim of this study is to test whether disorder specific biases towards negative and away from positive information result in altered memory formation and performance that could explain the prolonged course of the disease.

In **Chapter 4**, fMRI correlates of a visuospatial planning task are discussed. Visuospatial planning is an executive process that depends heavily on dorsal prefrontal integrity. Aim of this study is to test whether dorsal prefrontal involvement during executive processes is equally compromised in both

depression and anxiety, or whether it should be considered an MDD specific phenomenon.

In **Chapter 5,** we focus on the intrinsic organization of subcortical and cortical brain structures during an emotional word categorization task in MDD. As MDD is considered the result of complex deficient interactions between highly integrated networks of limbic-cortical regions, we aim to study intrinsic functional connectivity between both dorsal and lateral cortical regions on the one hand and subcortical and limbic regions on the other hand, while patients are engaged in an emotional task.

SECTION II

Chapter 6 focuses on the effects of childhood emotional trauma or abuse on regional brain volume across patients with depression and anxiety disorders. Early life trauma is one of the major risk factors for developing emotional disturbances. Aim of this study is to indicate a possible neuroanatomical vulnerability for developing depression and anxiety disorders resulting from early life abuse.

In **Chapter 7,** we present a study that focuses on the influence of the personality factors neuroticism and extraversion on brain structure. Neuroticism and extraversion are personality factors that have been associated with both increased and lower risk of developing mood and anxiety disorders. To avoid thepossible confounding effect of current psychopathology, we focus on personality correlates in healthy controls.

In **Chapter 8,** results are summarized and discussed. Also, recommendations will be provided for implementing (f)MRI in an observational study.

BOX I

INVOLVEMENT OF VENTRAL AND DORSAL REGIONS IN MDD AND ANXIETY DISORDERS: A SUMMARY

The amygdala, a limbic structure, is primarily associated with salience detection and processing of emotional cues, also in the context of memory processes (Hamilton & Gotlib, 2008). In MDD and panic disorder, the amygdala has found to show abnormal gray matter volume (Asami et al., 2009; Hamilton, Siemer, & Gotlib, 2008; Hayano et al., 2009; Massana et al., 2003; Wagner et al., 2008). An increased BOLD response during (masked) negative face viewing (Sheline et al., 2001) and a prolonged response during emotional word classification (Siegle et al., 2007) has been observed in MDD. In social anxiety disorders, the amygdala has also been associated with an elevated (Stein, Goldin, Sareen, Zorrilla, & Brown, 2002) and delayed response (Campbell et al., 2007) during negative (contemptuous and angry) face viewing. In both MDD and anxiety disorder, amygdala hyperactivation has been found to normalize after successful treatment (Ressler & Mayberg, 2007).

The hippocampus has been regarded as an affective state regulatory structure (Phillips, Drevets, Rauch, & Lane, 2003a) and has been repeatedly found to be reduced in volume in MDD (Campbell & MacQueen, 2006). The hippocampus is an important structure related to HPA-axis functioning, the primary stress-regulatory system of the human body, and is thought to be implicated in the feed-back loop on adrenal cortisol secretion (Jacobson & Sapolsky, 1991). Volumetric reductions in this region have been linked to the neurotoxic effects of excessive cortisol (McEwen, 2005; Sapolsky, 2000). Functionally, the hippocampus has been associated with contextual memory processes (McEwen, 2000), and reduced neurogenesis of hippocampal cells due to excessive cortisol may lead to compromised memory functions, as has been found in MDD (Zakzanis, Leach, & Kaplan, 1998). Although abnormal hippocampus activation is not a commonly observed phenomenon, the hippocampus has been found to abnormally inhibit dorsal prefrontal regions in MDD and at the same time facilitate ventral prefrontal regions (Hamilton, 2010). These abnormal projections may be at the base of depressive symptomatology. Also in the study of anxiety disorders, abnormal interactions of the hippocampus with the amygdala and cortical areas has been observed (Etkin et al., 2010; Etkin et al., 2009).

The insular cortex has extensive connections with the amygdala, hypothalamus, and peri-aquaductal gray, and has been associated with regulation of the autonomic nervous system (Etkin & Wager, 2007). The insula has been related to disgust processing (Phillips et al., 1997; Small, 2010), homeostatic regulation (Small, 2010), fear conditioning (Buchel, Dolan, Armony, & Friston, 1999), and anticipation of negative reward (Phelps et al., 2001). Abnormal insular

BOX I

functioning in MDD has been observed during aversive emotional paradigms (Surguladze et al., 2010) and is thought to reflect the somato-vegetative symptoms, such as abnormal sleep and appetite (Wiebking et al.,2010). Also, in social anxiety disorder, increased insular activation is among the most consistently observed phenomena during emotional paradigms and is associated with increased activation of a network related to producing fear responses to symptom provoking material (Etkin & Wager, 2007).

The ventral striatum, including the nucleus accumbens, and the ventro-medial PFC have been associated with reward processing, and abnormal activation of these regions in MDD has been associated with the MDD core symptom of anhedonia as reflected in decreased activation during processing of positive stimuli (Epstein et al., 2006), positive mood induction (Keedwell et al., 2005b), and with a failure to sustain activation over time during upregulation of positive emotional states (Heller et al., 2009). In the study of anxiety disorders, the ventral striatum has been a target for deep brain stimulation, an invasive therapy that aims to influence activation of specific brain targets in order to relieve symptom burden. Studies on the results of the effects of deep brain stimulation in anxiety disorders, including obsessive compulsive disorder, have proposed that the ventral striatum serves as a modulatory hub in the information flow between the amygdala and the basal ganglia, thalamus and PFC (Sturm et al., 2003).

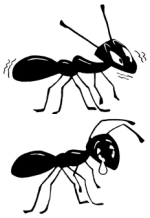
The subgenual ACC, the most ventral region of the cingulate cortex, has been associated with processing emotional and motivational information and the production of affective states (Bush, Luu, & Posner, 2000; Phillips et al., 2003a). In MDD, reduced volume of the subgenual ACC has been observed (Botteron, Raichle, Drevets, Heath, & Todd, 2002) and blunted regional blood flow in the subgenual ACC has been observed during mood induction, even in remitted (recovered) MDD patients (Liotti, Mayberg, McGinnis, Brannan, & Jerabek, 2002). The subgenual ACC has been linked to the treatment response to cognitive behavioral therapy in both MDD and social anxiety disorder (Ressler & Mayberg, 2007).

The orbitofrontal cortex has been associated with behavioral inhibition, impulsivity, decision making, and interpreting other people's mood (Elliott, Zahn, Deakin, & Anderson, 2010; Milad & Rauch, 2007). The orbitofrontal cortex has been related to the autonomic regulation of emotional behaviour (Phillips et al., 2003a) and reward related behaviour (Milad & Rauch, 2007). Related to mood regulation, the orbitofrontal cortex has been found to exert inhibitory control over amygdalar activation (Milad & Rauch, 2007). In MDD, orbitofrontal cortex metabolism has been related to symptom improvement after successful treatment (Brody et al., 1999). In the study of anxiety disorders, orbitofrontal functioning has been linked to a failure to inhibit inappropriate anxiety and fear

BOX I

responses ((Milad & Rauch, 2007).

Dorsal prefrontal cortex (PFC) regions, including the dorsolateral PFC, dorsal ACC, inferior frontal gyrus (IFG), and dorsomedial PFC have been predominantly linked to executive and attentional control functions, such as sustained attention (dorsal ACC), selective attention (IFG) and executive processes (dorsolateral PFC), during working memory tasks, switching tasks, planning tasks, and verbal fluency tasks; tasks that all have been found to be mildly impaired in MDD (Fossati et al., 2002). Verbal fluency and switch functions also have been found to depend on involvement of the orbitofrontal cortex. In the study of MDD, both increased and decreased activation of these regions have been observed (Fossati et al., 2002). In patients with social anxiety disorder, viewing of negative (disgust) facial expressions has been associated with increased ACC activation (Amir et al., 2005). In generalized anxiety disorder, failure of the ACC to dampen the amygdalar response has been associated with a failure to regulate emotional conflict (Etkin et al., 2010). Thus, next to executive and attentional control, dorsal PFC regions have been thought to exert top-down control over the ventral emotional appraisal regions, possibly via the ACC and medial PFC (Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007).



CHAPTER 2

REGIONAL BRAIN VOLUME IN DEPRESSION AND ANXIETY DISORDERS

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ARCHIVES OF GENERAL PSYCHIATRY, 2010; 67(10): 1002-1011

SUMMARY

Background: Major depressive disorder (MDD), panic disorder, and social anxiety disorder are among the most prevalent and frequently co-occuring psychiatric disorders in adults and may have, at least in part, a common etiology. In the present study we aimed to identify the unique and shared neuroanatomical profile of depression and anxiety, controlling for illness severity, medication use, sex, age of onset, and recurrence.

Methods: Sixty-eight patients with MDD, 88 patients with MDD and comorbid anxiety disorders, 68 patients with panic disorder and/or social anxiety disorder and/or generalized anxiety disorders, and 65 healthy controls were recruited from the Netherlands Study of Depression and Anxiety. Volumetric Magnetic Resonance Imaging was conducted for Voxel Based Morphometry analyses. We tested voxel wise for the effects of diagnosis, age of onset, and recurrence on gray matter density. Post hoc we studied the effects of use of medication, illness severity, and gender.

Results: We demonstrated lower gray matter volumes of the rostral anterior cingulate gyrus (ACC), extending into the dorsal ACC in MDD, comorbid depression-anxiety, and anxiety disorders without comorbid MDD, independent of illness severity, sex, and medication use. Furthermore, we demonstrated reduced right lateral inferior frontal volumes in MDD and reduced left middle/superior temporal volume in patients with anxiety disorders. Also, patients with onset of depression before 18 years of age showed lower volumes of the subgenual prefrontal cortex.

Discussion: Our findings indicate that reduced volume of the ventral anterior cingulate gyrus is a generic effect in depression and anxiety disorders, which is present independent of illness severity, medication use, and sex. This generic effect supports the notion of a shared etiology and may reflect a common symptom dimension related to altered emotional processing. Specific involvement of the inferior frontal cortex in MDD and lateral temporal cortex in anxiety disorders without MDD, on the other hand, may reflect disorder-specific symptom clusters. Early onset of depression is associated with a distinct neuroanatomical profile that may represent a vulnerability marker of depressive disorder.

Major depressive disorder (MDD), panic disorder, generalized anxiety disorder, and social anxiety disorder are among the most prevalent and most frequently co-occurring psychiatric disorders in adults. Estimations of the comorbidity of depression and anxiety range from 10% to more than 50% (Gorman, 1996; Gorman & Coplan, 1996; Ressler & Mayberg, 2007; Roy-Byrne et al., 2000). Therefore, it has been suggested that depression and anxiety have a similar etiology, also because they respond to the same treatment strategies (Ressler & Mayberg, 2007). The comorbid condition of depression and anxiety, however, may differ from MDD in clinical course and characteristics because it has been associated with worse outcome (Bruce et al., 2005; Gorman, 1996; Gorman & Coplan, 1996; Roy-Byrne et al., 2000; Rush et al., 2005) and more severe psychopathology (Kessler et al., 2005; Roy-Byrne et al., 2000; Rush et al., 2005). Also, the onset of anxiety often precedes the onset of the first depressive episode (Parker et al., 1999). However, in studies focusing on the neurobiology of depression and anxiety, comorbidity is rarely explicitly studied.

Major Depressive Disorder has frequently been associated with stress system dysregulation, as reflected by abnormal hypothalamus-pituitary-adrenal–axis function (Belmaker & Agam, 2008), of which the glucocorticoid cortisol is an end product. An abnormal glucocorticoid response has been associated with volumetric changes in the hippocampus, amygdala, and prefrontal cortex in animal studies (McEwen, 2005). These brain structures are rich in glucocorticoid receptors and are therefore a target for the putative neurotoxic action of excess glucocorticoids/cortisol. However, altered volumes of the hippocampus, amygdala, and prefrontal regions are likely to result from other pathogenetic mechanisms as well because abnormal cortisol levels have not been consistently found in MDD (Chida & Steptoe, 2009).

A number of neuroimaging studies of MDD have shown altered volumes in structures related to hypothalamus-pituitary-adrenal–axis function, emotion perception, and regulation, i.e. the hippocampus (Frodl et al., 2006; Lange & Irle, 2004; Mervaala et al., 2000; Sheline, Gado, & Kraemer, 2003; Wagner et al., 2008; Weniger, Lange, & Irle, 2006), amygdala (Hamilton et al., 2008; Sheline, Gado, & Price, 1998; Wagner et al., 2008), striatum (Hickie et al., 2007), medial prefrontal cortex (Bremner et al., 2002; Frodl et al., 2006; Wagner et al., 2008), and anterior cingulate gyrus (ACC) (Botteron et al., 2002; Caetano et al., 2006; Drevets et al., 1997; Hastings, Parsey, Oquendo, Arango, & Mann, 2004; Tang et al., 2007; Vasic, Walter, Höse, & Wolf, 2008; Yucel et al., 2008). Most studies have reported decreased volumes in these structures (Koolschijn et al., 2009), although findings have not been wholly consistent (Bremner et al., 2002; Caetano et al., 2001; Frodl et al., 2008a; Frodl et al., 2002; Frodl et al., 2003; Hastings et al., 2004; Koolschijn et al., 2009; Lange & Irle, 2004; Rusch, Abercrombie, Oakes, Schaefer, & Davidson, 2001; Vakili et al., 2000; Vasic et al., 2008; Weniger et al., 2006). Importantly, only few studies have explicitly controlled for comorbidity of anxiety, although morphometric changes have

been identified in anxiety disorders as well (Damsa, Kosel, & Moussally, 2009; Radua et al., 2010). Whereas volumetric studies in social anxiety disorder and generalized anxiety disorder have been rare, studies in panic disorder have fairly consistently found altered volumes of the amygdala (Asami et al., 2009; Hayano et al., 2009; Massana et al., 2003), insular cortex (Asami et al., 2009; Uchida et al., 2008), dorsomedial prefrontal cortex (Asami et al., 2009), and ACC (Asami et al., 2008; Asami et al., 2009; Uchida et al., 2008). In addition, altered brainstem (Asami et al., 2008; Protopopescu et al., 2006), orbitofrontal cortex (Asami et al., 2009; Mohlman et al., 2009), and superior temporal volumes (Asami et al., 2009; Yoo et al., 2005) have been implicated in the neuropathology of panic disorder. However, results of these studies may have been similarly confounded by the presence of comorbid depression (Asami et al., 2008; Uchida et al., 2008). In summary, volumetric studies appear to indicate specific involvement of the hippocampus in depression,the insular cortex and superior temporal areas in anxiety disorders, and prefrontal and amygdalar areas in both depression and anxiety disorders. Moreover, to our knowledge, no study to date studied the unique and common neuroanatomical profile of depression and anxiety.

In this cross-sectional study, we investigated the shared and unique neuroanatomical profile of depression and anxiety, controlling for the effects of illness severity, use of selective serotonin reuptake inhibitors (SSRIs), and sex as potential confounders (Campbell & MacQueen, 2006). We also investigated the effects of recurrence of depression and age at onset, reflecting changes associated with prolonged illness duration (McKinnon, Yucel, Nazarov, & MacQueen, 2009) or increased vulnerability to depression and anxiety (MacMaster et al., 2006; MacMillan et al., 2003). Based on previous studies, we hypothesized that patients with MDD with or without comorbid anxiety disorders (panic disorder, social anxiety disorder, generalized anxiety disorder) would show decreased volumes in the hippocampus, amygdala, ACC, and medial prefrontal cortex. In addition, we predicted decreased volumes in the ACC, amygdala, insula, and superior temporal gyrus in patients with an anxiety disorder with or without comorbid MDD.

METHODS

PARTICIPANTS

Participants were recruited from the Netherlands Study of Depression and Anxiety (NESDA), a large-scale, multi-site, longitudinal, observational cohort study. The design has been described in detail elsewhere (Penninx et al., 2008). In short, NESDA was designed to be representative of those with depressive and anxiety disorders in different health care settings and stages of developmental history. Therefore, the sample is stratified for setting (community, primary care and specialized mental health) and set up to include a range of psychopathology.

Of the 2981 NESDA respondents (main sample), participants aged between 18 and 57 years were asked to participate in the NESDA neuroimaging study if

they met the DSM-IV criteria for a half year diagnosis of MDD and/or anxiety disorder (panic disorder, social anxiety disorder and/or generalized anxiety disorder) or no lifetime DSM-IV diagnosis (i.e., healthy controls). Personality disorders were not screened for and so were not used in the inclusion/exclusion criteria, although persons with known personality disorders (through information from clinics or through self-report) were not included in NESDA. Exclusion criteria for patients were the presence of axis-I disorders other than MDD, panic disorder, social anxiety disorder, generalized anxiety disorder and any use of psychotropic medication other than a stable use of SSRIs or infrequent benzodiazepine use (i.e., equivalent to 2 doses of 10 mg of oxazepam 3 times per week or use within 48 hrs prior to scanning). Exclusion criteria for participants were the presence or history of major internal or neurological disorder (e.g., contusio cerebri with loss of consciousness>15 min, diabetes mellitus type I), dependency or recent abuse (past year) of alcohol and/or drugs, hypertension, and general MRI contraindications. Diagnoses were established using the structured Composite International Diagnostic Interview (CIDI), according to DSM-IV algorithms were established using the structured Composite International Diagnostic Interview (CIDI) – lifetime version 2.1 (Robins et al., 1988), given by a trained interviewer. Controls were currently free of, and had never met criteria for, depressive or anxiety disorders or any other axis-I disorder and were not taking any psychotropic drugs.

Overall, 301 native Dutch speaking participants (MRI-sample; 235 patients and 66 controls) were included and underwent magnetic resonance imaging in one of the three participating centers: Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen. The Ethical Review Boards of each participating center approved this study. All participants provided written informed consent after receiving written information.

ADDITIONAL PSYCHIATRIC MEASUREMENTS

Severity of depression and anxiety at the day of scanning was assessed using Dutch versions of the Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988), the Montgomery Åsberg Depression Rating Scale (Montgomery & Åsberg, 1979), the Inventory of Depressive Symptomology (Rush et al., 1986) and the Fear Questionaire (Marks & Mathews, 1979). Fear Questionnaire ratings were obtained from 243 subjects only.

IMAGE ACQUISITION

Imaging data were acquired using Philips 3-Tesla magnetic resonance imaging systems (Best, The Netherlands) located at the departments of Radiology of Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen, equipped with a SENSE-8 (Leiden University Medical Center and University Medical Center Groningen) or a SENSE-6 (Academic Medical Center Amsterdam) channel head coil. For each

subject, anatomical images were obtained using a sagittal 3-dimensional gradient-echo T1-weighted sequence (time to repetition=9 milliseconds, echo time =3.5 milliseconds; matrix 256x256; voxel size, 1x1x1mm; 170 slices, duration: 4.5 minutes).

STATISTICAL ANALYSIS

Demographic and clinical data were analyzed using SPSS 16.0 (SPSS Inc., Il, USA). Significance was set at $p<.05$, and post hoc paired tests were Bonferroni corrected for multiple comparisons.

Imaging data were analyzed using an optimized Voxel Based Morphometry (VBM) approach, following the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL (Ashburner, 2007)) using Statistical Parametric Mapping software (SPM5) implemented in Matlab 7.1.0. (The MathWorks Inc., MA, USA). Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) is a fully deformable method that is effectively unconstrained by number of degrees of freedom. It has proven good registration accuracy and has been recommended in favor of standard SPM normalization or the SPM unified segmentation approaches for whole brain and regional analysis without segmenting regions of interest (Yassa & Stark, 2009).

Preprocessing of VBM-DARTEL included (1) manually reorientation of the images to the anterior commissure; (2) segmentation of the anatomical images into gray matter, white matter and cerebrospinal fluid using the standard segmentation option implemented in SPM5; (3) applying the DARTEL approach for registration, normalization, and modulation, leaving the images in DARTEL space (in this approach, a DARTEL template is created based on the deformation fields that are produced during the segmentation procedure; next, all individual deformation fields were registered to this template); (4) smoothing of the gray matter and white matter images using an 8-mm full width at half maximum Gaussian kernel to increase signal to noise ratio and to maximize comparability with published VBM studies of depression and anxiety (Frodl et al., 2008b; Tang et al., 2007; Vasic et al., 2008; Wagner et al., 2008; Yoo et al., 2005). In the resulting images, each voxel represents an absolute amount of brain volume, equivalent to the brain volume per unit prior to normalization.

Based on previous articles on volumetric differences in MDD and anxiety disorders, we set the following a priori regions of interest: hippocampus, amygdala, medial PFC, orbitofrontal cortex, ACC, superior temporal gyrus, and insula. For the regions of interest, we set a threshold of $p<.001$, uncorrected, with an extent cluster threshold of 50 contiguous voxels. To further protect against type 1 error, small volume correction (SVC) was applied for the main comparison (effect of diagnosis) by centering a sphere of 16-mm around the peak voxel. The resulting volumes of interest had to meet $p<.05$, corrected for Family Wise Error (FWE) rate at the voxel level, to be considered significant. For non-regions of interest, a voxel level threshold of $p<.05$ whole brain FWE

corrected was set a priori.

Next, data were analyzed in the context of the general linear model (Friston et al., 1995). For our main comparison, we performed a 1x4 factorial analysis with group as random factor over all subjects. To test for effects of depression severity, anxiety severity, SSRI use, and sex, the mean signals of significant clusters were calculated and exported to SPSS. To test for the effects of depression severity, we divided the MDD and comorbid depression-anxiety patients into three subgroups (remitted, mild, moderate to severe), based on their current Montgomery Åsberg Depression Rating Scale score (Muller, Szegedi, Wetzel, & Benkert, 2000). In addition, a whole-brain voxel-wise analysis was performed in SPM to test for the effect of early (<18 year) vs. late (≥18 year) onset of depressive symptoms in MDD and comorbid depression-anxiety and for onset of anxiety symptoms within comorbid depression-anxiety and anxiety disorders without MDD, as compared with controls. Also, a whole-brain voxel-wise analysis was performed to test for effects of recurrent vs. a single major depressive episode in MDD and comorbid depression-anxiety.

Age, gray matter total volumes, and center (by means of two dummy variables) were entered as covariates in each comparison. White matter images were only used to verify whether white matter volume changes occurred in the same regions as gray matter volume changes. For each SPM comparison, groups were matched for age, sex, scan site, and handedness. A description of the total sample is given in the results section. Description of matching procedure and resulting samples for the additional analyses (exclusion of SSRI users, age of onset, recurrence) can be found in the supplemental material.

To achieve maximal sensitivity, optimize voxel residual smoothness estimation, and exclude false positives in non-gray matter tissue, voxel wise comparisons were masked using a comparison-specific explicit optimal threshold gray matter mask created using the Masking toolbox (Ridgway et al., 2009). To preserve optimal normalization accuracy, we left the normalized, modulated, and smoothed images in DARTEL space. Therefore, coordinates are not equivalent to Montreal Neurological Institute (MNI) coordinates. All regions are identified using the detailed brain atlas of Talairach and Tournoux (1988).

RESULTS

SAMPLE DESCRIPTIVE

Data from ten participants were excluded because of poor image quality. In addition, data from two controls were excluded because they had Montgomery Åsberg Depression Rating Scale scores >8 (Muller et al., 2000). We formed four groups based on Composite International Diagnostic Interview half-year diagnoses. Our final sample consisted of 289 subjects: 68 patients with MDD (MDD group), 88 with MDD and one or more comorbid anxiety disorder (CDA group: MDD and panic disorder and/or social anxiety disorder and/or generalized anxiety disorder), 68 with one or more anxiety disorder (panic

disorder, social anxiety disorder, generalized anxiety disorder) but no MDD (ANX group), and 65 controls.

Table 1 lists the sample characteristics. Groups were matched for sex, handedness, distribution of participants scanned over sites, and age, but not on education. The MDD and comorbid depression-anxiety group both had fewer years of education than controls (MDD: U=1370.5, p<.008; CDA: U=1594, p<.008). Furthermore, the comorbid depression-anxiety group included more SSRI users than the MDD and ANX groups. A main effect of group was found on Montgomery Åsberg Depression Rating Scale, Inventory of Depressive Symptomatology, Beck Anxiety Inventory, and Fear Questionnaire score. All diagnostic groups showed higher Montgomery Åsberg Depression Rating Scale, Inventory of Depressive Symptomatology, Beck Anxiety Inventory, and Fear Questionnaire total scores than controls (all z>-4.82; all p<.008). In addition, the comorbid depression-anxiety group reported higher Montgomery Åsberg Depression Rating Scale and Inventory of Depressive Symptomatology scores than the MDD and ANX group, and higher Beck Anxiety Inventory scores than the MDD group (all z>-2.9, p<.008).

Between the NESDA baseline interview (T1) and the magnetic resonance imaging session (T2), depressive scores decreased in all diagnostic groups (Inventory of Depressive Symptomatology; t>3.45; p<.001). The MDD group showed an additional decrease in Beck Anxiety Inventory scores (t_{65}=3.15, p=.002). Post hoc tests showed that currently remitted and mildly depressed subgroups but not the moderately to severely depressed subgroups, showed lowered depressive scores at the time of scanning than at time of baseline interview: At T1, all groups showed moderate/severe depressive scores. The MDD and comorbid depression-anxiety groups did not differ in onset at the first MDD episode, and the comorbid depression-anxiety and ANX groups did not differ in age at onset of the first anxiety disorder. Within the comorbid depression-anxiety group, onset of anxiety generally preceded onset of the first depressive episode (Z=-5.22, p<.001).

VBM RESULTS

Groups did not differ in total gray matter and white matter volumes (gray matter $F_{3,284}$=.25; p=.34 and white matter $F_{3,284}$=.25; p=.86). Lower regional gray matter density of the left rostral ACC (Brodmann area [BA] 24b/c and 32; [1]) was observed in patients compared with controls, extending into the dorsal ACC (BA 32') (Figure 1a). Voxel based comparison of the MDD, comorbid depression-anxiety, and ANX group with controls showed that the rostral and dorsal ACC reduction was most robust in the comorbid depression-anxiety group and was borderline significant in the MDD and ANX groups (MDD: MNI coordinates: [x=0 y=32 z=-11], Z=2.99; p=.001; ANX: MNI coordinates: [x=0 y=41 z=1], Z=3.08; p=.001). Gray matter results are listed in Table 2. The region surrounding the rostral and dorsal ACC gray matter reductions showed white matter volumetric reductions as well (Table 2).

1 For identifying subregions of the ACC, we used the definition described by Bush et al. (2000).

TABLE 1: CLINICAL CHARACTERISTICS OF THE TOTAL SAMPLE (N=289)

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; ANX= anxiety without MDD; HC= healthy controls; a=17 MDD+GAD, 17 MDD+PD, 9 MDD+PD+GAD, 9 MDD+SAD, 15 MDD+SAD+GAD, 12 MDD+PD+SAD, 9 MDD+PD+SAD+GAD; b=20 PD, 2 PD+GAD, 25 SAD, 3 SAD+GAD, 14 PD+SAD, 4 PD+SAD+GAD (of which 18 PD w/o AGO & 22 PD w/ AGO) & 22 PD w/ AGO). H= Kruskal Wallis non parametric multiple sample test; U= Mann-Whitney non-parametric 2 sample test; X^2 = Chi-square test for categorical variables; df=degrees of freedom; SSRI= Selective Serotonin Reuptake Inhibitor; T1: baseline measurement; T2: MRI-measurement; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; IDS: Inventory of depressive symptomatology; FQ: Fear Questionnaire; rem=remitted depressive scores (MADRS: 0-8), mild=mild depressive scores: (MADRS 9-8), mod_sev=moderate to severe depressive scores (MADRS>19); interval= interval between T1 and T2; GM=grey matter total volume; WM=white mattter total volume.

		MDD	**CDA a**	**ANX b**	**HC**	*H*	*F*	*U*	X^2	*df*	*p*	
N		68	88	68	65							
gender	male/female; N	24/44	29/59	18/50	24/41				*1.93*	*3*	*.59*	
scan site	amc/lumc/umcg; N	18/26/24	28/35/25	21/20/27	27/26/12				*.23*	*3*	*.97*	
handedness	left/right; N	6/62	6/82	5/63	5/60				*9.7*	*6*	*.14*	
SSRI use	yes/no; N	18/50	40/48	21/47	0/65				*6.88*	*2*	*.03*	*
age	in years; mean ± sd	37.16 ± 10.24	37.27 ± 10.64	35.96 ± 9.45	40.54 ± 9.71	*7.24*				*3*	*.07*	
education	in years; mean ± sd	12.67 ± 2.91	11.62 ± 3.13	13.11 ± 3.21	14.28 ± 2.86	*26.13*				*3*	*<.001*	*
MADRS	total score; mean ± sd	13.01 ± 9.18	19.94 ± 9.16	10.93 ± 8.66	1.05 ± 1.86	*147.73*				*3*	*<.001*	*
	range	0 – 39	0 – 49	0 – 35	0 – 7							
	rem/mild/mod_sev; N	24/25/19	9/35/43	-	-				*15.68*	*2*	*<.001*	*
IDS T1	total score; mean ± sd	27.68 ± 9.96	33.02 ± 11.51	22.79 ± 11.91	5.14 ± 3.51	*150*				*3*	*<.001*	*
IDS T2	total score; mean ± sd	19.85 ± 11.86	29.49 ± 11.16	19.26 ± 10.81	3.79 ± 3.58	*144.01*				*3*	*<.001*	*
	range	1 – 57	5 – 57	4 – 49	0 - 17							
BAI T1	total score; mean ± sd	11.68 ± 8.86	18.41 ± 9.10	15.22 ± 9.9	1.89 ± 3.11	*137.33*				*3*	*<.001*	*
BAI T2	total score; mean ± sd	8.95 ± 8.2	18.23 ± 8.97	14.12 ± 9.60	2.19 ± 2.57	*125.30*				*3*	*<.001*	*
	range	0 – 50	1 – 46	0 – 42	0 – 10							
FQ	total score; mean ± sd	21.1 ± 15.39	36.35 ± 19.09	37.17 ± 20.48	9.05 ± 7.71	*44.41*				*3*	*<.001*	*
	range	0 – 79	6 – 88	3 – 84	0 – 29							
interval	in days; mean ± sd	71.4 ± 59.1	57.9 ± 49.5	69.9 ± 33.2	63.7 ± 28.8		*1.47*			*3*	*.22*	
onset MDD	age in years; mean ± sd	25.62 ± 10.36	23.40 ± 11.38	-	-			*2505.5*			*.13*	
onset ANX	age in years; mean ± sd	-	17.67 ± 10.27	15.47 ± 11.27	-			*2156*			*.20*	
recurrence MDD	single/recurrent episode; N	29/39	39/49	-	-							
ANX	diagnosis in lifetime; N	21	87	68	0							
	diagnosis in past year; N	9	87	68	0							
MDD	diagnsois in lifetime; N	68	87	37	0							
	diagnosis in past year; N	68	87	4	0							
GM	ml; mean ± sd	728.7 ± 67.64	729.98 ± 75.11	739.98 ± 76.95	725.48 ± 76.58		*.48*			*3,29*	*.70*	
WM	ml; mean ± sd	486.12 ± 63	500.81 ± 64.76	493.99 ± 64.38	489.19 ± 63.66		*.78*			*3,29*	*.78*	

Furthermore, in the MDD group, gray matter volume reductions in the right inferior frontal cortex were observed (Figure 1b). The ANX group showed less left middle and superior temporal gyrus volume compared to controls (Figure 1c). In both regions, white matter reductions were observed as well. The reverse contrasts (MDD, CDA, and ANX groups > controls) did not reveal significant clusters.

EFFECTS OF ILLNESS SEVERITY, SEX, AND SSRI-USE

Analysis with SPSS showed that the rostral/dorsal ACC gray matter volume reduction occurred in all diagnostic groups relative to controls. (all $p < .05$, Bonferroni corrected), whereas the right inferior frontal gyrus and middle/superior temporal gyrus volume reductions were specific to the MDD and ANX groups, respectively ($p<.05$, Bonferroni corrected).

No effect of depressive state was observed within group on ACC (MDD: $F_{2,66}=.98$; $p=.38$; CDA: $F_{2,80}=.09$; $p=.91$) or inferior frontal gyrus volume within MDD ($F_{2,67}=2.14$, $p=.13$). Adding Beck Anxiety Inventory and Fear Questionnaire scores to these models did not change the results (rostral/dorsal ACC: MDD: $F_{2,64}=.51$, $p=.61$; CDA: $F_{2,78}=.1$, $p=.91$; inferior frontal gyrus: $F_{2,64}=2.06$, $p=.14$).

Beck Anxiety Inventory and Fear Questionnaire scores were not predictive of rostral/dorsal ACC volume in patients (BAI: β=05; $p=.22$; FQ: β=-.06; $p=.1$), of inferior frontal gyrus volumes in MDD (BAI: β=-.02; $p=.85$; FQ: β=.14; $p=.24$), and of middle/superior temporal gyrus volumes in patients with anxiety disorders (BAI: β=-.06; $p=.52$; FQ: β=.02; $p=.86$). No interaction of sex and diagnosis was observed in any region (rostral/dorsal ACC: $F_{3,287}=1.46$; $p=.23$; inferior frontal gyrus: $F_{3,187}=.56$; $p=.64$; middle/superior temporal gyrus: $F_{2,287}=1.6$; $p=.19$) and omission of the SSRI users from the analysis did not affect the results. Table S-1 lists the sample characteristics of the SSRI-free diagnostic groups.

AGE AT ONSET

A voxel-wise, whole-brain analysis showed that patients with early onset of depression (MDD and comorbid depression-anxiety) had lower gray matter volumes of the right subgenual ACC (BA 25) extending into the medial orbitofrontal gyrus compared with controls (Table 2c, Figure 1d), and no effect of sex was observed. No volumetric differences were observed in the subgenual ACC in persons with late-onset depression vs. controls, and no white matter reductions occurred in this region. Also, no effect of early vs. late onset of the first anxiety disorder was observed in the ANX and comorbid depression-anxiety group. Table S-2 lists the sample characteristics of the early vs. late onset subgroups.

A voxel-wise, whole-brain analysis showed no effect of single vs. recurrent depressive episodes in the MDD and comorbid depression-anxiety group. Table S-3 lists the sample characteristics of the single vs. recurrent episode MDD groups.

FIGURE 1: VBM EFFECTS

A) main effect of patients < healthy controls showing gray matter reductions in the rostral and dorsal anterior cingulate gyrus; B) lower right inferior frontal gyrus volume in MDD as compared to HC; C) lower right middle/superior temporal gyrus volume in ANX as compared to HC; D) MDD patients (MDD and CDA) with onset of the first depressive episode before the age of 18 are characterized by lower subgenual OFC volumes than HC. All effects are displayed at p<.005, uncorrected.

A full-color image can be found on the supplementary sheet.

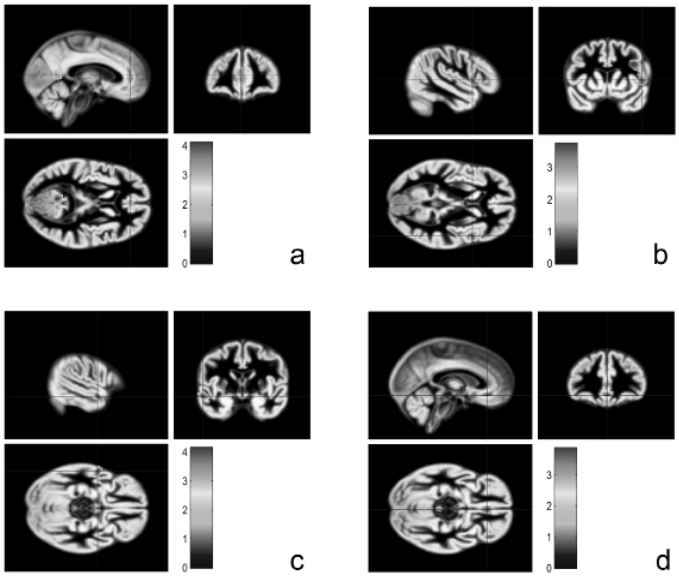

TABLE 2: VBM RESULTS

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; ANX= anxiety without MDD; HC= healthy controls; SSRI= Selective Serotonin Reuptake Inhibitor; DARTEL-coordinate= coordinates of the voxel showing peak significance in mean DARTEL space, R/L= Right/Left hemisphere; BA= Brodmann area; k= cluster size; SVC= Small Volume Correction.

A: Gray matter group comparisons, including SSRI-users

					DARTEL-coordinate			
comparison	R/L	BA	region	k	x	y	z	Z-value
pt < HC	L	24b/c/32	rostral anterior cingulate gyrus	152	0	42	4	3.54
	L	32'	dorsal anterior cingulate gyrus		-2	39	16	3.52
MDD<HC	R	44	inferior frontal gyrus	178	36	11	27	3.69
	R	45	inferior frontal gyrus	115	47	11	4	3.56
CDA<HC	R	24b/c/32	rostal anterior cingulate gyrus	83	2	42	6	3.50
ANX<HC	L	21	middle/superior temporal gyrus	181	-53	-3	-12	4.07

Statistics, coordinates and cluster sizes of the comparisons between pt (patients; MDD, CDA and ANX), and HC. SVC corrected at *p* <.05. SPM smoothness 11.2, 11.8, 11.3 mm; 698.7 resels.

B: White matter group comparisons, including SSRI-users

			k	DARTEL-coordinate			Z-value	
comparison	R/L	region		x	y	z		
pt < HC	R	rostral anterior cingulate gyrus	407	9	42	10	4.32	*
	L	rostral anterior cingulate gyrus	11	-9	41	10	3.17	

Statistics, coordinates and cluster sizes of the comparisons between pt (patients; MDD, CDA and ANX), and HC. Uncorrected at *p* <.001; * SVC FWE corrected at *p* <.05; SPM smoothness 10.6, 10.9, 10.6 mm; 633.3 resels.

C: Gray matter comparisons of early onset vs late onset of MDD in MDD and CDA

					DARTEL-coordinate			
comparison	R/L	BA	region	k	x	y	z	Z-value
EO<HC	R	25/11	subgenual ACC/ orbitofrontal gyru	127	2	32	-11	3.77

Statistics, coordinates and cluster sizes of the comparisons between early onset (EO) MDD and CDA vs. HC. SVC corrected at *p* <.05. SPM smoothness 11.2, 11.8, 11.3 mm; 690.5 resels.

DISCUSSION

In this study we investigated the neuroanatomical characteristics of depression and anxiety in a large sample of outpatients with MDD, panic disorder, social anxiety disorder, and/or generalized anxiety disorder. We used a whole brain, DARTEL-VBM approach, tested explicitly for the effects of comorbidity of depression and anxiety, and controlled for the effects of illness severity, SSRI use, and sex. In addition, we tested voxel-wise for the effects of age at onset and recurrence of depression.

We demonstrated lower gray matter volumes of the rostral ACC, extending into the dorsal ACC in patients with mood and/or anxiety disorders. This rostral ACC decrease occurred in patients with MDD, comorbid depression-anxiety, and anxiety disorders without comorbid MDD, independent of depressive state or anxiety severity, and no effect of SSRI use or sex was observed. Also, white matter reductions occurred in the region bordering the gray matter ACC reduction. Furthermore, we demonstrated reduced right inferior frontal gyrus volumes in MDD and reduced left middle/superior temporal gyrus volume in anxiety disorders compared with healthy controls. In addition, depressed subjects (MDD and comorbid depression-anxiety) with onset of the first depressive episode before 18 years of age showed lower volumes in the subgenual ACC, extending into the medial orbitofrontal gyrus. Finally, patients with MDD and comorbid depression-anxiety with recurrent episode depression showed no volumetric differences compared with a single episode of depression.

To our knowledge, this is the first study to examine the neuroanatomical (i.e. neuroradiological) correlates of both depression and anxiety while explicitly testing for the effects of their co-occurrence. Our findings indicate that reduced volume of the ACC, and more specifically the regions of the ACC that are part of the rostral-ventral affective subdivision (Bush et al., 2000) is a generic effect in depression and anxiety disorders, present independently of depressive state or anxiety severity. This generic ACC reduction supports the notion of a shared etiology in depression and anxiety and may reflect a common pathophysiological mechanism related to altered emotion processing. The rostral ACC region has been found to be primarily involved in salience assessments of emotional and motivational information, while the dorsal ACC has been implicated in effortful processing (Bush et al., 2000), motivational processes, and regulating negative mood (Mak, Wong, Han, & Lee, 2009). The ventral part of the ACC, (including the rostral ACC) has been associated with executive inhibition (Matthews et al., 2009), induced sadness (Liotti et al., 2002; Wang et al., 2008), and negative emotion processing (Shin et al., 2000) in both patients with MDD and controls. The volume reduction observed in this study most likely reflects loss of glial cell density and neuronal size (Cotter, Mackay, Landau, Kerwin, & Everall, 2001), and may be the result of hypothalamus-pituitary-adrenal axis dysregulation (Belmaker & Agam, 2008). The rostral-ventral affective subdivision of the ACC has extensive connections with the orbitofrontal cortex, amygdala, and anterior

insula (Devinsky, Morrell, & Vogt, 1995) and therefore is an important hub in emotion perception and regulation. Also, abnormal ACC morphometry has been associated with worse outcome and/or worse treatment response in MDD (Chen et al., 2007; Frodl et al., 2008c; Yucel et al., 2008).

The results are in concordance with previous imaging studies that have demonstrated affective ACC abnormalities in depression and anxiety disorders, however without consistently controlling for comorbidity (Asami et al., 2009; Botteron et al., 2002; Drevets et al., 1997; Tang et al., 2007; Yucel et al., 2009). Although our comorbid depression-anxiety group displayed more severe depressive and anxiety related pathology, the two depressive groups were characterized by a similar rostral ACC gray matter volume reduction. Also, within groups, no associations between illness severity and ACC volume were observed. The latter finding appears to be at odds with studies that did report a negative correlation between illness severity and ACC volumes (Frodl et al., 2008c; Yucel et al., 2009). For example, Frodl et al. (2008c) in their sample of inpatients who were receiving medication, demonstrated a moderate correlation of depression severity with total right ACC volume but not left total ACC, or subregions within the ACC. However, our findings are in agreement with studies that also failed to demonstrate a relation between illness severity and ACC volume (Caetano et al., 2006; Vasic et al., 2008; Yucel et al., 2008), as was confirmed in a recent meta-analysis (Koolschijn et al., 2009). In this study, we further demonstrated rostral ACC volume reductions even in (recently) remitted patients with depression, consistent with findings of abnormal rostral ACC activation following mood induction in remitted patients (Liotti et al., 2002). Finally, our negative findings regarding SSRI use on ACC volume are in agreement with work of Asami et al (2008). Recent animal studies also indicated that the neurogenesis promoting effects of SSRIs may only be achieved in youth, not in adulthood or old age (Couillard-Despres et al., 2009; Navailles, Hof, & Schmauss, 2008).

In addition to our rostral ACC findings, we also demonstrated reduced right inferior frontal gyrus gray matter volumes in MDD. This finding is in agreement with earlier VBM results of both decreased inferior frontal gyrus concentration (Vasic et al., 2008) and density (Frodl et al., 2008a)[2] in inpatients with MDD in which comorbid anxiety disorders were excluded as well. Data from the present study indicate that this finding is specific for patients with MDD without comorbid anxiety disorders, independent of depression severity and SSRI use. The right inferior frontal gyrus has been implicated in inhibitory processes relevant to executive performance (Matthews et al., 2009; Wang et al., 2008), selective response suppression (Forstmann, van den Wildenberg, & Ridderinkhof, 2008) and cognitive processes related to negative affect (Lieberman et al., 2004), functions that are likely to be impaired in MDD. Therefore, reduced right inferior frontal gyrus volume may represent a neuroanatomical basis for these abnormalities. The left middle/superior temporal reduction in anxiety disorders is also in agreement with previous research (Uchida et al., 2003; Vythilingam et

2 Concentration refers to the proportion of gray matter in each voxel, in which the changes in voxel size have not been accounted for, i.e., the voxel value has not been modulated with the Jacobian determinant derived from the spatial normalization. This does not allow for comparison of the absolute voxel value (Good et al., 2001).

al., 2000) and has repeatedly been linked to the pathophysiology of panic disorder, presumably reflecting impaired evaluation of interoceptive information (Uchida et al., 2008) and altered threat processing (Phillips et al., 1998), as has been shown in functional neuroimaging studies. Depressed subjects (MDD and comorbid depression-anxiety) with onset of the first depressive episode before 18 years of age showed lower gray matter volumes of the subgenual ACC relative to controls, extending into the medial orbitofrontal, an effect that was not observed in patients with onset of the first depressive episode after 18 years of age. This finding is in line with results of Botteron and colleagues (2002), who reported subgenual ACC volume decreases in adolescent-onset MDD, although no direct comparison with late-onset MDD was made. The subgenual ACC volume reduction may represent an early neurobiological lesion resulting in increased vulnerablility to depressed mood, as abnormal subgenual ACC activity during sad mood induction through autobiographical episodes, even after full remission of depression, has been proposed as a trait marker for depression (Liotti et al., 2002). Also, the subgenual ACC has been implicated in fear extinction and emotion regulation and appears to serve as a regulatory hub between the dorsolateral PFC and amygdala, modulating responsiveness in the latter (Delgado, Nearing, Ledoux, & Phelps, 2008). Disruption of this area may therefore lead to altered emotion processing. Finally, subgenual ACC volume reduction may reflect a genetically determined predisposition to MDD because early onset depression is associated with increased familial risk of MDD (Jaffee et al., 2002). This hypothesis has received empirical support from findings of Nolan and coworkers (2002). Moreover, Drevets et al. (1997) reported subgenual ACC reductions in a predominantly familial MDD group in adulthood. Although it may be argued that subgenual ACC reduction merely reflects longer disease duration, this suggestion conflicts with the observed subgenual ACC reduction in the adolescent female cohort of Botteron and colleagues (2002).

In this study, we failed to demonstrate hippocampal and amygdalar volume reductions in MDD, panic disorder, social anxiety disorder and generalized anxiety disorder. Post hoc analyses revealed hippocampal volume reduction only at z=2.17 (p=.02, uncorrected), far below our a priori threshold, and no amygdala volume changes were observed. One may argue that VBM techniques are less sensitive to detect volumetric alternations in small structures or that non-linear registration methods are less sensitive to pick up shape-related alterations compared with region of interest segmentation approaches. It should be noted that post mortem studies did not found support of apoptosis, massive cell loss, or loss of plasticity in the human hippocampus (Campbell & MacQueen, 2004; Swaab, Bao, & Lucassen, 2005), although reports have been conflicting (Stockmeier et al., 2004). Moreover, post mortem studies have mainly included the brains of patients who committed suicide, a subgroup that may not be representative of patients with MDD. Also, most of our depressed patients, although representative of our local outpatient population, were not severely depressed, and therefore did not show hippocampal atrophy.

However, hippocampal reductions were also absent in our severely depressed subgroups (data not shown). In their meta-analysis, MacKinnon et al. (2009) showed that hippocampal atrophy is more likely to occur in pediatric or elderly samples with recurrent MDD. Therefore, decreased hippocampal volume is considered the result of the disease process, as supported by the longitudinal findings of Frodl et al (2008b). Regarding the amygdala, Hamilton et al. (2008) concluded in their meta-analysis that volume of this region s dependent on medication use in MDD. However, this conclusion is not supported by our data; we did not observe amygdalar atrophy in our analysis nor depressed and anxious patients who did not take an SSRI.

In this study, we were unable to identify gray matter reductions outside our a priori regions of interest that survive whole-brain corrections for multiple comparisons. We did, however, observe reductions in the bilateral posterior cingulate cortex (BA 30/23; Z=4.04; p<.001 uncorrected) in addition to the rostral/dorsal ACC reductions described previously in our patient groups. Because most imaging studies to date have focused on frontal and subcortical regions, this region was not included in our a priori regions of interest. However, inclusion of the posterior cingulate cortex as region of interest in future imaging studies will likely be useful in further unraveling the complex neurodynamics of depression and anxiety. Notably, this region has been included in the default mode network as one of the highest energy consuming regions and has been associated with larger deactivations during emotional facial processing (Gentili et al., 2009) and threat processing in anxious patients (Zhao et al., 2007). The posterior cingulate cortex has shown negative connectivity with the amygdala (i.e. increased posterior cingulate cortex activation is correlated with decreased amygdalar activation) and positive connectivity with the dorsal ACC (BA 32) (Stein et al., 2007), indicating that the posterior cingulate cortex is also involved in regulatory interactions with the amygdala, directly and via the ACC.

This study has a number of strengths. First, we were able to include large samples of patients and controls who were extensively screened and phenotyped according to the NESDA protocol; therefore, we could define subgroups of patients based on clinical comorbidity. Second, only stable SSRI use was allowed in our study, and less than half of our patients were taking antidepressant medication at the time of scanning. Consequently, we were able to control for the effects of SSRI on regional brain volume. Third, we used a whole brain approach (VBM-DARTEL) that is rater-unbiased in its segmentation. Therefore, we did not restrict our analysis to only a limited number of brain structures but were able to detect volumetric changes across the brain and to verify if gray matter changes were accompanied by white matter changes in the same region. Voxel-based methods have been found to show satisfactory correlations with manual segmentation approaches (Asami et al., 2008; Uchida et al., 2008; Zhao et al., 2007).

Several potential limitations should also be noted. First, because the epidemiological NESDA cohort was recruited through general practitioners

and outpatient clinics, we may not have been fully able to capture the most severe end of the depressive spectrum. Second, patients with MDD and comorbid depression-anxiety differed slightly from controls in years of education. However, adding years of education as a covariate did not change the results of the main comparison; if anything, the rostral ACC reduction was more robust. Third, assessment of onset and recurrence of depression was based on self-report, which theoretically may have resulted in both underdiagnosis and overdiagnosis of past depression and anxiety. However, this would have biased our results to the null and therefore have led to underestimation of the true associations. Fourth, although persons with identified posttraumatic stress disorder where not included in the NESDA sample, subjects were not systematically screened for it. Therefore, we tested (post hoc) for the effect of trauma on ACC volume because data regarding the experience of emotional and physical trauma were available but no effect of self-reported trauma was observed on ACC volume and no interaction with diagnosis was observed (See the supplemental material for detailed information). Fifth, although similar Philips 3 tesla systems were used at each site in this multi-center study, variability in image acquisition may have occurred owing to minor differences in hardware (receiver coil) and timing of software upgrades. However, no diagnosis x scan site bias occurred. Moreover, reliability of multiscanner VBM has been proven good (Stonnington et al., 2008).

In conclusion, our results suggest a generic involvement of the ventral-rostral ACC in (comorbid) MDD, panic disorder, social anxiety disorder, and generalized anxiety disorder, extending into the dorsal ACC that is independent of symptom severity. Although our results do not directly address pathogenetic mechanisms involved in depression and anxiety, they support the notion of a shared pathogenetic mechanism in these disorders that may reflect impaired emotion processing and regulation, presumably through intricate connections of the ventral ACC with other limbic structures (i.e., amygdala, orbitofrontal cortex, and anterior insula) that have been implicated in mood regulation models as well. Psychometric and functional imaging studies focusing on the common and distinct symptom profiles of depression and anxiety, may further aid in unravelling the common and distinct phenomenological and neurobiological correlates of these disorders. In addition to this generic ACC effect, we showed disorder specific involvement of the right inferior frontal gyrus in MDD, and superior temporal gyrus in panic disorder and/or social anxiety disorder and/or generalized anxiety disorder. Longitudinal studies and prospective studies should clarify whether these volumetric abnormalities are the result of the disease process or represent a vulnerability factor for the development of depression and anxiety in adulthood.

ACKNOWLEDGEMENT

The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Quality of Healthcare (IQ healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute). We thank Aart Nederveen, Wouter Teeuwisse, and Thijs van Osch for technical assistance.

M.J. van Tol had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

EFFECTS OF SSRIs: DESCRIPTION OF GROUP

After omission of the SSRI using participants, groups were matched on age, gender, scan site, and handedness. Our renewed sample consisted of 207 subjects, i.e. 50 MDD, 48 CDA, 47 ANX, and 62 HC. Table S1 lists all characteristics for the medication free sample. Groups were comparable on all clinical characteristics to the groups that include the SSRI-using participants. Also, no effect of group exists with respect to total gray matter ($F_{3,203}$=.34, p=.80) and total white matter ($F_{3,203}$=.18, p=.91).

AGE OF ONSET

Groups were matched on age, edu, gender and handedness. Within diagnostic groups, early onset (EO) did not differ in MADRS and BAI scores. Clinical characteristics are listed in Table S2. Gray matter images were entered in a one-way ANOVA with group as independent variable and age, gender, gray matter totals, and center as covariates.

RECURRENCE

MDD and CDA were pooled and splitted into a single episode MDD group and a recurrent episode MDD group. Groups (single, recurrent, and healthy controls) were matched on age, gender and handedness. Groups differed in years of education, MADRS scores and BAI scores (edu: $F_{2,204}$=.14.19, p<.001; MADRS: $F_{2,204}$=74.8, p<.001; BAI: $F_{2;204}$=45.11, p<.001). Post hoc tests showed that healthy controls had more years of education and lower scores on MADRS and BAI than single and recurrent MDD. Clinical characteristics are listed in Table S3. Gray matter images were entered in a one-way ANOVA with group as independent variable and age, gender, gray matter totals, and center as covariates.

EFFECT OF TRAUMA ON ACC VOLUMES

Data regarding the experience of emotional and physical trauma were available (assessed with the Nemesis trauma interview; De Graaf et al, 2002). In our sample of 289 subjects, 55.9 % of MDD, 69.3% of CDA, 57.4% of ANX, and 38.5 % of healthy controls reported to have ever experienced emotional or physical trauma in their lives (ranging from 'once' to 'very often'). To test for the effect of trauma on ACC volumes, we set up ANCOVAs with gray matter ACC volume as dependent factor, and diagnosis and trauma (yes/no) as independent factor. Age, gray matter totals, and scan center were entered as covariates. No effect of self-reported trauma was observed on ACC volume ($F_{1,277}$=.37, p=.54], and no interaction with diagnosis was observed ($F_{3,277}$=.37, p=,26). The experience of a physical or emotional trauma more than once, occurred in 34 % of MDD, 46% of CDA, 40% of ANX, and 17% of the healthy controls before the age of 16. Again, (severe) childhood trauma was not associated with ACC volume when compared to subjects that never experienced trauma($F_{1,215}$=1.22, p=.27), and no interaction with diagnosis was observed ($F_{3,215}$=.84, p=.48).

TABLE S-1: CLINICAL CHARACTERISTICS OF THE SSRI FREE SAMPLE (N=207)

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; ANX= anxiety without MDD; HC= healthy controls; c=9 MDD+GAD, 12 MDD+PD, 5 MDD+PD+GAD, 7 MDD+SAD, 7 MDD+SAD+GAD, 5 MDD+PD+SAD, 3 MDD+PD+SAD+GAD; d= 16 PD, 2 PD+GAD, 20 SAD, 7 PD+SAD, 2 PD+SAD+GAD; SSRI = Selective Serotonin Reuptake Inhibitor; T1: baseline mearurement; T2: MRI-measurement; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; IDS: Inventory of depressive symptomatology; FQ: Fear Questionnaire; rem= remitted depressive scores (MADRS: 0-8), mild= mild depressive scores: (MADRS 9-18), mod_sev= moderate to severe depressive scores (MADRS>19); GM= grey matter total volume; WM= white mattter total volume.

		MDD	CDA c	ANX d	HC
N		50	48	47	62
gender	male/female; N	20/30	12/36	13/34	23/39
scan site	amc/lumc/umcg; N	12/20/18	16/21/11	14/15/18	26/25/11
handedness	left/right; N	6/44	4/44	3/44	5/57
History of SSRI use	1/2/3/4 *; N	7/3/4/36	5/7/5/31	3/7/3/34	
age	in years; mean ± sd	36.22 ± 10.02	36.58 ± 10.9	35.11 ± 9.85	39.89 ± 9.46
education	in years; mean ± sd	12.56 ± 2.45	12.04 ± 3.4	13.23 ± 3.27	14.34 ± 2.85
MADRS	total score; mean ± sd	12.56 ± 9.78	20.17 ± 9.58	10.87 ± 8.76	1 ± 1.78
	range	0-39	2-43	0-35	0-7
	severity class: rem/mild/mod_sev; N	19/18/13	6/17/25	-	-
IDS T1	total score; mean ± sd	27.54 ± 10.17	32.65 ± 10.61	22.51 ± 11.91	5.03 ± 3.52
IDS T2	total score; mean ± sd	20.58 ± 15.43	28.56 ± 11.15	19 ± 10.83	3.59 ± 3.64
	range	1 - 57	5 - 55	4 - 49	0 - 17
BAI T1	total score; mean ± sd	11.62 ± 9.38	17.90 ± 9.09	15.26 ± 9.73	1.84 ± 3.14
BAI T2	total score; mean ± sd	8.78 ± 8.59	17.94 ± 9.22	15.30 ± 10.28	1.84 ± 3.14
	range	0 - 50	1 - 46	0 - 42	0 - 10
FQ	total score; mean ± sd	20.58 ± 15.43	24.65 ± 21.47	29.98 ± 23.82	7.62 ± 8.10
	range	0 - 79	0 - 78	0 - 84	0 - 29
onset MDD	age in years; mean ± sd	25.26 ± 9.49	22.36 ± 11.72	-	-
onset ANX	age in years; mean ± sd	-	17.5 ± 10.54	15.38 ± 11.49	-
recurrence MDD	single/recurrent episode; N	21/29	21/27	-	-
ANX	diagnosis in lifetime; N	14	-	-	0
	diagnosis in past year; N	6	-	-	-
MDD	diagnsois in lifetime; N	-	-	25	0
	diagnosis in past year; N	-	-	1	-
GM	ml; mean ± sd	735.07 ± 57.90	725.2 ± 73.7	738.44 ± 79.77	728. 69 ± 75.26
WM	ml; mean ± sd	495.04 ± 60.85	489.65 ± 60.18	489.70 ± 67.78	486.55 ± 59.83

TABLE S-2: CLINICAL CHARACTERISTICS OF MATCHED EARLY AND LATE DEPRESSION ONSET GROUPS VS. HC

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; HC= healthy controls; EO= early onset MDD (onset of first episode before age of 18); LO= late onset MDD (onset of first episode after age of 18); SSRI= Selective Serotonin Reuptake Inhibitor; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; GM= gray matter total volume; WM= white mattter total volume.

		MDD		CDA		HC
		EO	LO	EO	LO	
N		23	38	32	40	48
gender	male/female; N	8/15	12/26	8/24	14/26	17/31
scan site	amc/lumc/umcg; N	4/8/11	12/16/10	10/11/11	16/14/10	19/22/7
handedness	left/right; N	1/22	5/33	2/30	3/37	5/43
SSRI use	current yes/no; N	7/16	7/31	14/18	21/19	0/48
age	in years: mean ± sd	34 ± 10.5	37 ± 8.1	32.4 ± 9.9	37 ± 9.6	37.7 ± 8.9
education	in years: mean ± sd	13 ± 2.9	12.3 ± 2.6	12.3 ± 2.6	12.6 ± 3.1	13.7 ± 2.2
MADRS	total score: mean ± sd	14.4 ± 9.4	13.2 ± 9.4	19.7 ± 8.8	19.1 ± 9.7	.8 ± 1.6
BAI	total score: mean ± sd	9.2 ± 7.2	8.9 ± 9.1	20.3 ± 8.7	16.3 ± 9.1	1.9 ± 2.6
onset MDD	age in years: mean ± sd	15.17 ± 2.55	30.13 ± 7.32	13.22 ± 3.52	28.18 ± 7.37	-
GM	in ml: mean ± sd	742.2±65.8	724.7 ± 68.8	746.5 ± 69.3	732.9 ± 78.9	738.4 ± 75.4
WM	in ml: mean ± sd	467.1 ± 62.2	491.1 ± 62.6	485.6 ± 59.6	509.5 ± 71.1	486.4 ± 58.8

TABLE S-3: CLINICAL CHARACTERISTIC OF MATCHED SINGLE EPISODE VS. RECURRENT EPISODE MDD

MDD= Major Depressive Disorder; CDA= Comorbid MDD and anxiety; HC= healthy controls; SSRI= Selective Serotonin Reuptake Inhibitor; MADRS: Montgomery Åsberg Depression Rating Scale; BAI: Beck Anxiety Inventory; GM= gray matter total volume; WM= white mattter total volume.

		MDD + CDA		HC
		single	recurrent	
N		68	85	58
gender	male/female; N	27/41	24/61	21/37
scan site	amc/lumc/umcg; N	19/35/14	26/26/33	17/26/22
handedness	left/right; N	5/63	7/78	5/53
diagn	MDD/CDA; N	29/39	36/49	-
SSRI	current use yes/no; N	26/42	30/55	-
age	in years; mean ± sd	37.37 ± 10.13	37.51 ± 9.60	38.9 ± 8.96
education	in years; mean ± sd	13.08 ± 3.12	12.45 ± 3.11	14.59 ± 2.6
MADRS	total score: mean ± sd	10.31 ± 11.11	16.14 ± 10.1	.98 ± 1.83
BAI	total score: mean ± sd	9.37 ± 10.4	13.2 ± 9.2	1.96 ± 2.46
GM	ml; mean ± sd	732.34 ± 77.97	727.94 ± 63.91	730.83 ± 75.06
WM	ml; mean ± sd	492.33 ± 65.12	491.09 ± 61.52	486.67 ± 60.76

CHAPTER 3

EMOTIONAL WORD ENCODING AND RECOGNITION IN DEPRESSION AND ANXIETY DISORDERS

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SUMMARY

Background: Major depressive disorder (MDD), panic disorder, social anxiety disorder, and generalized anxiety disorder are among the most prevalent and frequently co-occurring psychiatric disorders in adults and may be characterized by a common deficiency in processing of emotional information. We aimed to identify the unique and shared functional magnetic resonance imaging correlates of emotional word memory processes in depression and anxiety, controlling for comorbidity and illness severity.

Methods: Fifty-one patients with MDD (MDD), 59 with comorbid MDD and anxiety, 56 patients with a diagnosis of an anxiety disorder (panic disorder, social anxiety disorder and/or generalized anxiety disorder) without MDD and 49 controls performed an emotional word encoding and recognition paradigm during functional magnetic resonance imaging. We tested for the effects of current psychopathological status on the Blood-oxygen-level-dependent response and performance during task execution. Post-hoc we tested for the effects of illness severity, regional brain volume, and anti-depressant use.

Results: Patients with MDD, comorbid depression-anxiety, and anxiety disorders without MDD showed a common hypo-response in the right hippocampus during positive (>neutral) word encoding compared with controls. Also, MDD showed depressive state-independent hyperactivation of the insula, and depressive state-dependent hyperactivation of the amygdala, striatum, and prefrontal cortex during negative encoding. Recognition was associated with increased activation of prefrontal regions, related to illness severity in comorbid depression-anxiety and anxiety without MDD. Overall, effects were unaffected by SSRI use and regional brain volume.

Conclusion: Our findings indicate that hippocampal blunting during positive word encoding is a generic effect in depression and anxiety disorders, independent of illness severity, medication use, and regional volume, that may constitute a common vulnerability for MDD and anxiety disorders. MDD is additionally characterized by increased sensitivity for negative words as reflected in increased insular, amygdalar, striatal, and prefrontal response during encoding. The present results emphasize the current distinction between MDD and anxiety disorders (with and without comorbid MDD) with respect to processing of mood-congruent information, although a generic hippocampal hypo-response to positive words may mark a general insensitiveness to positive information in both MDD and anxiety disorders.

Major depressive disorder (MDD), panic disorder, social anxiety disorder, and generalized anxiety disorder are among the most prevalent and most frequently co-occurring psychiatric disorders in adults (Gorman, 1996; Ressler & Mayberg, 2007). Because of this high comorbidity and because MDD and anxiety disorders respond to the same treatment strategies, it has been suggested that they may share a similar etiology (Ressler & Mayberg, 2007). Recently, we reported on shared anatomical abnormalities in the anterior cingulate cortex in MDD and anxiety disorders (van Tol et al., 2010) supporting this suggestion. In contrast, it has been proposed that MDD with and MDD without comorbid anxiety should be considered distinct diagnostic groups in view of their course trajectories (Penninx et al., 2011). However, in studies investigating the neurobiology of depression and anxiety, comorbidity is rarely explicitly studied.

Major Depressive Disorder, panic disorder, social anxiety disorder, and generalized anxiety disorder have been associated with similar biases in processing syndrome specific information such as negative, threat, or stress eliciting words or pictures (Grant & Beck, 2006; Leppanen, 2006; Musa, Lepine, Clark, Mansell, & Ehlers, 2003; van den Heuvel et al., 2005b; Watkins, Martin, & Stern, 2000). Also, MDD has been associated with abnormalities in processing mood-incongruent (i.e. positive) information (Burt, Zembar, & Niederehe, 1995), reflective of anhedonia (i.e. loss of the capacity to experience pleasure), the MDD core-symptom next to lowered or sad mood. These biases could lead to biased memory formation (Coles & Heimberg, 2002; Coles, Turk, & Heimberg, 2007; Wagner et al., 1998), that might reinforce negative mood and could contribute to the course of the disorder (Elliott et al., 2002). Whether anxiety disorders are also associated with memory biases for positive information is unclear. Moreover, whether comorbid depression-anxiety resembles MDD or anxiety disorders in performance and regional brain activation during emotional memory processes, or should be considered a sum of its parts, is to our knowledge, unknown.

Neuroanatomical models of MDD propose that symptoms such as lowered mood may result from dysregulation of subcortical and (para)limbic regions on the one hand, and dorsal and lateral cortical regions on the other hand (Mayberg, 1997; Phillips et al., 2003b). (Para)limbic regions, such as the amygdala, insula, and ventromedial prefrontal regions, have been associated with emotional appraisal of information. Dorsal and lateral cortical regions, including dorsomedial-, dorsolateral-, and ventrolateral prefrontal (PFC) regions, are predominantly linked to regulatory control over subcortical and (para)limbic regions, possibly via the medial PFC and anterior cingulate cortex (ACC) (Johnstone et al., 2007). Neuroimaging studies in MDD have predominantly focused on processing of negative information, and demonstrated differential involvement of the amygdala, hippocampus, ACC, lateral PFC (Bremner, Vythilingam, Vermetten, & Charney, 2007; Bremner, Vythilingam, Vermetten,

Vaccarino, & Charney, 2004; Hamilton & Gotlib, 2008; Roberson-Nay et al., 2006), supporting the hypothesis of altered involvement of subcortical and prefrontal regions for memory processing of mood-congruent information. Yet, few studies have explicitly tested for the effect of mood-incongruent content on memory formation in MDD, although a blunted response in areas linked to reward processing such as the ventromedial PFC (Elliott et al., 2002; Keedwell, Andrew, Williams, Brammer, & Phillips, 2005a), ventrolateral PFC (Keedwell et al., 2005a), and ventral striatum (Epstein et al., 2006; Heller et al., 2009) has been associated with positive information processing. Only van Wingen et al. (2009) studied memory for positive and neutral faces in MDD. They demonstrated altered involvement of the amygdala and fusiform gyrus during memory formation, and differential involvement of the amygdala, fusiform gyrus, inferior frontal gyrus, orbitofrontal cortex, and posterior cingulate gyrus during retrieval. In panic disorder, social anxiety disorder, and generalized anxiety disorders, studies focusing on the processing of (social) threat related material reported increased prefrontal (Maddock, Buonocore, Kile, & Garrett, 2003; Monk et al., 2006; Nitschke et al., 2009; van den Heuvel et al., 2005b), hippocampal (van den Heuvel et al., 2005b),and amygdalar (Nitschke et al., 2009; Stein et al., 2002; van den Heuvel et al., 2005b) activation, but effects on emotional memory formation have, to our knowledge, not been studied yet.

In the present study, we investigated unique and shared functional magnetic resonance imaging (fMRI) correlates of mood-congruent and mood-incongruent information processing in MDD and frequently co-occurring anxiety disorders during an emotional word memory paradigm. Based on the overlap in their phenomenology, neurobiology, and information processing bias, and to capitalize on our sample size, we incorporated the anxiety disorders within one group. We focused on involvement of (para)limbic 'appraisal' and dorsal and lateral cortical 'regulatory' brain regions during hypothesized biased memory formation and recollection, while testing for the effects of the comorbidity of depression and anxiety disorders. We hypothesized better recognition performance for negative words (relative to neutral), and worse recognition performance for positive words (relative to neutral) in MDD compared with controls. Furthermore, in MDD, we hypothesized increased activation of the amygdala, hippocampus, insula, and decreased activation of prefrontal regions during encoding and recognition of negative information, whereas decreased activation of amygdala, hippocampus, striatal and medial PFC regions was expected during positive word encoding and recognition, relative to controls. We investigated whether this pattern generalizes to anxiety disorders with and without comorbid MDD and to what extent emotional word memory is affected by severity of depression and anxiety.

PARTICIPANTS

Three-hundred-one native Dutch speaking participants (233 outpatients with a half year diagnosis of MDD and/or PD and/or SAD and/or GAD, and 68 controls) recruited from the observational Netherlands Study of Depression and Anxiety (NESDA) were included and underwent magnetic resonance imaging in the Leiden University Medical Center (LUMC), Academic Medical Center (AMC) Amsterdam, or University Medical Center Groningen (UMCG). The Ethical Review Boards of each participating center approved this study. All participants provided written informed consent. Detailed information on participant recruitment and inclusion criteria can be found in the supplemental material.

TASK PARADIGM

We employed an event-related, subject-paced, word encoding- and recognition paradigm (Daselaar, Veltman, Rombouts, Raaijmakers, & Jonker, 2003). During the encoding part, participants were asked to classify 40 positive, 40 negative, and 40 neutral words according to their valence. Words were presented pseudo-randomized together with 40 baseline trials in 20 blocks of eight words. After a retention interval of ten minutes, participants were asked to complete a word recognition task. This task consisted of the 120 old encoding target words and 120 new distracter words, and 40 baselines. During baseline trials participants had to indicate the direction of arrows (<<left,<<middle>>,right>>). Words were presented pseudo-randomized in 20 blocks of 14 words. Subjects had to indicate whether they have 'seen' (i.e.,remembered) the words previously, 'probably have seen it' ('know'), or 'haven't seen it' (rejection). Participants' responses and response times were registered through two magnet-compatible button boxes. A detailed description of the task is given in the supplemental material.

Before and after the word encoding-recognition task, we monitored anxiety levels using a Visual Analogue Scale (VAS) (Huskisson, 1974) ranging from zero to 100.

ADDITIONAL PSYCHIATRIC MEASUREMENTS

Severity of depression and anxiety at the day of scanning was assessed using Dutch versions of the Beck Anxiety Inventory (BAI) (Beck et al., 1988), the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery & Åsberg, 1979), the Inventory of Depressive Symptomology (IDS) (Rush et al., 1986), and the Fear Questionnaire (FQ) (Marks & Mathews, 1979).

IMAGE ACQUISITION

Imaging data were acquired using Philips 3-tesla MRI-systems (Best, The Netherlands) located at the LUMC, AMC, and UMCG, equipped with SENSE-8 (LUMC and UMCG) and SENSE-6 (AMC) channel head coils. For each subject, echo-planar images were obtained using a T2*-weighted gradient echo sequence (repetition time [TR]=2300ms; echo time [TE]=30ms [UMCG: 28 ms], matrix size: 96x96 [UMCG: 64x64], 35 axial slices [UMCG: 39], interleaved

acquisition, 2.29x2.29mm in-plane resolution [UMCG: 3x3mm], 3mm slice thickness). Anatomical imaging included a sagittal 3-dimensional gradient-echo T1-weighted sequence (TR=9ms, TE=3.5ms; matrix 256x256; voxel size: 1x1x1mm; 170 slices).

STATISTICAL ANALYSIS

Psychometric and performance data were analyzed with SPSS (SPSS Inc, Chicago, Illinois, USA). Functional imaging data were preprocessed and analyzed using Statistical Parametric Mapping software (SPM5; http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 7.1.0. (The MathWorks Inc., Natick, MA, USA). To test for effects of the diagnosis, groups were formed based on current psychopathology and comorbidity. To test for the effects of depressive state, we formed subgroups based on current IDS score: 1) remitted (IDS 0-13), 2) mildly depressed (IDS 14-25), and 3) moderately to severely depressed (IDS>25) (Muller et al., 2000). Age and education were added to each model to account for variance related to these factors.

Performance

Abbreviations and descriptions of performance indices are listed in Table 1. Proportions Correctly Recognized words (pCREC), proportions False Alarms (pFA), and old/new discriminant accuracy (d';=pCREC - pFA) were calculated, overall and per valence (i.e. positive, negative, neutral).

Effects of diagnosis on recognition performance were analyzed for pCREC_all, pFA_all, and d'_all using ANCOVAs. Repeated-Measures analyses of covariance (ANCOVAs) were performed to test for interaction of valence and diagnosis, and valence and depressive state, on task performance. Deviation response times [(pos/neg) – neutral] of SCR and CREC were calculated and entered in multivariate analyses of covariance (MANCOVAs) to test for effects of diagnosis/depressive state on response times. Age and years of education were entered as covariates in each analysis. Significance for behavioral analyses was set at $p<.05$, and post hoc paired tests (T-test or Mann-Whitney [U]) were Bonferroni-corrected for multiple comparisons (p_{crit}).

Imaging data

Preprocessing included reorientation of the functional images to the anterior commissure, slice time correction, image realignment, registration of the T1-scan to the mean image, warping to Montreal Neurological Institute (MNI)-space as defined by the SPM5 T1-template, reslicing to 3x3x3mm voxels and spatial smoothing using an 8-mm FWHM Gaussian kernel. Subject movement greater than 3mm in more than one direction resulted in exclusion of the data. Next, data were analyzed in the context of the General Linear Model (Friston et al., 1995). Haemodynamic responses to each stimulus were modeled with a delta function convolved with a synthetic haemodynamic response function and modulated using response times. The model included regressors for

Abbreviations

Encoding phase		
SCR	Subsequent Correct Recognition	a word, presented during the encoding phase, that is correctly recognized during the subsequent recognition phase
Recognition phase		
CREC	Correct RECognition	correct recognition of a previously encoded word
CREJ	Correct REJection	correct recognition of a newly presented word as a new word
FA	False Alarm	incorrect indication of a newly presented word as a previously encoded word
MISS	Miss	incorrect indication of a previously encoded word as a new word
PROB	Probably seen/known	indication that a word is 'probably seen'
All		
p/prop	proportion	
rt	response time in seconds	
BL	baseline	
d'	old/new discrimination accuracy	(pCREC)-(pFA)
Affixes		
_all	all words, irrespective of valence	
_pos	positive words	
_neg	negative words	
_neu	neutral words	

encoding[1] and recognition[2] parameters. In addition, filler words, error- and no-response trials were included as a regressor of no interest. Low-frequency noise was removed by applying a high-pass filter (cut-off: 128s) to the fMRI time-series at each voxel. Owing to the small proportion of recognition trials that were responded to with a 'know' response, these responses were treated as 'remembered' and consequently added to either CREC or FA.

Following the summary statistics approach, contrast images for 'SCR_pos > SCR_neu', 'SCR_neg > SCR_neu', 'CREC_pos >CREC_neu', and 'CREC_neg> CREC_neu', were calculated per subject on a voxel-by-voxel basis and entered into second-level analyses for between-group comparisons (MANCOVA) with age and education as covariates. Additionally, 'center' was added as a regressor by means of two dummy variables. We repeated the analysis after omission of the SSRI-users. We contrasted positive and negative SCR/CREC trials to neutral SCR/CREC trials to avoid inclusion of signal related to familiarity processes which might be expected when contrasting against the repetitive lower level baseline. We tested for the effects of anxiety severity on encoding and recognition related activity by performing a linear regression analysis with BAI scores as regressor of interest, and age, IDS scores, and center as covariates, masked with a binary mask derived from the relevant main effect at $p<.05$.

We defined the following areas of interest: hippocampus, amygdala, dorsal medial PFC (Brodmann area (BA) 8 and 9), ventromedial PFC (BA 10), dorsolateral PFC (BA 8, 9 and 46), OFC (BA 11), IFG (BA 44, 45, and 47), ACC (BA 32 and 24), striatum, and insula (BA 13).

The main effects of task are reported at a threshold of $p<.05$, corrected for Family Wise Error (FWE) at the voxel level, unless specified otherwise. To restrict

1 SCR_pos, SCR_neg, SCR_neu, SMISS_pos, SMISS_neg, SMISS_neu, BL. (SMISS=subsequently missed)

2 CREC_pos, CREC_neg, CREC_neu, CREJ_pos, CREJ_neg, CREJ_neu, FA_pos, FA_neg, FA_neu, MISS_pos, MISS_neg, MISS_neu, BL. (CREJ=correct rejections; MISS=misses)

the search for significant effects to voxels which were identified in the main effect, group comparisons were masked with the orthogonal relevant main effect (across groups) at p<.05 (uncorrected). Group by task interaction effects (F-tests) were inspected at p<.005, uncorrected and post-hoc t-tests had to meet p<.05, FWE voxel wise corrected for the spatial extent of the volume of interest, to be considered significant. For this small volume correction, we used the Automated Anatomical Labeling atlas (Maldjian, Laurienti, Kraft, & Burdette, 2003), implemented in the WFU-Pick Atlas toolbox (Wake Forest University School of Medicine). For non-ROIs, a voxel level threshold of p<.05 FWE whole brain corrected was set a priori.

The statistical toolbox Biological Parametric Mapping (Wake Forest University School of Medicine, 2010) was used to test whether between-group effects were affected by variations in regional gray matter volume (Casanova et al., 2007). We used individual modulated gray matter images derived from the T1 scans (for a description and results of the optimized voxel based morphometry procedure, see van Tol et al. (2010)). Gray matter images were normalized to standard MNI space and resliced to 3x3x3 mm voxels.

SAMPLE CHARACTERISTICS

Group characteristics are listed in Table 2. Our final sample for the present report consisted of 215 participants, 51 with a diagnosis of MDD and no anxiety disorders (MDD), 59 patients with MDD and anxiety disorder(s) (comorbid depression-anxiety [CDA]), 56 patients with one or more anxiety disorder (panic disorder, social anxiety disorder, generalized anxiety disorder), but no MDD (ANX), and 49 controls. A description of excluded data sets is given in the supplemental material. Importantly, no selective drop out of data over diagnosis was observed ($X^2(3)=1.39$; $p=.71$).

Groups were matched for age, education (years), sex, handedness, and scan center. Patient groups did not differ in number of patients using an SSRI. All patient groups showed higher scores on the Montgomery Åsberg Depression Rating Scale (MADRS) scores, Inventory of Depressive Symptomology (IDS), Beck Anxiety Inventory (BAI), and Fear Questionnaire (FQ) than controls (all: U>34;p<.001). Additionally, the comorbid depression-anxiety group showed higher depression (MADRS and IDS) and anxiety scores (BAI) than the MDD and ANX groups, both at the time of scanning (T2) and at the time of the NESDA baseline interview (T1) (all: U>425; P<.001). Also, the comorbid depression-anxiety group showed higher FQ scores than the MDD group (U=486; p<.001), but not than the ANX group (U=1026.5; p=.94). Within diagnostic groups, SSRI-users did not differ from antidepressant non-users in scan center, sex, age, education, scan interval, onset and severity of depression and anxiety, and recurrence of depressive episodes in MDD and comorbid depression-anxiety patients- (see Table S-1). Finally, within the depressed groups (MDD and

TABLE 2: CLINICAL CHARACTERISTICS OF THE TOTAL SAMPLE (N=215)

Depressive Symptomatology; BAI=Becks Anxiety Inventory; FQ=Fear Questionnaire; VAS=Visual Analogue Score; pre: before word encoding task; post: after word recognition task; T1=time of NESDA baseline measurement; T2=time of MRI measurement; interval=interval between T1 and T2.
Available data: MDD: IDS_T2 available of 49 patients, BAI_T2 available of 48 patients, FQ_T2 available of 45 patients, MADRS available of 48 patients, VAS available of 50 patients; CDA: IDS_T2 available of 58 patients, BAI_T2 available of 57 patients, FQ_T2 available of 46 patients, MADRS available of 57 patients, IDS/BAI/FQ_T1 available of 57 patients; ANX: IDS_T2 available of 54 patients, BAI_T2 available of 53 patients, FQ_T2 available of 45 patients, MADRS available of 54 patients; HC: IDS_T2 available of 48 participants, BAI_T2 available of 48 participants, FQ available of 44 participants.

		MDD	**CDA a**	**ANX b**	**HC**	*H*	*F*	*U*	X^2	*df*	*p*
N		51	59	56	49						
gender	male/female; N	20/31	21/38	14/42	19/30				*3.17*	*3*	*.366*
scan site	amc/lumc/umcg; N	15/18/18	21/24/14	16/16/24	18/21/10				*8.32*	*6*	*.215*
handedness	left/right; N	5/46	3/56	5/51	4/45				*.98*	*3*	*.806*
SSRI use	yes/no; N	11/38	23/35	15/37	-				*3.83*	*2*	*.15*
age	in years; mean ± sd	36.37 ± 10.14	36.47 ± 11.22	34.73 ± 8.78	38.94 ± 9.97		*1.53*			*3,214*	*.21*
education	in years; mean ± sd	12.96 ± 2.71	12.49 ± 2.92	13.04 ± 3.4	13.8 ± 2.54		*1.80*			*3,214*	*.15*
MADRS	total score; mean ± sd	12.58 ± 8.94	19.26 ± 8.77	11.11 ± 8.38	.88 ± 1.55	*110.97*				*3*	*<.001*
	range	0-33	0-43	0-35	0-6						
IDS T1	total score; mean ± sd	27.0 ± 9.87	32.68 ± 9.35	23.55 ± 11.02	5.19 ± 3.47	*112.7*				*3*	*<.001*
IDS T2	total score; mean ± sd	17.78 ± 10.26	28.67 ± 10.29	19.46 ± 10.14	4 ± 3.71	*110.85*				*3*	*<.001*
	range	1-39	5-55	4-49	0-17						
	rem/mild/mod_sev; N	19/17/15	3/21/35	-	-						
BAI T1	total score; mean ± sd	10.37 ± 6.44	19.44 ± 8.07	15.33 ± 8.98	1.81 ± 3.14	*113.64*				*3*	*<.001*
BAI T2	total score; mean ± sd	7.69 ± 6.14	18.46 ± 8.96	14.28 ± 9.43	2.23 ± 2.55	*105.27*				*3*	*<.001*
	range	0-26	1-46	1-42	0-10						
FQ	total score; mean ± sd	18.78 ± 12.96	35.39 ± 18.62	36.09 ± 21.41	9.16 ± 7.57	*68.57*				*3*	*<.001*
	range	0-45	6-78	3-84	0-29						
VAS_pre	mean ± sd	25.52 ± 22.07	34.44 ± 22.21	38.18 ± 25.19	23.98 ± 20.55	*12.92*				*3*	*.005*
VAS_post	mean ± sd	18.14 ± 18.11	26.63 ± 21.66	29.89 ± 21.86	17.12 ± 16.40	*14.24*				*3*	*.003*
interval	in days; mean ± sd	59.48 ± 28.59	62.85 ± 33.78	74.62 ± 35.38	65.47 ± 28.16	*6.43*				*3*	*.09*
onset MDD	age in years; mean ± sd	26.51 ± 10.22	24.19 ± 11.2	-	-	*1.85*				*1*	*.18*
onset ANX	age in years; mean ± sd	-	19.24 ± 10.99	14.85 ± 11.06	-	*5.73*				*1*	*.02*
recurrence MDD	single/recurrent episode; N	20/31	25/34	-	-				*.11*	*1*	*.737*
ANX (PD, SAD, GAD)	diagnosis in lifetime: N	10	59	56	0						-
	diagnosis in past year: N	2	59	56	0						-
MDD	diagnosis in lifetime: N	51	59	35	0						-
	diagnosis in past year: N	51	59	4	0						-

comorbid depression-anxiety), the remitted, mildly and moderately to severely depressed groups did not differ in age, education, and sex. See Table S-2 for characteristics of the MDD and comorbid depression-anxiety subgroups. Between T1 and T2, the remitted and mildly depressed subgroups showed a decrease in symptom scores; currently moderately to severely depressed groups showed stable symptom scores.

BEHAVIORAL RESULTS

Classification behavior, memory performance, and response times are summarized in Table S-3.

Classification behavior: An interaction of diagnosis and valence was observed on classification behavior ($F_{6,388}$=3.67,*p*=.001,η^2=.05): during the encoding task, MDD classified fewer words as positive and more words as neutral, compared with controls. This effect was observed trend-wise in the ANX group, but not in comorbid depression-anxiety group. No effect of group on classification of negative words was observed.

Recognition accuracy: No effect of diagnosis was found on memory performance (all F<.74;*p*>.53) and no effect of group x valence was observed for correctly recognized words, false alarms and d' (all F<.84;P>.54).

Response times: Analysis of response times revealed an interaction of diagnosis and valence on response times ($F_{6,418}$=2.41;*p*=.03,η^2=.03): controls showed shorter response times when encoding positive words than MDD patients. This effect was observed independent of illness severity in the MDD group, and in the moderately and severely depressed comorbid depression-anxiety group[3]. Also, MDD patients showed longer response times when recognizing positive than neutral words, an effect that was absent in controls. No effect of valence on response times was observed in anxiety disorders without MDD, and results were unaffected by anxiety severity. Results are summarized in Figure-1. Results remained unchanged after omission of the SSRI-users.

3 Only three CDA patients were remitted at time of scanning (see Table 2). Therefore, we left the remitted CDA group out of the severity analyses.

FMRI RESULTS

FWE corrected main effects of the contrast 'SCR_pos>SCR_neu' and 'SCR_neg>SCR_neu' reflecting encoding related activity are listed in Table S-4. At a more liberal voxel-threshold of *p*<.001, uncorrected, the left ventral putamen/striatum, right hippocampus, amygdala, insula, and bilateral fusiform gyri also showed activation during encoding. No whole brain FWE corrected effects of 'CREC_pos>CREC_neu' and 'CREC_neg>CREC_neu', reflecting recognition related activity, were observed. At *p*<.001, uncorrected, emotional recognition was associated with activation of the left inferior frontal gyrus and dorsomedial PFC. Interactions of task and diagnosis and effects of illness severity are listed in Table 3.

FIGURE 1: RESPONSE TIMES PLOTS

Error bars showing mean ± se for response times in seconds (s) for SCR per valence for MDD, CDA, ANC, and HC (top figure); CREC per valence for MDD, CDA, ANC, and HC (middle figure); SCR per valence for CDA_mild, CDA_mod/sev, and HC (bottom figure).

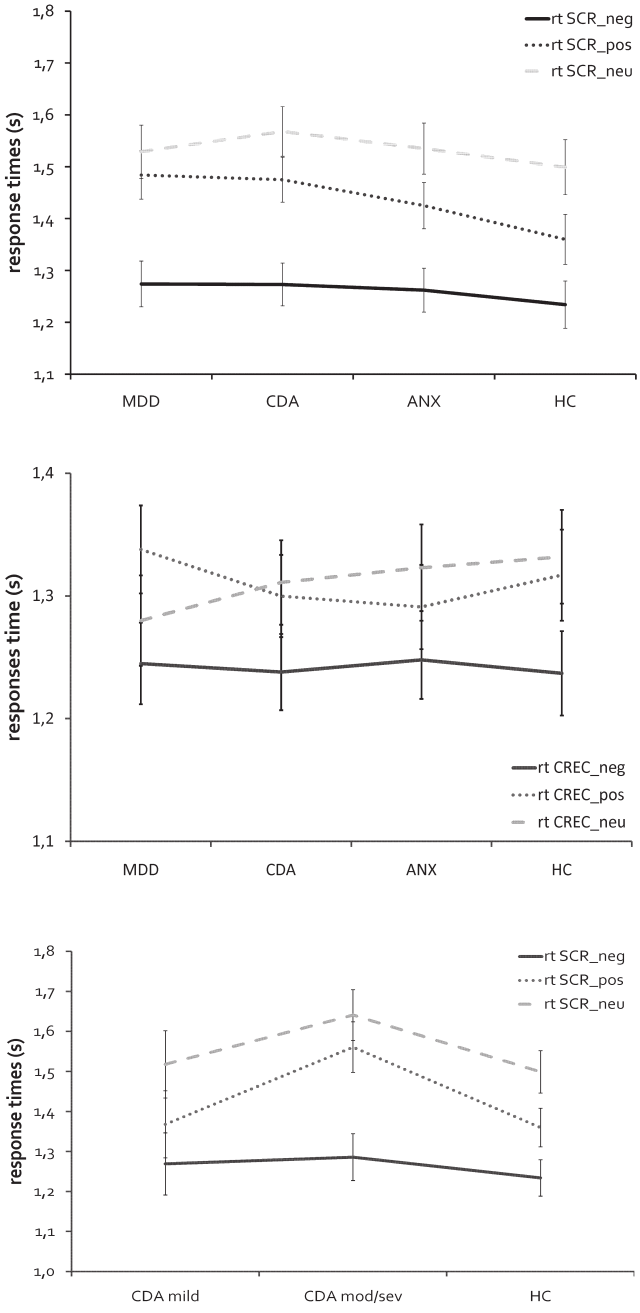


TABLE 3: MAIN EFFECTS OF DIAGNOSIS AND EFFECTS OF ILLNESS SEVERITY ON EMOTIONAL MEMORY

for the contrasts 'SCR_pos > SCR_neu', 'SCR_neg > SCR_neu', 'CREC_pos > CREC_neu', and CREC_neg > CREC_neu. Results are reported at $p_{FWE} < .05$, corrected for volume of Anatomic Automatic Label (AAL); BA = Brodmann Area; side=hemisphere; L=left; R=right. [] gray matter volume corrected Z-values and corresponding *p*-values are printed between brackets. ** significant at *p*<.001, uncorrected

Emotional word encoding

positive words > neutral words; encoding

			MNI-coordinates					
regions	side	BA	x	y	z	T	Z	P_{SVC_FWE}
MDD<HC								
hippocampus	R	n/a	33	-18	-12	4.19 [4.17]	4.14 [4.08]	.003 [.005]
ANX<HC								
hippocampus	R	n/a	33	-24	-9	3.80 [3.77]	3.76 [3.70]	.027 [0.034]
MDD_mod/sev>HC								
anterior cingulate cortex	L	24	-3	3	39	3.75	3.65	.03

negative words > neutral words; encoding

			MNI-coordinates					
regions	side	BA	x	y	z	T	Z	P_{SVC_FWE}
MDD>HC								
insula	L	13	-33	12	-15	3.52 [3.33]	3.49 [3.23]	.048 [.11]
MDD_mod/sev>HC								
anterior hippocampus/amygdala (lateral)	R	n/a	33	-9	-18	3.36	3.29	.045
caudate head	L	n/a	-6	12	0	3.54	3.46	.028
superior prefrontal gyrus	R	8	24	18	48	3.47	3.39	.15 **
putamen	R	n/a	33	9	0	3.33	3.26	.052 **

Emotional word recognition

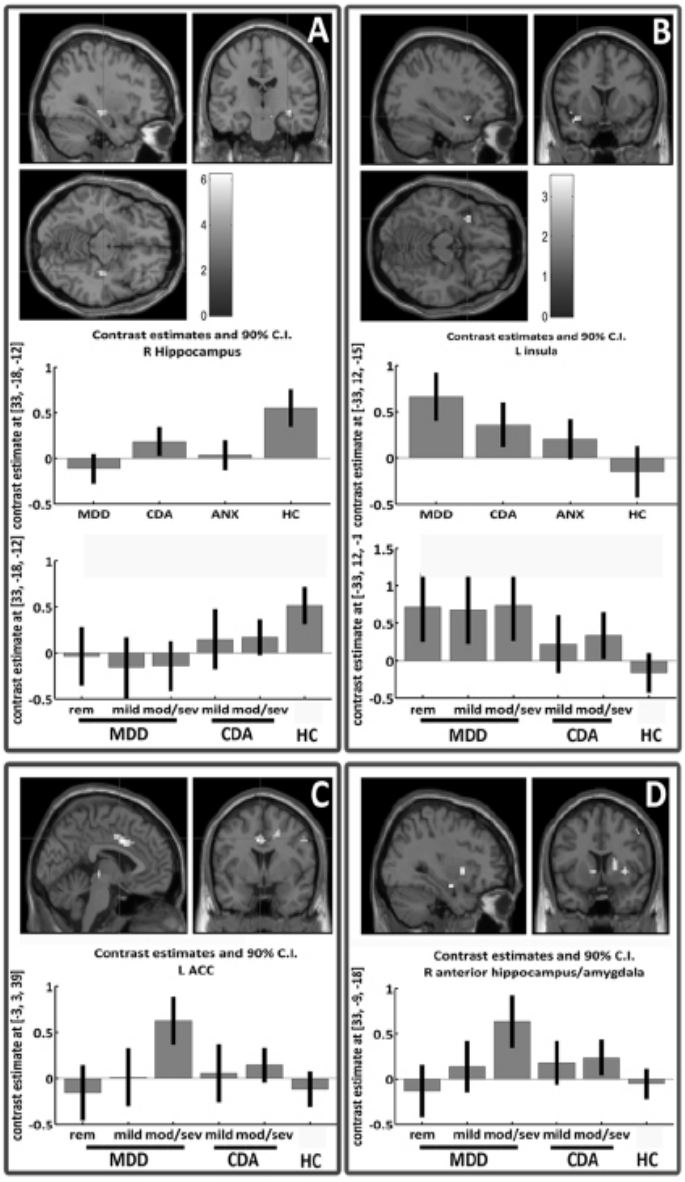
positive words > neutral words; recognition

			MNI-coordinates					
regions	side	BA	x	y	z	T	Z	P_{SVC_FWE}
ANX>MDD								
inferior frontal gyrus	L	45	-54	18	21	4.29 [3.76]	4.24 [3.69]	.004 [.045]
CDA_mod/sev>HC								
middle frontal gyrus	L	10	-30	48	3	3.94	3.84	.023
middle frontal gyrus	L	8	-45	12	45	3.71	3.62	.046

negative words > neutral words; recognition

			MNI-coordinates					
regions	side	BA	x	y	z	T	Z	P_{SVC_FWE}
negative correlation of BAI within CDA								
middle/inferior frontal gyrus	R	46	36	42	15	5.38	4.75	.001

FIGURE 2:
EFFECTS OF DIAGNOSIS AND ILLNESS SEVERITY ON ENCODING



A) Effects of groups (F statistics) on 'SCR_pos>SCR_neu' overlayed (top) and 90% confidence intervals (C.I.) centered at the right hippocampus showing contrast estimates of diagnosis (middle) and depression severity subgroups within MDD and CDA, and HC (bottom);
B) Effect of MDD>HC on 'SCR_neg>SCR_neu' (Z-statistics) (top) and 90% confidence intervals (C.I.) centered at the left ventral insula showing effects of group (middle) and effects of depression severity within MDD and CDA, and HC (bottom);
C) Effects of MDD_mod/sev> HC on 'SCR_pos>SCR_neu' (Z-statistics) (top) and 90% confidence intervals (C.I.) centered at the left ACC showing contrast estimates of depression severity subgroups within MDD and CDA, and HC (bottom);
D) Effects of MDD_mod/sev> HC on SCR_neg>SCR_neu' (Z-statistics) (top) and 90% confidence intervals (C.I.) centered at the right anterior hippocampus/amygdala showing contrast estimates of depression severity subgroups within MDD and CDA, and HC (bottom). Plots centered at the left caudate nucleus, right putamen, and right superior PFC showed similar effects (plots not shown).
Z- and F-statistics are displayed on mean T1 SPM5 template at $p<.005$, uncorrected. Effects on positive encoding are displayed in the left boxes, effects on negative encoding in the right boxes.

ENCODING

Effect of diagnosis

A group by valence interaction was observed in the right hippocampus ($F_{3,418}$=7.21, Z=3.72). Post-hoc t-tests demonstrated that patients showed hypoactivation of the right hippocampus compared with controls during positive encoding only, and was most strongly observed in the MDD and ANX groups (See Figure-2A). Comorbid depression-anxiety showed this hypoactivation below threshold (Z=2.7, p_{FWE}=.21, $p_{uncorrected}$=.004). The hippocampal hypoactivation was not explained by regional volume, illness severity (Figure-2A_bottom), and SSRI use. Also, within the moderately to severely depressed MDD and CDA groups, hippocampal hypoactivation did not vary as a function of depression severity (r<|.14|, *p*>41). Next, we tested for the main effect of diagnosis on positive and negative word encoding separately. In addition to the hippocampal effect during positive encoding, an effect of group was observed on negative encoding in the left insula ($F_{3,418}$=4.34, Z=2.58). Post-hoc t-test showed increased insular activation in MDD relative to controls (Figure-2B), that was observed independent of SSRI-use and illness severity (Figure-2B_bottom), also within severity groups. This effect was not observed during positive word encoding.

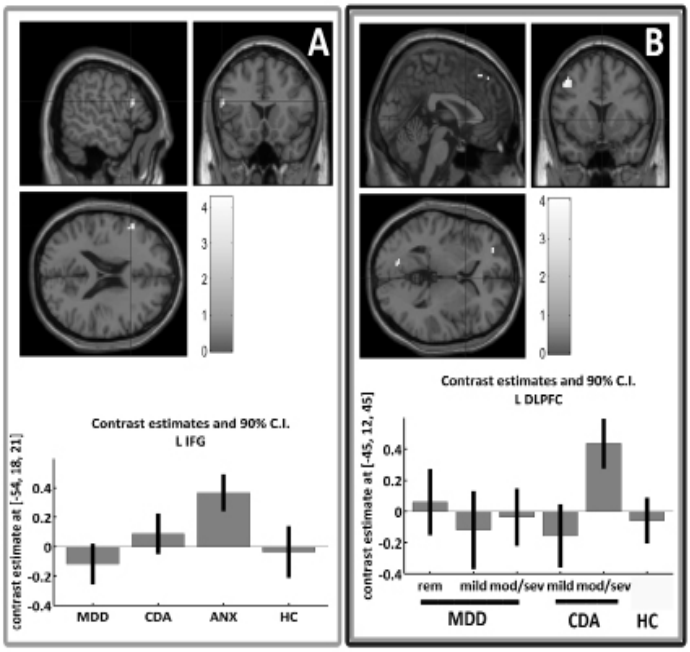
Effects of illness severity

Positive word encoding: Within MDD, an effect of depression severity was observed in the left ACC (BA 32; $F_{3,142}$=5.54, Z=3.02). A post-hoc t-test showed that compared to controls, moderately to severely depressed MDD patients showed increased ACC activation, whereas remitted and mild MDD patients and the comorbid depression-anxiety subgroups did not show this activation difference (Figure-2C). No effect of depression severity was observed in the comorbid depression-anxiety and ANX groups, and no correlation of anxiety severity was observed within groups. Moreover, within moderately to severely depressed MDD and comorbid depression-anxiety groups, ACC activation did not vary as a function of depression severity (r<-.19, *p*>.51).

Negative word encoding: An effect of depression severity on negative word encoding was observed in the right anterior hippocampus/amygdala, left caudate nucleus, right putamen, and right superior PFC (all $F_{3,142}$>4.47, Z>2.58; *p*<.005; Figure-2D): Post-hoc t-tests showed that moderately to severely depressed MDD patients showed hyperactivation of these regions compared with controls, although the superior PFC and right putaminal hyperactivation were observed subthreshold at p_{FWE}=.15 and p_{FWE}=.052, respectively. Within the moderately to severely depressed group, activation in these regions was unrelated to IDS and BAI scores (all r<|.36|, *p*>.20). No effect of depression and/or anxiety severity was observed within anxiety disorders, with or without comorbid MDD.

FIGURE 3:
EFFECTS OF DIAGNOSIS AND ILLNESS SEVERITY ON RECOGNITION

A) Effects of ANX>MDD on 'CREC_pos>CREC_neu' (Z-statistics) (top) and 90% confidence intervals (C.I.) centered in the left inferior frontal gyrus showing mean response per group (bottom);
B) Effects of CDA_mod/sev>HC on 'CREC_pos>CREC_neu' (Z-statistics) (top) and 90% confidence intervals (C.I.) centered in the left DLPFC (bottom) showing contrast estimates of depression severity subgroups within MDD and CDA, and controls. The frontopolar hyperactivation was observed on 'CREC_neg>CREC_neu' as well.
Z- and F-statistics are displayed on mean T1 SPM5 template at p<.005, uncorrected.



RECOGNITION

Effect of diagnosis

No significant interaction of diagnosis x valence occurred at the set threshold. For exploratory purposes, we tested for the effects of diagnosis on positive and negative word recognition separately. An effect of group was observed on positive word recognition in the left inferior frontal gyrus ($F_{3,418}$=6.91, Z=3.62; p<.001; Figure-3A). Post-hoc t-tests demonstrated that the ANX group showed hyperactivation of this region compared with MDD, and trend-wise compared with controls (Z=3.06; p_{FWE}=.17, $p_{uncorrected}$=.001). No effect of diagnosis was observed on negative word recognition.

Effects of illness severity

Within the comorbid depression-anxiety group, a main effect of illness severity on positive word recognition was observed on left superior PFC and left frontal pole activation ($F_{2,142}$>7.78, Z>3.23; Figure-3C): the moderately to severely depressed group showed increased activation of these regions compared with controls. However, this fronto-polar effect was also observed during negative word recognition, although subthreshold at p_{FWE}=.07. Also, within the comorbid depression-anxiety group, a negative correlation of Beck Anxiety Inventory (BAI) scores and activation of the right dorsolateral PFC was observed when Inventory of Depressive Symptomatology (IDS) scores were added to the

model (Figure-3B). No effect of illness severity was observed within the MDD and ANX group on positive or negative word recognition.

DISCUSSION

In this study, we investigated the unique and shared functional MRI correlates of mood-congruent and mood-incongruent word encoding and recognition in patients with MDD and frequent co-occurring anxiety disorders, explicitly testing for the effects of their comorbidity, and at the same time controlling for symptom severity, regional brain volume, and SSRI use.

Overall, we found no evidence for the hypothesized mood-congruent and mood-incongruent memory biases, as indicated by equal recognition accuracy for emotional words across diagnostic groups and controls. We demonstrated a depression related effect on response times during the encoding of positive words, unrelated to depressive state in MDD without comorbid anxiety, but related to current depressive state in patients with comorbid depressed-anxiety. Anxiety disorders without MDD were not associated with biases in memory or processing speed. Imaging results in MDD largely confirmed our hypothesis of differential involvement of (para)limbic and dorsal and lateral cortical areas during mood-congruent vs. mood-incongruent word encoding. Our results of increased processing times for positive information in MDD and currently depressed comorbid depression-anxiety are in concordance with the mood- incongruent bias theory as proposed by Bower (1981) and suggest that positive content impedes encoding and recognition in MDD.

In anxiety, no memory bias towards positive or negative material was found, consistent with previous studies (Coles & Heimberg, 2002). The absence of a mood-congruent bias in anxiety may be attributed to the low self-relevance of the words presented in our experiment, as previous research in panic disorder and generalized anxiety disorder demonstrated an attentional bias only when threat words with high self-relevance were presented (Coles et al., 2007). The absence of biases on recognition performance in mood and anxiety disorders has been described before (Banos, Medina, & Pascual, 2001; Bremner et al., 2004; van Wingen et al., 2009), although not consistently (Bradley et al., 1995; Epstein et al., 2006; Hamilton & Gotlib, 2008). Differences in findings could result from memory processes examined (e.g., strategic linking vs. priming; recognition vs. free recall) and task specifications, for instance length of the retention interval between encoding and recognition.

Our imaging result of a hypo-response during positive encoding in the hippocampus in both patients with MDD and anxiety disorders, and trend-wise in patients with comorbid depression-anxiety, compared with controls was unexplained by regional volume, SSRI use, and illness severity and therefore indicates a depression-anxiety generic phenomenon during encoding of positive words. Given the important role of the hippocampus in episodic

memory (Squire, Stark, & Clark, 2004), recollection of previously learned material (Daselaar, Fleck, & Cabeza, 2006), and context-based memory (Davachi, Mitchell, & Wagner, 2003; Ranganath et al., 2004), our results suggest decreased contextual coupling during semantic classification and simultaneous encoding of positive words in both depression and anxiety disorders. Possibly, mood-incongruent information is insufficiently recognized as positive information and subsequently connected less efficiently to (less) available memory 'nodes', as reflected in fewer words classified as positive, hippocampal hypo-activation, and longer response times. Hippocampal blunting in untreated patients with MDD during a neutral episodic memory task has been described before (Bremner et al., 2004). We now demonstrated hippocampal blunting during positive encoding in MDD and patients with anxiety disorders other than posttraumatic stress disorder. Also, we demonstrated that this hippocampal blunting is not specific to the moderately/severely depressive state, but is observed in the mild and (newly) remitted state as well. This finding supports previous behavioral studies that demonstrated a state independent memory bias in MDD for positive stimuli only, whereas biases towards negative stimuli appeared state dependent phenomena (Bradley & Mathews, 1988; Teasdale & Dent, 1987).

In addition, over-recruitment of the dorsal ACC during encoding of positive information was specifically observed in moderately and severely depressed MDD patients, and not in mild or remitted depressed patients. This over-recruitment of the dorsal cognitive subdivision of the ACC (Bush et al., 2000) may be interpreted as recruitment of extra attentional resources to classify mood- incongruent words in currently depressed patients, as this effect was not observed during encoding of negative words. As positive words might not be associated with a personally relevant state (Lemogne et al., 2009) in currently depressed patients, additional ACC resources might be called on to resolve this classification conflict (Kerns et al., 2004).

Related to encoding of negative words, exploratory analyses indicated a a trait-like effect of MDD was observed in the left ventral insula, that may reflect a general increased sensitiveness for negative information (Surguladze et al., 2010). It has been suggested that abnormal insula functioning in MDD reflects the presence of somato-vegetative symptoms and indicates an abnormal sense of the self (Wiebking et al., 2010). In addition, a depressive state dependent hyperactivation of the right anterior hippocampus/lateral amygdala, caudate head, and trend-wise right putamen, and superior frontal cortex was observed during encoding of negative words only, results that are in line with the report of Hamilton and Gotlib (2008). This finding suggests that an abnormal amygdalar response is related to depressive state, and the co-activation of the superior PFC and striatum implies increased recruitment of the dorsolateral PFC circuit (Mega & Cummings, 1994) that may mark a state dependent sensitivity for encoding negative information. Surprisingly, increased amygdalar/striatal/PFC activation was not observed in moderately to severely depressed comorbid

depression-anxiety patients. The finding of normal subcortical and PFC activity during encoding of mood-congruent stimuli in comorbid depression-anxiety is in concordance with behavioral studies (Grant & Beck, 2006; Musa et al., 2003; Tarsia, Power, & Sanavio, 2003), and it has been suggested that the presence of depressive symptoms in anxious patients abolishes the attentional bias. A possible explanation is that depression and anxiety are associated with hypo- and hyper-vigilant responses (Posner, Russell, & Peterson, 2005), respectively, when processing negative information, resulting in a net null effect.

In MDD, recognition of positive and negative words was unaffected in terms of BOLD responsiveness. Exploratory analyses in CDA patients indicated increased activation of left frontal pole and DLPFC during both negative and positive word recognition associated with depression severity. Moreover, in anxiety disorders without MDD left inferior frontal hyperactivation was observed during the recognition of positive words compared with MDD patients, and trend-wise compared with controls. This inferior frontal gyrus over-recruitment may indicate an increased need for attentional resources for correct response selection (Thompson-Schill, D'Esposito, Aguirre, & Farah, 1997), possibly due to less efficient encoding, as reflected in the hippocampal blunting during positive word encoding.

No effect of trait anxiety or depression was observed on recognition of negative words. However, as a function of depressive state, CDA patients showed increased activation of left frontal pole and DLPFC during both negative and positive word recognition. This valence non-specific effect suggests an increased demand for attentional and executive control during word recognition to maintain adequate task performance (van den Heuvel et al., 2003; Wagner, Koch, Reichenbach, Sauer, & Schlösser, 2006), related to illness severity. The moderately to severely CDA patients were characterized by higher depression and anxiety severity score than the moderately to severely MDD patients. This difference in illness severity may explain why we did not observe increased frontal recruitment during recognition in MDD patients without comorbid anxiety disorders.

In general our results were largely unaffected by variations in regional gray matter volume and inclusion of SSRI users, and no difference in activation in the reported regions was observed between SSRI-users and medication free patients (data not shown). Effects of SSRI treatment on amygdalar reactivity have been previously reported by Sheline et al. (2001) This latter result may be alternatively interpreted as an effect of successful treatment (i.e., remission), and therefore in line with our findings: only currently depressed MDD, and not remitted patients, showed increased anterior hippocampal and amygdalar responses to negative information.

In this study we included large and representative outpatient groups, excluded insufficient performers, could test for state and trait like effects, and were able to correct for possible confounds such as SSRI use and regional volume. However, several potential limitations should also be noted. First, the NESDA

strategy of recruiting through general practitioners and outpatient clinics across a wide range of disease severity and duration is likely to result in a representative sample but may also have increased variability (i.e., noise), without fully capturing the most severe end of the depressive spectrum. Second, on average, only approx. 15 per cent of the presented words were 'forgotten' (i.e., MISS). Therefore, the contrast 'recognized>forgotten' was underpowered and could not be calculated. Hence, caution should be taken when interpreting the results as purely reflecting memory related activity. However, we only analyzed signal related to events with a high probability of reflecting successful encoding and recognition related activity (i.e. SCR/CREC), and concentrated on valence effects during successful encoding and recognition which are likely to be subtle. Also, our strategy to control for type I error using an individual ROI approach rather than correcting for the overall search volume, although customary in imaging research, should be considered a potential limitation. Third, the negative words used in our study were not selected based on their relevance for mood and specific anxiety disorders, but had a negative connotation in general. We therefore could not examine encoding and recognition effects of disorder specific and non-specific negative words. Fourth, although similar 3-tesla systems were used at each site in this multi-center study, variability in image acquisition may have occurred due to minor differences in hardware (receiver coil), imaging parameters, and timing of software upgrades, but no systematic scanning site x diagnosis bias occurred. Finally, we aggregated the anxiety disorders in order to capitalize on our sample size and to compare the neural profile of these anxiety disorders with those observed in comorbid depression-anxiety and MDD. Therefore, no conclusions regarding the specific neural patterns of individual anxiety disorders within the ANX group could be drawn.

In conclusion, our results indicate that abnormal encoding of positive information is a shared feature of depression and anxiety, whereas abnormal encoding of negative information is a unique feature of MDD without comorbid anxiety. Hippocampal blunting during positive word encoding appeared as a common and state-independent neurophysiological phenomenon in depression and anxiety that may mark a general insensitiveness to positive information. At the same time, MDD is characterized by increased reactivity for negative words, reflected in an increased insular, amygdalar, striatal, and PFC response. With respect to their functional MRI profile during negative word processing, MDD with and without comorbid anxiety disorders should be considered separate groups. Possibly, comorbid depression-anxiety is primarily characterized by anxiety related functional neuropathology since the onset of anxiety disorders often precedes the manifestation of the first episode of MDD (Parker et al., 1999). Future studies should investigate whether individuals at high risk for depression and anxiety are characterized by abnormal processing of positive stimuli, as this may constitute a risk factor for mood and anxiety to be considered in prevention programs. Furthermore, we underline that current comorbid anxiety disorders should be controlled for when studying processing

of mood-congruent information in MDD.

ACKNOWLEDGEMENT

The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Quality of Healthcare (IQ healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute). We thank Aart Nederveen, Wouter Teeuwisse, and Thijs van Osch for technical assistance.

M.J. van Tol had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PARTICIPANT RECRUITMENT AND INCLUSION CRITERIA

Participants were recruited from NESDA (Netherlands Study of Depression and Anxiety), a large-scale multi-site longitudinal observational cohort study (Penninx et al., 2008). The rationale, methods, and recruitment have been described in detail elsewhere (Penninx et al., 2008). Out of the 2981 NESDA respondents (main sample, T1), participants aged between 18 and 57 years were asked to participate in the NESDA neuroimaging study (T2) if they met DSM-IV criteria for a half-year diagnosis of MDD and/or anxiety disorder (PD, SAD and/or GAD), or no lifetime DSM-IV diagnosis (i.e. healthy controls). At this interval, no specific intervention occurred. Exclusion criteria for patients were 1) the presence of axis-I disorders other than MDD, PD, SAD or GAD, 2) any use of psychotropic medication other than a stable use of selective serotonin reuptake inhibitors (SSRIs) or infrequent benzodiazepine use (i.e., equivalent to 2 doses of 10 mg of oxazepam 3 times per week or use within 48 hrs prior to scanning). Exclusion criteria for all NESDA-participants were: 3) the presence or history of major internal or neurological disorder 4) dependency or recent abuse (past year) of alcohol and/or drugs, 5) hypertension, 6) general MR-contraindications. Diagnoses were established using the structured Composite International Diagnostic Interview (CIDI) (Robins et al., 1988), administered by a trained interviewer. Healthy controls were currently free of, and had never met criteria for, depressive or anxiety disorders or any other axis-I disorder and were not taking any psychotropic drugs.

Complete word encoding and recognition data (EPIs and e-prime output) were not available of 15 participants. Data of another 61 participants were excluded because of 1) bad quality of the EPI data acquired during encoding and/or recognition (n=22), 2) movement >3mm (n=6), 3) not enough coverage of the hippocampus and amygdala (n=4), 4) loss of voxels in the first level mask, owing to large inter-hemispheric frontal space (n=1), 5) very low discriminant power (i.e. d'=<.1; n=17) or >40 missing responses (n=7), indicating unreliable task involvement, 6) medication use (n=2; 1x mirtazepine, 1x corticosteroids), 7) MADRS scores of HC (n=2) that were indicative of possible depressive psychopathology (Muller et al., 2000). To obtain a good match on both age and education, data of another ten subjects (CDA patients: n=7; HC: n=3) had to be excluded. Characteristics of the CDA patients were: male/female: 2/5; mean (sd) age: 41.1 (4.8); mean (sd) years of education: 6.7 (1.6); mean (sd) IDS score: 24.7 (11.38), mean (sd) BAI: 13.7 (8.3). Of the three excluded healthy controls, 2 were female, mean (sd) age: 49.7 (4.2); mean (sd) years of education: 18 (0).

TASK PARADIGM

We employed an event-related, subject-paced, implicit word encoding- and recognition paradigm (Daselaar et al., 2003), programmed in E-prime software (Psychological Software Tools, Pittsburgh, PA). During the encoding part 40 positive, 40 negative, and 40 neutral words, and 40 baseline trials were presented in 20 blocks of eight words. Words were presented with an average ISI of 1026 ms (minimum, 1018 ms; maximum, 1035 ms). Within each block, two negative words, two positive words, two neutral words and two baseline trials were presented randomized. Over valence (i.e. negative, neutral, positive), words were matched for length (ranging from three to twelve letters) and frequency of occurrence in the Dutch language. The task was paced by the subject, but each word was presented with a maximal duration of 5 sec. During each stimulus presentation, response options were displayed at the bottom of the screen. Subject had to indicate whether they thought the word presented was positive, negative, or neutral to them. Baseline (BL) words were '<<left', '<<middle>>', and 'right>>' and participants were instructed to press the corresponding button. To protect against primacy and recency effects three filler (1 positive, 1 negative, 1 neutral) words were presented at the start and end of the encoding task. These filler words were not part of the subsequent recognition task. The recognition test phase consisted of the 120 old encoding target words and 120 new distracter words, and 40 baselines. Again, words were presented pseudo-randomized in 20 blocks of 14 words, each block containing two old and two new negative words, two old and two new positive words, two old and two new neutral words, and two baseline trials. 'Old' and 'new' words were matched on complexity, word length, and emotional intensity. Subjects had to indicate whether they have 'seen' (i.e. remembered) the words previously, 'probably have seen it' (i.e. know), or 'haven't seen it' (rejection). Interval between the encoding task and recognition task was 10 minutes. Participants' responses and response times were registered through two magnet-compatible button boxes. No feedback regarding the answers was provided.

The words encoding and recognition task was administered as part of a larger functional and structural imaging study, results of which will be reported elsewhere. The words task was administered as the second task in the session in all participants.

TABLE S-1: SSRI USERS VS SSRI-NON USERS WITHIN MDD, CDA, AND ANX

MADRS=Montgomery Asberg Depression Rating Scale; IDS=Inventory Depressive Symptomatology; BAI=Becks Anxiety Inventory; FQ=Fear Questionnaire; VAS=Visual Analogue Score; pre: before word encoding task; post: after word recognition task; T1=time of NESDA baseline measurement; T2=time of MRI measurement; interval=interval between T1 and T2.

		MDD		CDA		ANX	
		SSRI users	SSRI free subjects	SSRI users	SSRI free subjects	SSRI users	SSRI free subjects
N		12	39	24	35	17	39
gender	male/female; N	3/9	17/22	12/12	9/26	3/14	11/28
scan site	amc/lumc/umcg; N	5/4/3	10/14/15	8/10/6	13/14/8	5/4/8	11/12/16
SSRI use past	yes/no; N	n/a	6	n/a	5	n/a	3
age	in years; mean ± sd	39.6 ± 10	35.4 ± 10.1	36.9 ± 11.3	36.2 ± 11.4	36.5 ± 8.5	34 ± 8.9
education	in years; mean ± sd	13.4 ± 3.5	12.8 ± 1.5	12.1 ± 3	12.8 ± 2.9	12.4 ± 3.7	13.3 ± 3.3
MADRS	total score; mean ± sd	13.5 ± 7.7	12.3 ± 9.4	16.9 ± 7.6	21 ± 9.2	11 ± 8	11.2 ± 8.7
IDS T1	" "	25 ± 9.8	27.6 ± 9.9	30.1 ± 10.3	34.4 ± 8.4	24.3 ± 9.6	23.3 ± 11.7
IDS T2	" "	18.9 ± 7.6	17.4 ± 11.1	27.4 ± 9.4	29.5 ± 9.8	19.3 ± 9.2	19.5 ± 10.7
BAI T1	" "	10.3 ± 7.3	10.4 ± 6.2	19.2 ± 7.9	19.6 ± 8.3	15.9 ± 11.5	15.1 ± 7.9
BAI T2	" "	6.8 ± 5.4	8 ± 6.4	17.6 ± 1.9	19.1 ± 9.7	11.6 ± 7.8	15.6 ± 10
VAS_pre	mean ± sd	16.8 ± 18.5	28.3 ± 22.6	30.3 ± 17.4	37.3 ± 24.9	35.2 ± 26.4	39.5 ± 24.9
VAS_post	mean ± sd	19.1 ± 18.9	17.8 ± 18.1	21.6 ± 15.6	30.1 ± 24.6	28.1 ± 20	30.7 ± 22.4
interval	in days; mean ± sd	52.4 ± 22.4	61.7 ± 30.2	58.7 ± 21.3	65.7 ± 40.2	68.9 ± 37	77.1 ± 34.8
onset MDD	age in years; mean ± sd	28.9 ± 11.5	25.7 ± 9.8	24 ± 9.9	24.3 ± 12.2	19.6 ± 5.1	19.8 ± 6.6
onset ANX	age in years; mean ± sd	13 ± 5.6	19.1 ± 14.3	17 ± 12.2	17.2 ± 12.4	22.1 ± 13.3	17.4 ± 10.9
recurrence MDD	single/recurrent episode; N	6/6	14/25	11/13	14/21	n/a	n/a

TABLE S-2: CLINICAL CHARACTERISTICS IN MDD AND CDA SUBGROUPS, BASED ON CURRENT IDS SCORES

MADRS=Montgomery Asberg Depression Rating Scale; IDS=Inventory of Depressive Symptomatology; BAI=Becks Anxiety Inventory; FQ=Fear Questionnaire; VAS=Visual Analogue Score; pre: before word encoding task; T1=time of NESDA baseline measurement; T2=time of MRI measurement; interval=interval between T1 and T2. Rem=remitted depressed state at T2; mild=mild depressed state at T2; mod/sev=moderately to severely depressed state at T2. * significant within-group difference $p<.05$ (non-parametric related samples test).

		MDD rem	MDD mild	MDD mod/sev	CDA rem	CDA mild	CDA mod/sev
N		19	17	15	3	21	35
gender	male/female; N	9/10	7/10	4/11	1/2	6/15	14/21
scan site	amc/lumc/umcg; N	2/7/10	9/6/2	4/5/6	2/0/1	8/8/5	11/16/8
handedness	left/right; N	1/18	3/14	1/14	0/3	0/21	3/32
SSRI use	yes/no; N	3/16	6/11	3/12	2/1	8/13	14/21
age	in years; mean ± sd	36.3 ± 12.3	38.7 ± 7.7	33.9 ± 9.6	37.7 ± 7.4	37.6 ± 11.9	35.7 ± 11.3
education	in years; mean ± sd	13 ± 2.8	12.8 ± 2.7	13.1 ± 2.8	16 ± 3.5	12.8 ± 2.8	12 ± 2.8
MADRS	total score; mean ± sd	4.6 ± 4.8	13.6 ± 6.8	22.1 ± 7.6	2 ± 2.8	14.3 ± 5.5	23.1 ± 7.9
IDS T1	total score; mean ± sd	24.4 ± 11.6 *	26.2 ± 8.5 *	31.1 ± 8.1	18 ± 12.12	29.5 ± 8.5 *	35.7 8
IDS T2	total score; mean ± sd	6.7 ± 3.5	19.3 ± 3.9	30.2 ± 3.7	7 ± 2.7	21.8 ± 2.9	34.5 ± 6.8
BAI T1	total score; mean ± sd	10.4 ± 7.4 *	9.3 ± 5.5 *	11.6 ± 6.4	13.3 ± 8.6	18.1 ± 9.5 *	20.7 ± 7
BAI T2	total score; mean ± sd	3.3 ± 2.6	7.9 ± 5	13 ± 6.4	3 ± 1.7	15.7 ± 7.2	21.5 ± 8.4
FQ	total score; mean ± sd	13.2 ± 10.9	22.9 ± 11.1	21.3 ± 15.6	17.5 ± 9.2	32.9 ± 17.7	27.9 ± 19.1
VAS_pre	mean ± sd	24.7 ± 16.7	20.6 ± 25.5	32.1 ± 23.4	23 ± 32.5	32 ± 20.2	36.9 ± 22.8
interval	in days; mean ± sd	67.8 ± 30.7	46.4 ± 15	64.3 ± 33.7	66.3 ± 21.7	58.7 ± 21.9	65.1 ± 40.2
onset MDD	age in years; mean ± sd	25.5 ± 12.1	29.1 ± 9.7	24.7 8.4	19 ± 6	26.1 ± 11.4	23.5 ± 11.4
onset ANX	age in years; mean ± sd	-	-	-	16 ± 8.9	21 ± 11	18.4 ± 11.3
recurrence MDD	single/recurrent episode; N	5/14	7/10	8/7	0/3	10/11	15/20

TABLE S-3: TASK PERFORMANCE

Classification behavior: number of words classified as positive, neutral, and negative during the encoding phase. Mean proportion correct and response times for encoding and recognition indices. Abbreviations can be found in Table 1. A one-way ANCOVA showed no effect of diagnosis on proportion PROB responses (M_{old_words}= .13, sd=.09; F=.42, *p*=.74; M_{new_words}=.14, sd=.09; F=.23, *p*=.88). Therefore, we added the PROB trials to the CREC or FA and recalculated the proportion CREC and FA (and d'). * In the word encoding task, 42 positive, 42 neutral and 42 negative words (including the filler words) were presented.

Word classification*

			MDD	CDA	ANX	HC
# words classified	pos	mean ± sd	39.32 ± 9.95	41.45 ± 11.03	39.51 ± 8.8	44.62 ± 9.82
	neu	"	46.52 ± 10.74	42.04 ± 11.84	44.61 ± 11.37	40.21 ± 9.89
	neg	"	39.79 ± 2.80	40.81 ± 5.06	41.28 ± 5.27	40.40 ± 4.51

Memory performance

			MDD	CDA	ANX	HC
CREC	_all	prop; mean ± sd	.85 ± .08	.84 ± .09	.84 ± .09	.82 ± .11
	_neg	"	.84 ± .10	.84 ± .11	.84 ± .10	.82 ± .13
	_pos	"	.85 ± .10	.86 ± .09	.85 ± .11	.83 ± .12
	_neu	"	.84 ± .11	.83 ± .12	.84 ± .10	.79 ± .14
FA	_all	"	.26 ± .12	.25 ± .14	.25 ± .10	.25 ± .13
	_neg	"	.32 ± .12	.31 ± .15	.31 ± .12	.33 ± .16
	_pos	"	.28 ± .15	.27 ± .18	.27 ± .13	.28 ± .18
	_neu	"	.19 ± .11	.18 ± .16	.16 ± .09	.15 ± .10
d'	_all	"	.58 ± .14	.59 ± .14	.60 ± .12	.56 ± .12
	_neg	"	.52 ± .12	.53 ± .13	.53 ± .12	.50 ± .12
	_pos	"	.57 ± .17	.59 ± .18	.58 ± .15	.56 ± .18
	_neu	"	.65 ± .16	.65 ± .17	.68 ± .14	.64 ± .15

Response times

			MDD	CDA	ANX	HC
rt_SCR	_neg	in sec: mean ± sd	1.27 ± .26	1.28 ± .35	1.26 ± .28	1.23 ± .36
	_pos	"	1.49 ± .35	1.48 ± .39	1.42 ± .27	1.36 ± .31
	_neu	"	1.53 ± .33	1.58 ± .41	1.53 ± .38	1.49 ± .36
rt_CREC	_neg	"	1.24 ± .21	1.24 ± .23	1.24 ± .24	1.25 ± .27
	_pos	"	1.34 ± .27	1.31 ± .26	1.29 ± .23	1.32 ± .27
	_neu	"	1.28 ± .22	1.31 ± .27	1.32 ± .27	1.34 ± .29
rt_CREJ	_neg	"	1.46 ± .30	1.46 ± .34	1.44 ± .28	1.47 ± .32
	_pos	"	1.5 ± .31	1.52 ± .33	1.52 ± .29	1.49 ± .32
	_neu	"	1.38 ± .26	1.36 ± .30	1.41 ± .25	1.38 ± .26
rtFA	_neg	"	1.50 ± .49	1.49 ± .43	1.48 ± .46	1.49 ± .51
	_pos	"	1.66 ± .59	1.69 ± .65	1.57 ± .55	1.74 ± .76
	_neu	"	1.54 ± .54	1.90 ± .71	1.68 ± .67	2.04 ± .83
rtMISS	_neg	"	1.79 ± .62	1.98 ± .80	1.92 ± .69	1.84 ± .60
	_pos	"	1.97 ± .68	1.89 ± .68	1.91 ± .73	1.99 ± .68
	_neu	"	1.66 ± .65	1.62 ± .55	1.69 ± .57	1.77 ± .63
rtBL_enc		"	.83 ± .17	.81 ± .16	.79 ± .14	.83 ± .24
rtBL_rec		"	.78 ± .13	.79 ± .16	.76 ± .13	.76 ± .14

TABLE S-4: MAIN EFFECTS OF ENCODING OF POSITIVE AND NEGATIVE (VS. NEUTRAL WORDS)

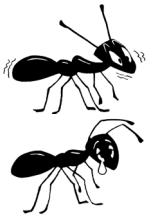
Main effects of contrast 'SCR_pos>SCR_neu' and 'SCR_neg>SCR_neu' across groups (p<.05, FWE whole brain corrected); BA = Brodmann Area; side=hemisphere; L=left; R=right.

Main effect of SCR_pos>SCR_neu (whole brain FWE corrected)

			MNI-coordinates				
Regions	side	BA	x	y	z	T	Z
Frontal							
inferior frontal gyrus	L	45/47	-51	21	0	6.95	6.95
inferior frontal gyrus	L	44	-57	9	18	5.17	5.17
anterior cingulate gyrus	L	32	-3	45	-3	6.56	6.56
medial superior frontal gyrus	L	10	-3	57	0	6.32	6.32
medial frontal gyrus	L	10	0	60	18	5.96	5.96
middle frontal gyrus	L	6	-33	-9	57	5.94	5.94
middle frontal gyrus	L	6	-45	3	51	5.43	5.43
middle/superior frontal gyrus	R	6	27	-3	57	5.13	5.13
inferior frontal gyrus	R	44	51	6	30	4.94	4.94
precentral gyrus	L	4	-30	-24	48	4.87	4.87
temporal/parietal							
superior temporal gyrus/middel temporal gyrus	L	22/39	-51	-54	18	7.36	7.36
superior temporal gyrus/gyrus supramarginalis	L	39/40	-57	-51	30	5.61	5.61
middle temporal gyrus	L	37	-51	-66	3	5.11	5.11
posterior cingulate gyrus	L	31	-6	-54	27	5.66	5.66
inferior parietal lobule	R	40	45	-30	42	5.44	5.44
inferior parietal lobule	L	40	-39	-39	42	5.30	5.30
inferior parietal lobule	L	40	-45	-33	42	5.21	5.21
inferior parietal lobule	L	40	-42	-33	57	5.27	5.27
middle temporal gyrus	L	21	-48	-30	-6	5.03	5.03
inferior parietal lobule	L	40	-33	-48	57	4.83	4.83
other (subcortical)							
caudate nucleus	L		-9	-3	12	4.94	4.94

Main effect of SCR_neg>SCR_neu (whole brain FWE corrected)

			MNI-coordinates				
Regions	side	BA	x	y	z	T	Z
Frontal							
middle frontal gyrus	L	6	-30	-6	54	6.42	6.26
medial frontal gyrus	L	9	-6	51	24	5.67	5.56
inferior frontal gyrus	R	44	48	9	30	5.14	5.06
inferior frontal gyrus	L	44	-48	9	27	5.10	5.02
precentral gyrus	L	6	-39	3	36	5.08	5.00
inferior frontal gyrus	R	44	57	15	24	4.94	4.87
Temporal/Parietal							
inferior parietal lobule	L	40	-42	-42	39	7.28	7.06
inferior parietal lobule	L	40	-33	-48	42	6.86	6.68
supramarginal gyrus	L	40	-57	-48	27	6.35	6.20
middle temporal gyrus	L	21	-48	9	-27	6.42	6.27
middle temporal gyrus	L	21	-54	-3	-18	6.00	5.88
middle temporal gyrus	L	21	-57	-24	-6	5.99	5.87
inferior parietal lobule	R	40	45	-33	42	5.59	5.49
posterior cingulate gyrus	L	31	0	-48	24	5.53	5.43
middle temporal gyrus	L	39	-48	-63	6	5.19	5.11
inferior parietal lobule	R	40	33	-51	51	5.02	4.95
Occipital							
middle occipital gyrus	R	18	48	-63	-6	5.20	5.11
occipital gyrus	R	19	39	-81	0	5.19	5.10
middle occipital gyrus	L	19	-30	-78	21	5.08	5.00



CHAPTER 4

FUNCTIONAL MRI CORRELATES OF VISUOSPATIAL PLANNING IN OUTPATIENT DEPRESSION AND ANXIETY

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ACTA PSYCHIATRICA SCANDINAVICA, 2011; E-publication ahead of print

SUMMARY

Background: Major depressive disorder (MDD) has been associated with executive dysfunction and related abnormal prefrontal activity, whereas the status of executive function (EF) in frequently co-occurring anxiety disorders and in comorbid depression-anxiety is unclear. We aimed to study functional MRI correlates of (visuospatial) planning in MDD and anxiety disorders, and to test for the effects of their comorbidity.

Methods: Functional-MRI was employed during performance of a parametric Tower of London task in outpatients with MDD (n=65), MDD with comorbid anxiety (n=82), or anxiety disorders without MDD (n=64), and controls (n=63).

Results: Moderately/severely depressed MDD patients showed increased left dorsolateral prefrontal activity as a function of task load, together with subtle slowing during task execution. In mildly depressed and remitted MDD patients, in anxiety patients and in patients with comorbid depression-anxiety, task performance was normal and no activation differences were observed. Medication use and regional brain volume were not associated with altered visuospatial planning.

Conclusion: Prefrontal hyperactivation during high planning demands is not a trait characteristic, but a state characteristic of MDD without comorbid anxiety, occurring independent of SSRI-use. Disturbances in planning or the related activation are probably not a feature of anxiety disorders with or without comorbid MDD, supporting the current distinction between anxiety disorders and depression.

Executive functions are essential for successful adaptation to daily life and comprise a complex set of cognitive function, dysregulation of which may result in changes in coordination, organization, and inhibition of behavior (Elliott, 2003). Executive deficits have been repeatedly associated with Major Depressive Disorder (MDD) (Rogers et al., 2004). However, MDD frequently co-occurs with anxiety disorders, such as panic disorder, and social anxiety disorder (Ressler & Mayberg, 2007). Executive impairments in these common anxiety disorders (panic disorder, social anxiety disorder, and generalized anxiety disorder) are less well established and findings may have been confounded by the presence of comorbid MDD (Kaplan et al., 2006). Notably, the comorbid condition of depression and anxiety disorders has been associated with more severe psychopathology (Kessler et al., 2005; Roy-Byrne et al., 2000; Rush et al., 2005), increased disability (Fichter, Quadflieg, Fischer, & Kohlboeck, 2010; Gorman, 1996; Spijker et al., 2004), and worse outcome compared with patients with only a diagnosis of MDD or anxiety disorders (Bruce et al., 2005; Gorman,1996; Gorman & Coplan, 1996; Melartin et al., 2004; Roy-Byrne et al., 2000; Rush et al., 2005). Nevertheless, the neural correlates of executive functions in comorbid depression-anxiety disorders have not been investigated yet.

A key aspect of executive functioning is planning (i.e. the process of making a plan when facing a problem, and performing the plan, while monitoring its execution), associated with recruitment of a dorsal prefrontal-parietal-striatal network during functional Magnetic Resonance Imaging (fMRI) in healthy controls as measured with the Tower of London visuospatial planning task (van den Heuvel et al., 2003; Wagner et al., 2006). In MDD, conflicting findings have been reported: Whereas two emission tomography studies (i.e., PET (Elliott et al., 1997) and SPECT (Goethals et al., 2005)) observed reduced prefrontal and subcortical perfusion coupled with impaired performance, in the only fMRI study to date increased dorsolateral PFC activation was found in depressed subjects relative to healthy controls (Fitzgerald et al., 2008). However, the size of the included depressed groups was rather small, limiting the power of the studies and the generalizability to the depressive population at large. Also, inconsistencies in results may have arisen owing to differences in scanning modalities and clinical characteristics of patient groups, including medication use and the presence of comorbidity.

In the present study, we aimed to investigate neural correlates of visuospatial planning in a large sample of outpatients with a half-year diagnosis of MDD, MDD with a comorbid anxiety disorder or an anxiety disorder without comorbid MDD, and in healthy controls. We also investigated whether illness severity (McDermott & Ebmeier, 2009) and use of antidepressant medication was associated with (abnormal) planning performance and its neural correlates. Executive processes including visuospatial planning have been shown to rely on cortical prefrontal cortex (PFC) regions, such as the ventrolateral PFC,

dorsolateral PFC, and anterior cingulate cortex (ACC) (van den Heuvel et al., 2003; Wagner, Koch, Reichenbach, Sauer, & Schlösser, 2006). Importantly, these regions have also been implicated in neuroanatomical models of mood regulation in psychiatric disorders (Drevets et al., 2008a; Phillips et al., 2003b) and some evidence exists that top-down regulatory brain regions are compromised in MDD (Heller et al., 2009; Johnstone et al., 2007). However, whether such deficient DLPFC and ACC involvement is implicated in the neuropathology of both depression and anxiety disorders has, to our knowledge, rarely been studied. Therefore, we aimed to examine executive function during a planning task in patients with MDD, anxiety disorders or comorbid depression-anxiety. We hypothesized that relative to healthy controls, outpatients with MDD with and without comorbid anxiety exhibit impaired visuospatial planning performance coupled with abnormal DLPFC and anterior cingulate cortex (ACC) activity, correlated with illness severity. Finally, we aimed to explore whether anxiety disorders were characterized by a similar pattern of visuospatial planning abnormalities.

PARTICIPANTS

Participants were recruited from the NESDA study (Netherlands Study of Depression and Anxiety), a large-scale, multi-site longitudinal observational cohort study (Penninx et al., 2008). NESDA has been designed to be representative of those with depressive and anxiety disorders in different health care settings and different stages of the developmental history of disorders (e.g., normal controls, high familial risk, subthreshold disorders, first and recurrent episodes). Therefore, the sample is stratified for setting (community, primary care, and specialized mental health) and set up to include a range of psychopathology. The rationale, methods, and recruitment have been described in detail elsewhere (Penninx et al., 2008).

Out of the 2981 NESDA respondents, participants aged between 18 and 57 years were asked to participate in the NESDA neuroimaging study if they met diagnostic and statistical manual for mental disorders (DSM) version IV criteria for MDD and/or anxiety disorder (panic disorder and/or social anxiety disorder and/or generalized anxiety disorder) during the last six months, or no lifetime DSM-IV diagnosis.

Exclusion criteria for patients were 1) the presence of axis-I disorders other than MDD, panic disorder, social anxiety disorder, generalized anxiety disorder, lifetime, 2) any use of psychotropic medication other than a stable use of SSRIs or infrequent benzodiazepine use (i.e., equivalent to 2x10 mg oxazepam 3 times a week, or use within 48 hrs prior to scanning). Healthy control participants had never met criteria for any DSM-IV disorder and were not taking any psychotropic drugs.

Exclusion criteria for all participants were: 3) presence of major internal and/or neurological disorders (including contusio cerebri), 4) dependency or recent

abuse (past year) of alcohol and/or drugs, 5) hypertension, 6) general MRI contraindications. Diagnoses according to DSM-IV algorithms were established using the structured Composite International Diagnostic Interview (CIDI) – lifetime version 2.1 (Robins et al., 1988), administered by a trained interviewer.

Overall, 301 native Dutch speaking participants (233 patients and 68 HC) were included and underwent magnetic resonance imaging in one of the three participating centers (i.e., Leiden University Medical Center [LUMC], Academic Medical Center [AMC], University of Amsterdam, and University Medical Center Groningen [UMCG]). The study was carried out in accordance with the Declaration of Helsinki. Also, the Ethical Review Boards of each participating center approved this study. All participants provided written informed consent after complete description of the study.

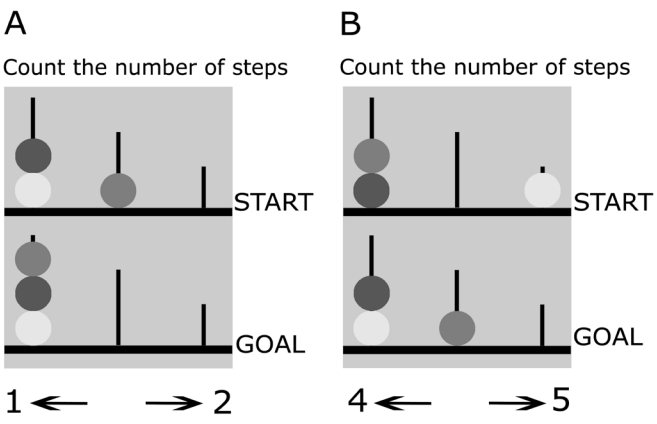
TASK PARADIGM

We used an event-related parametric version of the Tower of London, described in detail elsewhere (van den Heuvel et al., 2003). Briefly, participants were presented a starting configuration and a target configuration and were requested to work out the minimum number of steps (ranging from one to five) to reach the target (Figure 1). In the baseline condition, participants were instructed to count the number of blue and yellow beads. We used a pseudo-randomized, self-paced design with maximal response duration of 60s for each trial, presented using E-prime (Psychological Software Tools, Pittsburgh, PA, USA). Participants' responses and response times were registered through two magnet-compatible button boxes. No feedback regarding the answers was provided.

FIGURE 1: EXAMPLE OF THE TOWER OF LONDON

A) example of a one-step planning trial.

B) example of a four-step planning trial.



SCANNING PROCEDURE

Severity of depression and anxiety at the day of scanning was assessed using Beck Anxiety Inventory (Beck et al., 1988) and the Montgomery Åsberg Depression Rating Scale (Montgomery & Åsberg, 1979). During the scanning session, we monitored anxiety levels using a Visual Analogue Scale (Huskisson, 1974) ranging from 0 to 100. Prior to scanning, participants underwent a brief training session (10 trials).

The Tower of London paradigm was performed as part of a larger functional imaging session, including a verbal memory task, an emotional faces paradigm, and 'resting state' imaging, results of which will be reported elsewhere. The Tower of London paradigm was administered as the first paradigm in each session.

IMAGE ACQUISITION

Imaging data were acquired using Philips 3-Tesla magnetic resonance systems (Best, The Netherlands) located at the Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen, equipped with a SENSE-8 (Leiden University Medical Center and University Medical Center Groningen) and a SENSE-6 (Academic Medical Center) channel head coil, respectively. For each subject, echo-planar images (EPI) were obtained using a T2*-weighted gradient echo sequence (repetition time [TR]=2300 ms, echo time [TE]=30ms [University Medical Center Groningen: TE=28 ms], matrix size: 96x96 [University Medical Center Groningen: 64x64], 35 axial slices [University Medical Center Groningen: 39 slices], interleaved acquisition, 2.29x2.29mm in-plane resolution [University Medical Center Groningen: 3x3mm], 3mm slice thickness). Echo planar images were scanned parallel to the anterior-posterior commissure plane.

Anatomical imaging included a sagittal 3-dimensional gradient-echo T1-weighted sequence (TR=9 ms, TE=3.5 ms; matrix 256x256; voxel size: 1x1x1mm; 170 slices).

DATA ANALYSIS

Task performance and clinical characteristics

Psychometric and performance data were analyzed with SPSS (SPSS Inc., Chicago, Il, USA). If the data did not meet the assumptions required to perform parametric analyses, and log-transforming did not resolve these problems, the appropriate non-parametric test was performed (Kruskall-Wallis multiple independent sample test [H] and subsequent Mann-Whitney test for two independent samples [U]). Performance was analyzed by means of a Repeated-Measures-ANCOVA, using the proportion correct scores and mean response times per trial type as dependent factors, group (1) MDD without comorbid anxiety disorders [MDD], 2) comorbid depression-anxiety [CDA], 3) anxiety disorders [ANX], healthy contols [HC]) as between subject factor, and age as a

covariate. Significance for behavioral analyses was set at 5%, and post hoc paired tests were Bonferroni-corrected for multiple comparisons.

Image processing

Functional imaging data were preprocessed and analyzed using Statistical Parametric Mapping software (SPM5; http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 7.1.0 (The MathWorks Inc., MA, USA). Preprocessing included slice time correction, image realignment, registration of the T1-scan to the mean EPI, warping to MNI-space as defined by the SPM5 T1-template, reslicing to 3x3x3mm voxels and spatial smoothing using an 8mm FWHM Gaussian kernel. Subject movement greater than 3mm in any direction resulted in exclusion of the data from further analysis.

The fMRI experiment was modelled in an event related fashion with regressors (i.e. explanatory variables) made by convolving each event-related stimulus function (baseline, 1-5 step trials) with a canonical haemodynamic response function and modulated using reaction times. In addition, error and no-response trials were included as a regressor of no interest. Low-frequency noise was removed by applying a high-pass filter (cut-off of 128s) to the fMRI time-series at each voxel.

Main effects of task and between group comparisons

Following the summary statistics approach, contrast images for task load (with trial types 1-5 having weights [-1.5 –1 –0.5 1 2]) were calculated per subject on a voxel-by-voxel basis and entered into second-level analyses for between-group [MDD, CDA, ANX, HC] comparisons (1x4 ANCOVA), with age as covariate. Additionally, 'center' was added as a regressor by means of two dummy variables. We repeated this analysis after exclusion of the SSRI using patients and also directly compared the functional maps of SSRI using patients to SSRI-non using patients. To test for the impact of depression severity on planning activity we formed 3 clinical relevant subgroups (Muller et al., 2000) and compared them to HC: 1) remitted (MADRS 0-8), 2) mild (MADRS 9-18), 3) moderate/severe depressed (MADRS>18). We performed an ANCOVA with age and center as covariates within MDD, CDA, and HC, and repeated these analyses with BAI scores as additional covariate. Separately, we tested for the effects of anxiety severity within group by performing a linear regression analysis with BAI scores as regressor of interest, and age, MADRS score, and center as covariates, masked with a binary mask derived from the relevant main effect at p<.005, uncorrected.

The main effect of task is reported at a threshold of p<.05 whole brain corrected for Family Wise Error ($p_{FWE_wholebrain}$). For the between-group comparisons, we choose the dorsolateral PFC and anterior cingulate cortex (ACC) as regions of interest (ROI). To restrict the search for interaction effects to voxels which were identified in the main effect, group comparisons were masked with the orthogonal relevant main effect at p<.005 (uncorrected).

Group by task interaction effects were inspected at p<.001, uncorrected ($p_{uncorrected}$). To further protect against Type-I error, Small Volume Correction (SVC) was applied for the main comparison (effect of diagnosis), by centering a sphere with a 16mm radius around the peak voxel (i.e., twice the size of the smoothing kernel and equivalent to 17.2 ml). The resulting volumes of interest had to meet p<.05, FWE voxel corrected (p_{FWE_SVC}), to be considered significant. Non-ROIs had to meet p<.05, whole brain FWE corrected ($p_{FWE_wholebrain}$).

In a previous study, we reported decreased ACC volumes in both patients with MDD and anxiety disorders (van Tol et al., 2010). Therefore, we used the statistical toolbox Biological Parametric Mapping (BPM; Wake Forest University School of Medicine) to test whether between-group effects were affected by variations in regional grey matter volume (Casanova et al., 2007).

SAMPLE CHARACTERISTICS

Data from 12 participants were excluded because of poor imaging data quality or incomplete data. In addition, task involvement was considered insufficiently reliable when overall performance was below 75%, (including baseline accuracy) in order to maximize the likelihood of analyzing planning-related activity and to reduce possible bias due to non-task related processes. Distribution of sufficient/insufficient performance was similar over groups (MDD: 65/4; comorbid depression-anxiety: 82/3; panic disorder and/or social anxiety disorder: 64/4; healthy controls: 65/2, $X^2_{3,289}$= 1,12, p=.77). We excluded insufficient performers from further analysis (n=13). Finally, two healthy controls were excluded because of Montgomery Åsberg Depression Rating Scale scores >8 (Muller et al., 2000).

Our final sample consisted of 274 participants, i.e. 65 patients with a half-year diagnosis of MDD (MDD), 82 patients with MDD plus panic disorder and/or social anxiety disorder and/or generalized anxiety disorder (comorbid depression-anxiety [CDA]), 64 patients with panic disorder and/or social anxiety diosrder, and/or generalized anxiety disorder without MDD (ANX), and 63 healthy controls (HC). Group characteristics are listed in Table 1. All diagnostic groups showed higher Montgomery Åsberg Depression Rating Scale and Beck Anxiety Inventory scores than the healthy controls. In addition, comorbid depression-anxiety patients showed higher Montgomery Åsberg Depression Rating Scale and Beck Anxiety Inventory scores than patients with MDD or anxiety disorders, and patients with anxiety disorder showed higher Beck Anxiety Inventory scores than MDD (Montgomery Åsberg Depression Rating Scale: all z> -4.26, p<.001; Beck Anxiety Inventory: all z> -3.01,all p<.003). The sample was highly representative of the main NESDA sample (see supplemental material). Depression severity scores decreased during the interval between baseline assessments (T1) and subsequent MRI sessions (T2) (Table S-1). Splitting of the depressed groups into severity groups based on their symptom severity at T2

TABLE 1: DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF THE TOTAL SAMPLE (N=274)

MADRS= Montgomery Asberg Depressive Rating Scale; BAI: Beck Anxiety Inventory; rem=MADRS scores indicative of a remitted depressive state (0-8); mild=MADRS scores indicative of a mild depressive state (9-18); modsev=MADRS scores indicative of a moderate to severe depressive state(>18); 1yr diagn MDD: Diagnosis of MDD in the last year; lifetime MDD: life time diagnosis of MDD; lifetime ANX: life time diagnosis SAD/PD/GAD; T1= baseline assessment; T2=MRI measurement; interval= time between T1 and T2.
a: CDA =18 MDD+GAD, 17 MDD+PD, 9 MDD+PD+GAD, 8 MDD+SAD, 14 MDD+SAD+GAD, 8 MDD+PD+SAD, 8 MDD+PD+SAD+GAD; b: ANX=18 PD, 2 PD+GAD, 25 SAD, 3 SAD+GAD, 12 PD+SAD, 5 PD+SAD+GAD.

		MDD (n=65)		CDA a (N=82)		ANX b (N=64)		HC (N=63)	
Variable		N	%	N	%	N	%	N	%
male gender		24	36.9	28	34.2	18	28.1	24	38.1
scan site	amc/lumc/umcg	17/23/25	26.2/35.4/38.4	26/31/25	31.7/37.8/30.5	21/20/23	32.8/31.3/35.9	24/27/12	38.1/42.9/19.0
right handed		59	90.8	76	92.7	60	93.8	58	92.1
SSRI user		16	24.6	34	41.5	19	29.7	0	0
recurrent MDD		39	60.0	46	56.1	-	-	-	-
MADRS category	rem/mild/modsev	22/23/20	33.8/35.4/30.8	8/32/42	9.8/39.0/51.2	34/19/11	53.1/29.7/17.2	63/0/0	100/0/0
1 yr diagn MDD		65	100	82	100	5	7.8	0	0
lifetime MDD		65	100	82	100	36	56.3	0	0
lifetime ANX		20	30.8	82	100	64	100	0	0
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
age	years	37.08	10.44	36.93	10.65	35.49	9.05	40.05	9.51
education	years	12.67	2.91	11.62	3.13	13.11	3.21	14.25	2.75
MADRS	total score	13.43	9.10	20.21	9.33	10.48	8.62	1.10	1.86
BAI	total score	8.97	8.19	17.91	9.41	13.22	9.51	2.05	2.49
onset MDD	age in years	25.38	10.35	24.21	11.53	-	-	-	-
onset ANX	age in years	-	-	18.22	11.03	14.48	10.42	-	-
		range		**range**		**range**		**range**	
MADRS		0 - 39		0 - 49		0 - 35		0 - 7	
BAI		0 - 50		0 - 47		0 - 42		0 -10	

TABLE 2: DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF THE REMITTED, MILD, AND MODERATE/SEVERE MDD AND CDA SUBGROUPS

MADRS= Montgomery Asberg Depressive Rating Scale; BAI: Beck Anxiety Inventory; IDS= Inventory of Depressive Symptomatology; rem=MADRS scores indicative of a remitted depressive state (0-8); mild=MADRS scores indicative of a mild depressive state (9-18); modsev=MADRS scores indicative of a moderate to severe depressive state(>18); T1= baseline assessment; T2=MRI measurement; interval= time between T1 and T2. * = significant at $p<.05$, paired T-test.

		MDD_rem (n=22)		MDD_mild (n=23)		MDD_modsev (n=20)	
Variable		N	%	N	%	N	%
male gender		7	31.8	10	43.5	7	35.0
SSRI user		5	22.7	5	21.7	6	30.0
		Mean	SD	Mean	SD	Mean	SD
age	years	35.86	10.90	38.22	10.91	36.15	10.35
IDS T1	total score	25.10	11.15	26.39	7.64	34.06	1.84
IDS T2	total score	9.00	5.67 *	20.87	6.43 *	30.89	8.33
MADRS T2	total score	3.86	2.40	12.83	2.64	24.65	5.04
BAI T1	total score	10.48	7.71	11.83	7.11	14.05	12.13
BAI T2	total score	3.95	2.67 *	9.09	5.70	14.89	11.02
interval	days	64.62	28.5	60.52	36.22	74.44	54.36
		CDA_rem (n=8)		CDA_mild (n=32)		CDA_modsev (n=42)	
		N	%	N	%	N	%
male gender		1	12.5	9	28.1	17	40.5
SSRI user		3	37.5	16	50.0	15	35.7
		Mean	SD	Mean	SD	Mean	SD
age	years	36.12	6.64	37.42	12.28	36.45	10.12
IDS T1	total score	21.38	9.61	31.61	8.98	35.93	12.71
IDS T2	total score	11.25	5.95 *	24.29	7.93 *	36.15	9.52
MADRS T2	total score	5.38	3.02	14.10	2.52	27.55	6.11
BAI T1	total score	10.62	7.54	20.6	7.83	17.78	9.36
BAI T2	total score	7.25	5.04	18.07	6.47	20.75	9.76
interval	days	23.38	127.14	63.81	25.47	61.66	39.6

revealed that only the currently remitted and mildly depressed subgroups, but not the moderate/severe subgroups, showed this decrease in symptom severity (see Table 2).

Groups matched on gender, handedness, distribution of diagnosis over scan-center, and age (all $X^2< 7.23$, $p>.3$; age: $H_3= 6.36$, $p=.10$). SSRI use was not significantly different across patient groups ($X^2_2= 5.06$, $p=0.08$). Groups differed in years of education ($H_3=28.77$, $p<.001$), due to healthy controls being higher educated than MDD and comorbid depression-anxiety (HC>MDD: U=1361.5; HC>CDA: U=1292; all $p<.008$).

BEHAVIORAL RESULTS

Overall, no effect of diagnosis was observed on planning accuracy ($F_{7.77;699.66}$= 1.29, p=.25) and performance speed ($F_{4.99;449.26}$= .35, p=.86). During the task, patients with anxiety disorders reported higher levels of anxious arousal than healthy controls (ANX>HC: VAS: U=1457, z= -2.7, p<.008; all other comparisons: All z< -2.24, all p>.025) (Table 3).

IMAGING RESULTS

Brain activation patterns reflecting increasing task load were highly consistent over groups (Figure 2), involving clusters in bilateral DLPFC, frontopolar regions, superior frontal regions, lateral parietal cortices, and precuneus (Table S-2, reported at $p_{FWE_wholebrain}$<.05).

A main effect of diagnostic group was observed in the left DLPFC (Figure 3-A): MDD subjects showed increased activation in the left DLPFC relative to HC ([x=-39 y=36 z=30], BA 9/46, Z=3.59, p_{FWE_SVC}=.04). CDA showed increased left DLPFC activation as compared to HC that approached significance ([x=-39 y=36 z=30], BA 9/46, Z=2.69, $p_{uncorrected}$ =.004). No other group comparison revealed significant differences. Results were unaffected by regional brain volume differences.

Effects of SSRI's

We repeated our analysis over 205 SSRI-free participants. Groups were similar to the overall groups with respect to demographic and clinical characteristics. Exclusion of the SSRI using patients did not change behavioral and imaging results. In addition, we directly compared SSRI-using and SSRI-free subjects. Clinical characteristics were similar in SSRI-free and SSRI-using groups. No effect of SSRI use was found.

Effects of illness severity

Within the MDD and comorbid depression-anxiety[1] group, depressive state (remitted, mild, and moderate/severe) was unrelated to gender, scan center, and SSRI use.

Within MDD patients, illness severity was trend-wise associated with response times ($F_{2,58}$=3.07, p=.054, η^2=.1). Explorative post hoc t-tests showed that the moderately to severely depressed MDD patients showed increased response times during execution of all trial types, except for step-5 trials as compared to remitted patients (Step 1-4: all T_{38}= >.2.12, p<.04: Step 5: T_{38}=1.69, p=.1). Within patients with comorbid depression-anxiety, no effect of illness severity on response times was observed ($F_{1,68}$=.93, p=.34). Also, no effect of depression severity was observed on planning accuracy (MDD: $F_{2,60}$=1.05, p=.36; CDA: $F_{1,68}$=.87, p=.37).

A multivariate analysis of covariance (MANCOVA) with Beck Anxiety Inventory scores as fixed factor showed no effect of anxiety scores on performance and response times (all F<1.07, p>.32). Adding Montgomery Åsberg Depression

1 Only three CDA patients were remitted at time of scanning (see Table 2). Therefore, we left the remitted CDA group out of the severity analyses.

TABLE 3: TASK PERFORMANCE

VAS=Visual Analogue Scale. Baseline: baseline trials of the ToL task. Step 1 thru 5: ToL trials with the minimum amount of steps to achieve the goal configuration ranging from 1 thru 5. Total: total proportion of correct trials.

		MDD		CDA		ANX		HC	
Variable		Mean	SD	Mean	SD	Mean	SD	Mean	SD
VAS	total score	32.8	24.44	37.89	25.05	42.94	26.20	29.86	24.08
baseline	proportion correct	0.98	0.03	0.98	0.04	0.98	0.04	0.98	0.04
1 step	"	0.96	0.06	0.95	0.05	0.96	0.06	0.96	0.06
2 step	"	0.92	0.12	0.92	0.09	0.94	0.07	0.93	0.08
3 step	"	0.94	0.09	0.93	0.10	0.92	0.09	0.94	0.08
4 step	"	0.85	0.15	0.78	0.19	0.81	0.15	0.84	0.14
5 step	"	0.80	0.16	0.77	0.23	0.76	0.19	0.78	0.17
total	"	0.94	0.06	0.93	0.06	0.93	0.05	0.94	0.04
baseline	response time (s)	3.51	1.11	3.89	1.26	3.60	1.22	3.43	1.05
1 step	"	4.69	1.40	5.08	1.51	4.86	1.47	4.46	1.09
2 step	"	5.82	1.73	6.24	1.69	6.19	1.94	5.66	1.46
3 step	"	8.07	2.68	8.17	2.30	8.31	2.27	7.95	2.71
4 step	"	11.44	4.36	11.58	3.69	11.40	3.63	11.62	4.02
5 step	"	15.77	6.41	16.13	5.49	16.54	5.97	15.86	4.94

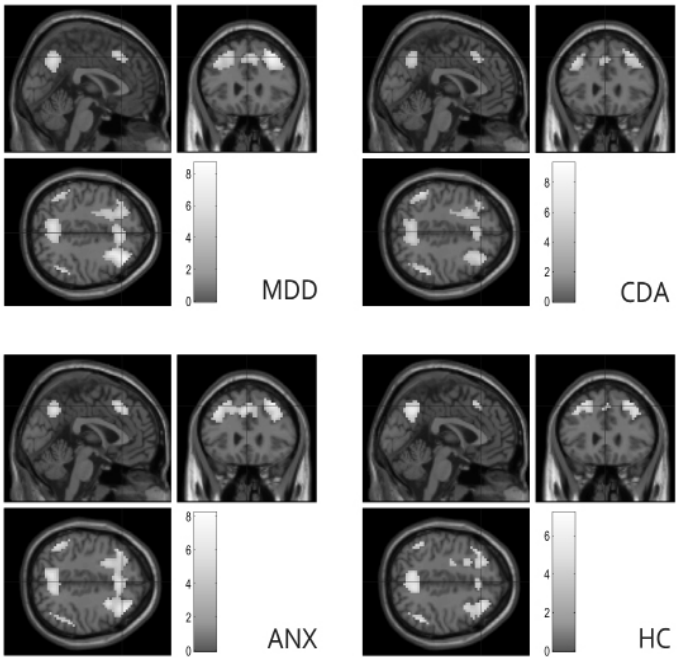
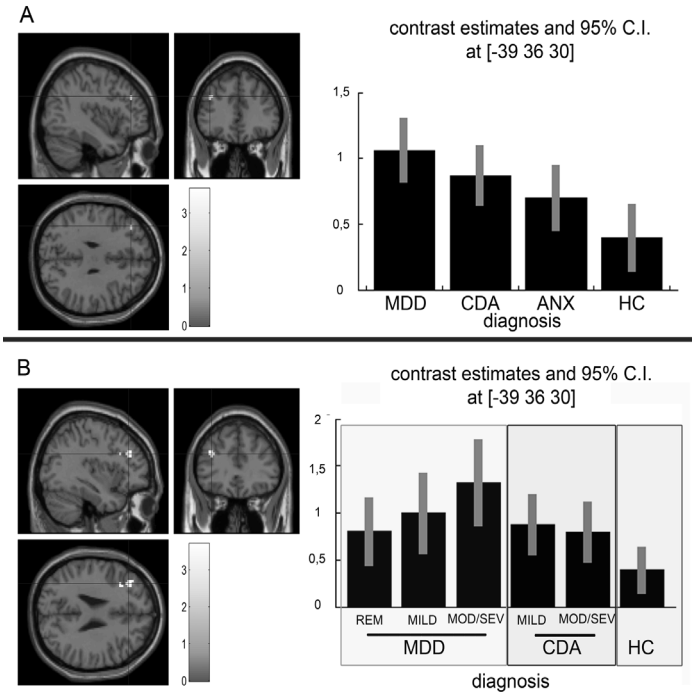


FIGURE 2: MAIN EFFECTS OF INCREASING TASK LOAD

Activation maps of the main effect of increasing task load within the four diagnostic groups (MDD: n=65; CDA: n=82; ANX: n=64; HC: n=63). Activations are displayed at $p<.001$, uncorrected.

FIGURE 3: BETWEEN-GROUP COMPARISONS

A) Left: Increased DLPFC activation as compared to HC. Right: Parameter estimates and 95% confidence intervals showing increased activation of the left DLPFC in MDD as compared HC, and trendwise in CDA; B) Left: Areas showing increased DLPFC activation in the moderately/severely depressed MDD patients as compared to HC. Right: Parameter estimates and 95% confidence intervals showing increased activation of the left DLPFC is specific for moderately/severely depressed MDD patients. Group difference is displayed at p<.001, uncorrected. Rem=remitted depressed patients; mild= mildly depressed patients; mod/sev=moderately/severely depressed patients



Rating Scale scores as a covariate or omitting the SSRI users from the analysis did not affect these results.

Imaging correlates of depression severity

The increased left dorsolateral PFC activation relative to healthy controls was found to be specific for the moderately and severely depressed MDD patients: MNI coordinates: [x=-36 y=33 z=27], BA 46/9, Z=3.62, p_{SVC}=.04 [see Figure3-B]. No differences in left dorsolateral PFC activation were observed between the mild and remitted MDD subgroups and healthy controls. Within comorbid depression-anxiety patients, no effect of depression severity on dorsolateral PFC activation was observed. Omission of the SSRI-users from the analysis did not affect these results. Adding Beck Anxiety Inventory scores to the models did not change the effects of depressive state on activation patterns.

Imaging correlates of anxiety severity

No effect of anxiety severity was observed on task related activity in MDD, CDA and ANX.

In the present study, we used a parametric visuospatial planning task (Tower of London) to investigate the neurophysiological correlates of increased planning load in a large sample of outpatients with MDD, MDD with comorbid anxiety disorders, and anxiety disorders only, compared with healthy controls. Anxiety disorders included in this study were panic disorder, social anxiety disorder, and generalized anxiety disorder. To our knowledge, this is the first functional neuroimaging study that was able to test for effects comorbidity of depression and anxiety, depressive state, and medication use on visuospatial planning and its neural correlates. Also, to our knowledge, this is the first functional neuroimaging study to investigate the neural correlates of executive function (i.e., planning) in PD and SAD, two highly prevalent anxiety disorders.

Taken together, our results indicate that depression with or without anxiety disorders is not associated with gross trait visuospatial planning impairments, but with subtle state abnormalities. Increased DLPFC activation coupled with subtle decreases in response speed during task execution was observed in MDD patients with moderate/severe depression only. MDD patients with mild or (newly) remitted depression showed similar performance and DLPFC activation as HC. This observation is in line with 18FDG-PET findings of Holthoff and colleagues (2004), who reported that remission was accompanied by dorsolateral and dorsomedial PFC (and cingulate) metabolism as compared with the active depressed state, although no direct comparison with healthy controls was made. Furthermore, we observed that in MDD with comorbid anxiety disorders increased left DLPFC activation occured subthreshold, independent of illness severity. In PD and SAD, normal performance and no planning related activation differences were observed, nor did we find significant correlations of anxiety severity in anxiety disorder patients with or without comorbid depression. Results were unrelated to variations in regional gray matter volume. Finally, no overall effects of SSRI-use were observed, consistent with reports in bipolar patients (Goswami et al., 2009).

Our finding of normal executive functioning related fronto-striatal activation in panic disorder and social anxiety disorder are consistent with those of Van den Heuvel and coworkers (2005b) during classical Stroop performance in panic disorder. In the present study we chose to capitalize on our sample size by incorporating all anxiety disorders within one group, which enabled us to identify their shared task-related activity patterns as compared with the comorbid group, in which MDD was often accompanied by more than one anxiety disorder. Taken together, these findings suggest that, in contrast to obsessive compulsive disorder (van den Heuvel et al., 2005a), executive function in panic disorder, social anxiety disorder, and generalized anxiety disorder is not abnormal.

Similar to Fitzgerald and coworkers (2007), we observed increased dorsolateral PFC activation during Tower of London performance in MDD, although in the contralateral hemisphere. The explanation for this lateralization difference

between the study of Fitzgerald and ours is unclear, but could result from a number of methodological issues, such as small sample size, the non-parametric task design, and the inclusion of error-trials in the study of Fitzgerald.

In the present study, task-related activity was assessed using a parametric load contrast, which is likely to be more specific for detecting task-related activity (Friston, Holmes, Poline, Price, & Frith, 1996). In addition, we could not replicate the findings of abnormal Tower of London performance coupled with decreased frontal-striatal activation as reported by Elliott and colleagues (1997). However, the sample size in this early PET study was small (n=6), and the use of psychotropic medication and presence of comorbid Axis-I disorders was not controlled for. Also, Elliott and coworkers (1997) included the functional maps of poorly performing subjects; consequently, the possibility that decreased prefrontal activation was due to non-engagement during task blocks cannot be ruled out. Finally, our results are not in line with the observations of Goethals and coworkers (2005), who observed slower Tower of London performance coupled with decreased prefrontal perfusion in nine medicated MDD patients relative to nine control subjects. However, these authors used a fixed-order dual-scan 99mTc- SPECT method difficult to compare with conventional PET- and fMRI paradigms.

This is the first study that explicitly investigated executive functioning in comorbid depression-anxiety disorder. Our clinical data appear to support the suggestion that patients with comorbid depression-anxiety differ from MDD patients in their clinical profile and course (Bruce et al., 2005; Kessler et al., 2005; Rush et al., 2005), as the comorbid depression-anxiety patients showed higher depression and anxiety symptom scores than MDD patients. Also, the number of remitted comorbid depression-anxiety patients did not increase between the NESDA baseline measurement (T1) and the MRI measurement (T2), whereas depression and anxiety severity decreased substantially between T1 and T2 in MDD patients; almost one-third of the MDD patients reached the remitted phase by the time of scanning (i.e. within two months on average). With respect to task performance, contrary to our expectations, CDA patients showed no executive deficits as measured with the Tower of London, but showed a trend of increased left DLPFC recruitment as a function of increased planning load. This discrepancy in findings may indicate that CDA is primarily characterized by anxiety related neuropathology (i.e. no planning deficits), as in most patients the anxiety disorder was the first to manifest. However, this suggestion should be further tested in future research. Nevertheless, our results indicate that with respect to neural correlates of visuospatial planning, the comorbid condition of depression and anxiety is not identical to the pattern observed in MDD, especially with regard to the effects of depression severity.

The present results of similar task performance (i.e., error rates) across groups appear to be at odds with a number of neuropsychological studies that showed executive deficits in MDD. Given that executive functions are implicated particularly in novel problem solving (Alvarez & Emory, 2006), the

Tower of London arguably has adequate face validity. In contrast, the construct validity and specificity of executive tasks like the frequently used Wisconsin Card Sorting Test and verbal fluency tasks has been called into question (Alvarez & Emory, 2006; Carpenter, Just, & Reichle, 2000). Interestingly, whereas previous Tower of London imaging studies (Elliott et al., 1997; Fitzgerald et al., 2007; Goethals et al., 2005) have reported abnormal performance in MDD, neuropsychological studies have shown mixed results: two studies reported impaired performance in middle-aged MDD subjects (Elliott et al., 1996; Naismith et al., 2003), but studies in younger subjects failed to demonstrate impaired Tower of London performance in MDD (Naismith, Longley, Scott, & Hickie, 2007; Porter, Gallagher, Thompson, & Young, 2003; Purcell, Maruff, Kyrios, & Pantelis, 1997). Behavioral results from the present study are therefore in agreement with these latter neuropsychological studies and may indicate that cognitive functioning is relatively unaffected in the outpatient population (Castaneda et al., 2008; Castaneda et al., 2010; Gladsjo et al., 1998; van Wingen et al., 2009).

Imaging results of the present study are partially in agreement with previous imaging studies in MDD using other executive tasks such as the Stroop task, working memory tasks (n-back and delayed match to sample) and verbal fluency tasks, although these tasks may be characterized as tasks of selective attention and behavioural inhibition as well. Most (Matsuo et al., 2007; Wagner et al., 2006; Walter, Vasic, Hose, Spitzer, & Wolf, 2007), but not all (Videbech et al., 2004) neuroimaging studies using working memory or Stroop tasks reported similar task performance in patients and controls. Increased (dorsal) lateral PFC (Matsuo et al., 2007; Wagner et al., 2006; Walter et al., 2007), frontopolar (Matsuo et al., 2007) and ACC (Wagner et al., 2006) activation has been reported during n-back and Stroop performance in MDD, although not consistently (Siegle et al., 2007). In contrast, two studies using a verbal fluency paradigm in MDD reported impaired performance coupled with increased (Okada, Okamoto, Morinobu, Yamawaki, & Yokota, 2003) or normal dorsolateral PFC activation (Videbech et al., 2003). However, verbal fluency tasks rely heavily on intact semantic memory, and therefore verbal fluency impairment may be reflective of a more generalized impairment than executive dysfunction (Henry & Crawford, 2005).

The present study has a number of strengths. We recruited a large and representative outpatient sample, which allowed us to compare several clinical groups to our healthy controls, and to investigate the effects of psychotropic drug use (SSRIs) and symptom severity within each clinical group. All patients were extensively screened, according to the NESDA protocol (Penninx et al., 2008). Moreover, our control subjects were also recruited through NESDA, were similarly screened and scan-naïve. Therefore, familiarity with researchers and/or experimental procedures could be ruled out as a potential confound. Furthermore, we used an event-related design, to decrease expectancy effects and to allow post hoc selection of correct trials; in addition, we excluded

participants that performed insufficiently, to minimize the risk of non-compliance as a confounder.

Several potential limitations should also be noted. First, since the epidemiological NESDA cohort was recruited through general practitioners and outpatient clinics, we may not have been fully able to capture the most severe end of the depressive spectrum. Second, MDD and comorbid depression-anxiety patients differed slightly from healthy controls in years of education, although neither performance nor task-associated brain activity was significantly correlated with education level (data not shown). Third, since we employed only one executive task, care needs to be taken when generalizing our results outside the executive function domains of planning (Kafer & Hunter, 1997; Unterrainer & Owen, 2006), problem solving (Unterrainer & Owen, 2006), and inhibition (Culbertson & Zillmer, 1998). Fourth, although similar 3-tesla systems were used at each site in this multi-center study, variability in image acquisition may have occurred due to minor differences in hardware (receiver coil), imaging parameters, and timing of software upgrades; also, scanning sessions were scheduled at different hours (between 8 am and 8 pm) across participating centers, but no systematic scanning time x diagnosis bias occurred.

Our findings of relatively increased prefrontal activation associated with task load in currently depressed patients are in agreement with previous studies in MDD and could be interpreted as reflecting less efficient recruitment of neural resources when faced with increasing task demands in MDD, a suggestion that is in line with neurocircuitry models of depression (i.e. Drevets et al., 2008; Mayberg, 1997; Clark, Chamberlain, Shakian, 2009). We also found that increased dorsolateral PFC recruitment in MDD is present only in the moderate to severe depressive state, but not in mildly depressed and remitted states, which suggests high resilience of the brain executive network and does not indicate permanent frontal damage. Also, since the increased recruitment of planning resources occurred in the context of normal planning accuracy, we need to be careful in interpreting our findings as supportive of the notion of executive dysfunction in MDD outpatients. Finally, our results of increased dorsolateral PFC activation in severe MDD, but not in severe comorbid depression-anxiety seem to indicate subtle differences in neurophysiological profiles in MDD patients with or without comorbid anxiety disorders. Therefore, we may conclude that prefrontal hyperactivation during high planning demands is not a trait characteristic, but a state characteristic of MDD, occurring independent of SSRI use; however, executive dysfunction is not a feature of anxiety disorders (i.e., panic disorder, social anxiety disorder, generalized anxiety disorder), supporting the current distinction between anxiety disorders and depression.

In conclusion, future longitudinal studies need to investigate the hypothesis that prefrontal over-recruitment during planning performance normalizes after recovery from depression in MDD without comorbid anxiety. Also, whereas the present results indicate that within the context of a neutral task executive

functions in MDD and anxiety disorders are not grossly abnormal, future research should investigate whether executive functions are compromised when processing emotional stimuli, to further elucidate the role of executive functions in controlling emotions in these disorders.

ACKNOWLEDGEMENT

The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Quality of Healthcare (IQ healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute). We thank Michiel de Ruiter for helpful comments during data analysis, Aart Nederveen, Wouter Teeuwisse, and Thijs van Osch for technical assistance.

COMPARISON OF THE CURRENT SAMPLE TO THE NESDA MAIN-SAMPLE

Groups were highly representative of the main NESDA sample with respect to diagnosis distribution, sexe distribution, depression severity and anxiety severity at the time of baseline measurement. Out of the 2981 included in the main sample, 1961 matched our diagnostic inclusion criteria and were aged between 18 and 57. Of these 1961, 19 % (66% female) fulfilled the criteria for our MDD group, 33 % (66% female) for CDA, 21% (70 % female) for ANX and 28% (67 % female) for the HC group. Finally, the total group of subjects in the MRI study consisted of 24 % (63% female) MDD, 30 % (66% female) CDA, 23 % (72% female) ANX and 23 % (62% female) HC. Although the sub sample of participants included in the MRI study were highly representative of the main sample at the time of baseline interview, at the time of scanning diagnostic groups showed lower depressive scores than at the time of baseline measurements (paired sample non-parametric test; MDD: $z=-5.0$; CDA: $z=-4.1$; ANX: $z=-3.7$; all $p<.001$, see table S2), but showed equivalent anxiety scores (mean # days between baseline measurement and MRI = 65 ± 45). Overall, patients groups included in the MRI study were on average two years younger. The sub sample of healthy controls were slightly older (+ 2.8) and higher educated (+ 1.4 years) than the HC in the main sample.

Variable		MDD (n=65) Mean	MDD (n=65) SD	CDA (n=82) Mean	CDA (n=82) SD	ANX (n=64) Mean	ANX (n=64) SD
IDS T1	total score	28.18	9.67	32.8	11.81	22.55	1.66
IDS T2	total score	19.76	11.03	29.06	11.83	18.67	10.70
BAI T1	total score	12.05	9.05	18.13	9.00	14.16	8.02
BAI T2	total score	9.13	8.27	18.33	9.06	13.60	9.50
interval	days	65.95	39.95	58.66	51.12	68.11	31.18

TABLE S-1: ILLNESS SEVERITY AT T1 AND T2 WITHIN PATIENT GROUPS

IDS= Inventory of Depressive Symptomatology; BAI= Beck's Anxiety Inventory; T1=time of baseline assessment; T2=time of MRI measurement; interval = time between T1 and T2.

Lobe	region	MNI coordinate x	MNI coordinate y	MNI coordinate z	Z value
Frontal					
Left	superior frontal	-21	12	54	>8
	middle frontal	-36	30	39	7.07
	middle frontal	-36	54	12	5.82
Right	superior frontal	27	12	54	>8
	middle frontal	33	24	48	>8
	medial frontal	3	24	45	6.54
Parietal					
Left	precuneus (BA 7)	-6	-63	42	7.70
	inferior parietal	-51	-51	42	7.24
	inferior parietal	-54	-42	42	6.58
Right	precuneus (BA 7)	9	-63	48	6.95
	precuneus (BA 7)	9	-60	57	6.89
	inferior parietal	51	-45	42	7.21
	inferior parietal	48	-54	42	6.79
Occipital					
Left	angular gyrus (BA	-42	-72	36	5.70
Right	angular gyrus (BA	42	-72	33	6.72

TABLE S-2: MAIN EFFECTS OF INCREASING TASK LOAD OVER ALL SUBJECT (N=274)

Main effects for the contrast 'increasing task load' are reported at $p<.05$, FWE-corrected. BA= Brodmann Area; side=hemisphere; L=left; R=right.

CHAPTER 5

WHOLE BRAIN FUNCTIONAL CONNECTIVITY DURING EMOTIONAL WORD CLASSIFICATION IN MEDICATION FREE MAJOR DEPRESSIVE DISORDER

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SUMMARY

Background: Evaluating emotional information involves coordinated activity of prefrontal and subcortical brain regions that may be compromised in Major Depressive Disorder (MDD). This study tested whether patients with MDD show altered functional connectivity during the processing of emotional information with a set of independently derived brain networks that in previous research have shown high correspondence with different task demands, including stimulus salience and emotional processing.

Methods: Twenty-five medication-free MDD patients and 25 right-handed matched controls performed an event-related, subject-paced emotional word evaluation task during functional magnetic resonance imaging. Data were analyzed with a dual regression approach using eight independently derived template independent component networks. First, a time series was estimated for each template per subject. Next, a subject-specific weighted spatial map of brain connectivity with that template was created based on the time series created in the first regression step. Individual spatial connectivity maps were used to evaluate between-group differences using permutation tests.

Results: In patients with MDD, we observed decreased functional connectivity of the medial prefrontal cortex, ventrolateral prefrontal cortex, and ventral striatum with a network that has previously been associated with stimulus salience and emotional task execution compared with controls. Within MDD, results were unrelated to illness severity, but related to the number of words evaluated as positive. No other independent component networks showed between-group differences.

Conclusion: The decreased connectivity of prefrontal and ventral-striatal regions with the salience network during emotional word evaluation confirms the hypothesis of a relative (para)limbic-cortical decoupling that may serve to explain abnormal emotional regulation in MDD.

INTRODUCTION

Failure to adaptively process emotional information is one of the key features of Major Depressive Disorder (MDD). The ability or tendency to continuously evaluate significant stimuli in our surroundings provides us with the ability to limit the magnitude or duration of negative emotions, thus allowing us to devote attention and resources to other important needs or goals. Equally, the capability to "lift oneself up" by evaluating stimuli in a positive manner provides one of the primary motivations needed in an active and productive day-to-day life. Anhedonia, a fundamental aspect of MDD, may in large part reflect an inability to engage in such positive emotional appraisal. Animal research and neuroimaging studies with humans indicate that adaptive appraisal of emotional stimuli depends on intact interactions between (para)limbic and subcortical brain structures, and dorsal and lateral prefrontal cortical (PFC) areas (Ochsner et al., 2004; Phillips et al., 2003a; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). A reduction in the coordinated engagement of such neural systems is one possible mechanism underlying MDD.

Processing external (and internal) emotional information involves the perception of a stimulus (e.g., word, picture, bodily sensation) and appraisal of this stimulus for its content, meaning, and significance. Any negative or positive emotional state resulting from this process of primary appraisal can subsequently be increased or decreased in amplitude through continuing reappraisal of the stimulus or situation. Circuits responsible for these processes may include perception areas (visual cortex, auditory cortex, sensory cortex), areas associated with initial evaluation of or conditioned responding to potentially salient information (amygdala, ventral striatum, insula) and areas supporting more elaborate cognitive appraisal and top-down control such as dorsal and lateral prefrontal cortex (Ochsner et al., 2009; Urry, van Reekum, Johnstone, & Davidson, 2009; Wager et al., 2008).

Functional magnetic resonance imaging (fMRI) studies that have specifically tested the connectivity of (para)limbic and prefrontal regions in MDD suggest abnormal connectivity of the amygdala (Anand et al., 2005; Chen et al., 2008; Cullen et al., 2009), thalamus (Anand et al., 2005), frontal regions (Lemogne et al., 2009; Sheline, Price, Yan, & Mintun, 2010) including the ACC (Anand et al., 2005; Cullen et al., 2009; Sheline et al., 2010) and parietal regions (Bluhm et al., 2009; Sheline et al., 2010; Zhou et al., 2010) during emotional paradigms (Anand et al., 2005; Chen et al., 2008; Lemogne et al., 2009) and without any externally cued task (the so called resting state) (Bluhm et al., 2009; Cullen et al., 2009; Sheline et al., 2010). However, these studies used seed-based analyses where signal from one brain region is correlated with signal from other voxels in the brain or a (set of) predefined brain region(s). Such approaches only allow to test for connectivity based upon a limited number of seed regions (e.g. the amygdala), potentially missing important information about the involvement of broadly interconnected regions throughout the brain. Further, choosing a seed region is rather arbitrary and may be biased when the seed region

is based at least partially on the data being analyzed (Kriegeskorte, Simmons, Bellgowan,& Baker, 2009). Independent component analysis (ICA), on the other hand, is a multivariate data-driven approach that allows studying whole-brain intrinsic connectivity. Using ICA, functional data is decomposed into a set of independent spatial maps and their associated time series which can then be compared between conditions. It is now established that a highly consistent set of component maps can be identified across multiple studies and human samples (Beckmann, DeLuca, Devlin, & Smith, 2005; Biswal et al., 2010; Damoiseaux et al., 2006). Recent evidence suggests that such independent component maps depict networks of brain regions that co-activate when particular categories of tasks or cognitive processes are being performed (Smith et al., 2009), including perception, motor processing, cognition, and emotional processing.

So far, whole brain ICA approaches for analyzing functional imaging data have been sparingly applied in MDD. There is some evidence, based on resting-state ICA that prefrontal networks are altered in MDD, though some studies have indicated increased (Greicius et al., 2007) while others have revealed decreased (Veer et al., 2010) connectivity. However, the lack of a specific task during resting-state studies means that brain activation and connectivity may be influenced by a variety of perceptual, emotional or cognitive processes preceding or during the resting state session (Harrison et al., 2008; Pyka et al., 2009; Waites, Stanislavsky, Abbott, & Jackson, 2005). Also, differences in cortico-limbic connectivity in MDD might become more apparent when studied during tasks that tap into cortico-limbic processes. So far, to our knowledge, no study attempted to investigate whole-brain connectivity during execution of an emotional task in MDD.

In this study we tested whether depressed patients showed different patterns of connectivity in distributed networks during emotional processing compared with controls, using a subset of independently derived brain networks that are involved in perception, and emotional appraisal and regulation. We used a set of template independent component networks (ICNs) derived from a resting state study using healthy individuals (Beckmann et al., 2005). These ICNs also show strong correspondence to a set of spatial components estimated in a meta-analysis of the BrainMap database, which consists of activation maps from nearly 30,000 subjects engaged in a wide variety of tasks (18). We hypothesized that prefrontal and limbic connectivity within these template networks, specifically those found previously to become active during emotion tasks, would be reduced in a group of individuals with MDD compared to a sample of matched healthy controls.

PARTICIPANTS

Twenty-five right-handed medication-free patients with a half-year diagnosis of MDD (16 female; age range: 20-52), with no past or present diagnosis of comorbid anxiety disorders were selected from the Netherlands Study of Depression and Anxiety (NESDA, see supplemental material) neuroimaging study. Diagnoses according to Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV) algorithms were established using the structured Composite International Diagnostic Interview (CIDI) – lifetime version 2.1 (Robins et al., 1988), administered by a trained interviewer. The control group consisted of 25 age, sex, handedness, scan-center and education matched healthy control participants also recruited through NESDA (HC; 17 female; age range: 21-54), who were currently free of, and had never met criteria for depressive or anxiety disorders or any other axis-I disorder, and were not taking any psychotropic drugs. Exclusion for all participants in this analysis were: 1) a history of major internal or neurological disorder, 2) dependency or recent abuse (past year) of alcohol and/or drugs, 3) hypertension, 4) left handedness, 5) general MR-contraindications.

TASK PARADIGM

We employed an event-related subject-paced word encoding paradigm (Daselaar et al., 2003), programmed in E-prime software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Forty positive, 40 negative, 40 neutral study words, and 40 baseline trials were presented in a pseudo-randomized order in 20 blocks of eight words. Each block consisted of two negative words, two positive words, two neutral words, and two control words. Words were matched for length (ranging from three to twelve letters) and frequency of use. The task was paced by the subject, but each word was presented with a maximum duration of 5 sec. Subjects had to indicate whether they thought the word presented was positive, negative, or neutral to them (response options were displayed at the bottom of the screen). Control words were '<< left', '<< middle>>', and 'right >>' and participants were instructed to press the corresponding button. This task was part of a word recognition paradigm. Therefore, the task started and ended with three filler words to protect for the 'primacy-recency' memory effect.

PROCEDURE

MR imaging was performed in one of the three participating centers, the Leiden University Medical Center (LUMC), Academic Medical Center (AMC), University of Amsterdam, or University Medical Center Groningen (UMCG). The Ethical Review Boards of each center approved this study. All participants provided written informed consent after having received written information about the study.

Severity of depression and anxiety on the day of scanning was assessed using Dutch versions of the Beck's Anxiety Inventory (Beck et al., 1988), the Montgomery Åsberg Depression Rating Scale (Montgomery & Asberg, 1979),

and the Inventory of Depressive Symptomatology (Rush et al., 1986). Before the word encoding task, self-rated anxiety levels were monitored using a Visual Analogue Scale ranging from 0 to 100. The words paradigm was performed as part of a larger functional imaging session, including a visuospatial planning task (van Tol et al., 2011), an emotional faces paradigm (Demenescu et al., 2011), and resting-state imaging (Veer et al., 2010).

IMAGE ACQUISITION

Imaging data were acquired using Philips 3-tesla MR-systems (Best, The Netherlands) located at the LUMC, AMC, and UMCG Neuroimaging Center, equipped with a SENSE-8 (LUMC and UMCG) and a SENSE-6 (AMC) channel head coil, respectively. For each subject, echo-planar images (EPI) were obtained using a T2*-weighted gradient echo sequence (repetition time [TR]=2300 ms, echo time [TE]=30ms [UMCG: TE=28 ms], matrix size: 96x96 [UMCG: 64x64], 35 axial slices [UMCG: 39 slices], interleaved acquisition, 2.29x2.29mm in-plane resolution [UMCG: 3x3mm], 3mm slice thickness). EPI's were scanned parallel to the anterior-posterior commissure plane. Scan session length was dependent on the subjects' pace of completing the task and varied between 128 and 240 volumes. Anatomical imaging included a sagittal 3D gradient-echo T1-weighted sequence (TR=9 ms, TE=3.5 ms; matrix 256x256; voxel size: 1x1x1mm; 170 slices).

STATISTICAL ANALYSIS

Imaging data acquired during the word encoding task were processed using FSL software (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl, (Smith et al., 2004)). Preprocessing of the fMRI images was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, implemented in MELODIC Version 3.10. The following processing steps were applied: motion correction, slice timing correction, non-brain removal, spatial smoothing using a Gaussian kernel of 5 mm full width at half minimum, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma= 150 s). Next, the functional images were registered to MNI-152 standard space (T1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) using a two-step registration from functional to high resolution structural T1 image to MNI template. Normalized 4D data sets were resampled to 4 mm isotropic voxels to reduce computational burden in the subsequent analysis steps.

Resampled and normalized data sets from both MDD patients and HCs were then entered into a dual regression analysis (Beckmann, Mackay, Filippini, & Smith, 2009) using the set of eight standard independent component networks (ICNs) as described in detail in Beckmann et al. (2005). In the dual regression procedure, two stages of multiple linear regression analyses are performed. 1) A time series is estimated for each ICN template per subject, using the subject's

functional data as the predicted variable and the set of spatial ICNs as predictors; 2) A subject-specific weighted spatial map is created using the time series created in [1] as predictors of the participant's functional data. The resulting spatial maps represent an unbiased measure of the degree to which BOLD signal fluctuations in each voxel covary with each ICN time series for each subject separately. More simply, each participant's spatial map for a given ICN can be considered a voxelwise map of the strength of functional connectivity with that ICN.

To assess main effects across both groups of functional connectivity within each ICN, individual spatial maps were included in a one-sample t-test performed separately for each ICN. To assess group differences in functional connectivity within each ICN, individual spatial maps were compared in an independent samples t-test performed separately for each ICN. All results are reported at $p<.05$, family wise error corrected for multiple comparisons using permutation tests (# permutations=5000) with cluster-mass thresholding (voxelwise threshold: z=2.3) (Hayasaka & Nichols, 2003).

Table 1 lists sample characteristics. MDD showed higher depression and anxiety scores than controls (MADRS, IDS, BAI: All U>38.5; all $p<.001$). Between the NESDA interview (Time_1) and the MRI-session (Time_2) depressive symptom ratings decreased in MDD (IDS, z=-3.35, $p<.001$). At the time of scanning, eight MDD patients were in the remitted stage. No group difference was observed in the number of volumes acquired during the self-paced task ($F_{1,49}=.69$, $p=.41$). An interaction of valence classification and diagnosis was observed ($F_{1,2;53,2}= 6.0$, $p=<.05$, $\eta^2=.12$): MDD patients indicated less words as 'positive' and more words as 'neutral' than the healthy controls (F>6.07, $p<.05$) (figure 1), independent of illness severity. No effect of diagnosis on classifying negative words was observed and no interaction of diagnosis and valence occurred on response times.

As a check of overall fit of data to the template ICNs, we assessed main effects across groups of functional connectivity with each ICN to evaluate the spatial correlation pattern per template ICN. Overall, mean spatial maps over both groups (i.e., one sample t-tests per component) closely matched the template ICNs, although the components often included additional regions compared with the templates.

Comparison of spatial maps between the two groups showed decreased connectivity of the bilateral medial PFC, nucleus accumbens, caudate nucleus, right orbitofrontal cortex (OFC), and right putamen with a template network including the ventrolateral-PFC, dorsolateral PFC, anterior cingulate gyrus, medial PFC, cerebellum, and cuneus (Figure 2) in MDD compared with controls. This network had the strongest loading on emotion-related tasks in the

BrainMap database (Smith et al., 2009) and has previously been described as the salience network (Seeley et al., 2007). Therefore, we will refer to this network as the salience network for the remainder of this report.

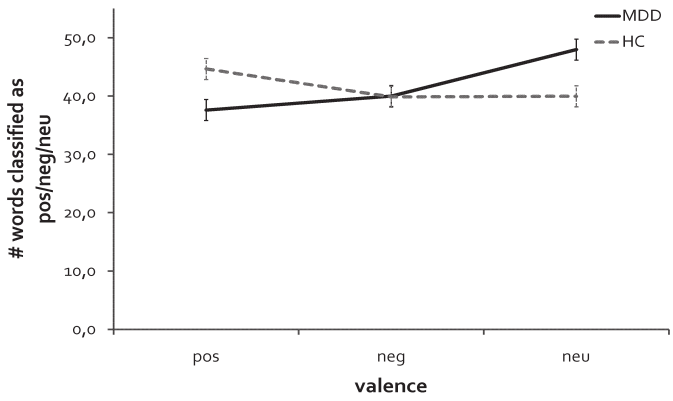
We exported the individual subject beta-values of the 8 sub-clusters (see Table 2) for this independent component network to SPSS to test for possible confounds. A one-way MANOVA confirmed the voxelwise analysis, with lower values in MDD than in controls ($F_{8,41}$=2.86, p<.05, η^2= .36). Adding age, education, and scan center (2 dummy variables) did not change the result ($F_{8,37}$=2.35, p<.05, η^2= .34). In a previous study, we reported on decreased inferior frontal gyrus and rostral anterior cingulate gyrus gray matter volumes in MDD (van Tol et al., 2010). As these regions are implicated in the salience network, we explored the relation between regional anterior cingulate cortex and inferior frontal gyrus volume and connectivity within the salience network. However, no correlation was observed ($r_{Kendall's\ tau}$<.11, p>.26). Moreover, connectivity of the medial PFC-striatal regions within the salience network was not associated with depression severity (IDS_T2:($r_{Kendall's\ tau}$=.02, p=.87).

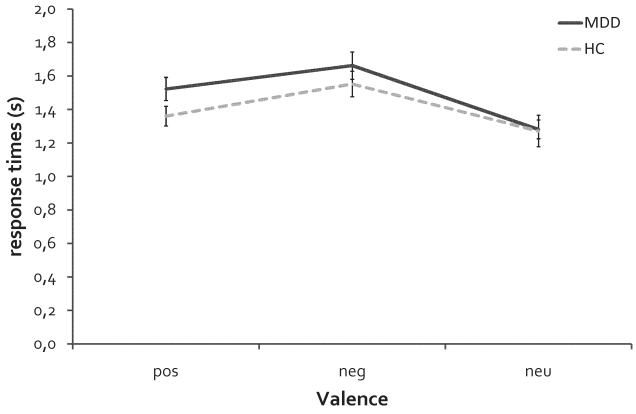
TABLE 1: SAMPLE CHARACTERISTICS

MDD: Major Depressive Disorder; HC: healthy controls; MADRS: Montgomery Åsberg Rating Scale; IDS: Inventory of Depressive Symptomatology; BAI: Beck Anxiety Inventory; VAS: Visual Analogue Scale; T1: time of the Netherlands Study of Depression and Anxiety baseline interview; T2: time of Magnetic Resonance Imaging session; # volumes: number of volumes acquired during the word classification task; # words_pos/neg/neu: number of words classified as positive, negative, or neutral; rt pos/neg/neu; response time of classifying positive/negative/neutral words.

		MDD (n=25)	HC (n=25)
female	n	16	17
amc/lumc/umcg	n	8/10/7	8/10/7
age	years; mean (sd)	33.9 (9.9)	37.2 (10)
education	years; mean (sd)	12.9 (2.6)	13.8 (2.3)
recurrent MDD	N	15	-
age of onset	years; mean (sd)	25 (11)	-
MADRS	mean (sd)	14.4 (10.2)	.9 (1.7)
	range	0 - 33	0 - 6
IDS_T2	mean (sd)	19.2 (11.9)	3.8 (3.3)
	range	2 - 39	0 - 11
IDS_T1	mean (sd)	28.5 (10.5	5.3 (2.8)
	range	2 -47	0 - 10
BAI	mean (sd)	8.5 (7)	2.3 (2.2)
	range	0 - 26	0 - 8
VAS	mean (sd)	24.8 (20.4)	24 (22.3)
	range	0 - 65	0 - 80
# volumes	mean (sd)	168.8 (18.8)	163.9 (22.3)
	range	128 - 207	138 - 240
classification behavior			
# words_pos	mean (sd)	37.6 (9.5)	44.6 (9.7)
# words_neg	mean (sd)	40 (2.5)	39.9 (6.8)
# words_neu	mean (sd)	48 (10.2)	40 (9.8)
rt pos	sec; mean (sd)	1.52 (.33)	1.36 (.28)
rt neg	sec; mean (sd)	1.28 (.27)	1.27 (.44)
rt neu	sec; mean (sd)	1.66 (.39)	1.55 (.36)

FIGURE 1:
plots showing mean and standard errors for number of words classified as positive, neutral or negative (top), and response times in seconds during classifying words as positive, negative, and neutral (bottom).





We then explored whether connectivity was related to word classification behavior and response times. Within MDD, connectivity of the medial PFC with the salience network was positively correlated with the number of words classified as positive (rKendall's tau=.33, p=.03). Connectivity in the fronto-striatal areas was unrelated to response times during classification of positive, neutral and negative words ($r_{Kendall's\ tau}$<.18, p>.25).

No significant between-group differences were observed for the other networks.

FIGURE 2:
SALIENCE NETWORK ACROSS PATIENTS AND CONTROLS AND BETWEEN GROUP DIFFERENCES

A) mean component across patients and controls together; B) differences between MDD and HC within the salience network showing decreased medial PFC, caudate nucleus, and nucleus accumbens connectivity in the MDD group. Results are displayed at $p<.05$, family wise error corrected. Right figures: mean and 95% confidence interval plots showing the mean connectivity strength in the nucleus accumbens (upper plot), caudate nucleus (middle plot), and medial PFC (bottom plot).
A full-color image can be found on the supplementary sheet.

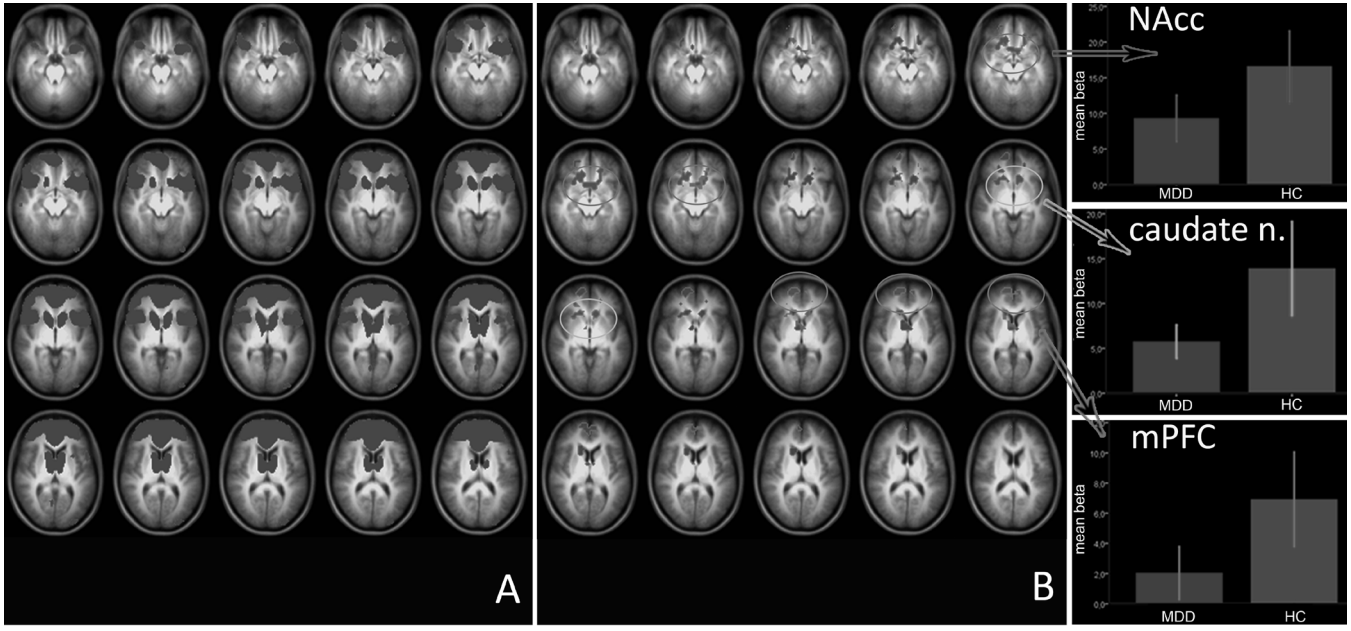


TABLE 2:
EFFECT OF DIAGNOSIS ON CONNECTIVITY WITH THE SALIENCE NETWORK: MDD<HC

L=left hemisphere; R=right hemisphere; MNI: Montreal Neurological Institute coordinate system. Results are reported at p<.05, Family wise error corrected for multiple comparisons using permutation tests (# permutations=5000) of cluster-mass (voxelwise threshold: z=2.3).

Location		Center of Gravity (MNI)			No. of voxels
		x	y	z	
R	orbitofrontal cortex	16	24	-4	102
L	nucleus accumbens	-12	20	-8	70
R	medial prefrontal gyrus, frontal poles	8	60	4	63
R	caudate nucleus	4	4	6	20
R	frontal pole/ventrolateral prefrontal gyrus	24	50	-12	7
L	medial/superior prefrontal gyrus	-20	44	0	3
R	ventral caudate nucleus	8	20	-4	1
R	putamen	20	12	-8	1

DISCUSSION

In this study we investigated whole-brain functional connectivity in MDD during execution of an emotional word classification task using a subset of eight independently derived independent component networks. We demonstrated decreased functional connectivity of the medial PFC, putamen, caudate nucleus, OFC, and ventral striatum (including the nucleus accumbens) within the salience network in MDD as compared with healthy control participants. Results were unexplained by age, education, sex, and brain volume of regions implicated in the neuroanatomical profile of MDD. Moreover, within MDD, decreased functional connectivity of the fronto-striatal regions was unrelated to illness severity. No differences in functional connectivity were observed within any other network.

These results confirm the hypothesis that abnormal intrinsic connectivity of cortical and subcortical and (para)limbic regions during emotional processing is part of the functional pathophysiology of MDD. Our results indicate that this decreased coupling might not be a state phenomenon of MDD, but appears to be a trait-like phenomenon that continues into the (newly) remitted phase. Moreover, the decreased subcortical-cortical coupling was observed within the salience network, a network that has been found to specifically correlate with subjective emotional ratings (Seeley et al., 2007).Furthermore, the salience network is the only work of the Smith et al. (2009) study that showed a high load on emotional processes and tasks in a large scale meta-analysis of the BrainMap database, but also loads on sensory perception, including pain perception, working memory, explicit memory, cognition, and action inhibition (Smith et al., 2009). These processes are highly relevant for MDD symptoms such as sad mood, failure to stop negative thoughts, attentional deficits, and altered bodily sensations related to appetite and temperature.

Interestingly, we observed relative decoupling of the medial prefrontal cortex and striatum within the salience network in MDD. The medial prefrontal cortex is thought to be an important region for regulating emotional states through cognitive appraisal (Johnstone et al., 2007; Ochsner et al., 2009), self-referential processing (Lemogne et al., 2009), and affective conflict processing (Ochsner, Hughes, Robertson, Cooper, & Gabrieli, 2009). Moreover, the strength of connectivity in the medial prefrontal cortex and striatum was associated with positive word evaluation. Therefore, our findings indicate that the distributed network involved in cognitive appraisal, self-referential processing, and affective conflict processing is compromised in MDD, potentially related to the processing of positive information. The medial PFC has numerous connections with other regions that have been implicated in normal and abnormal mood regulation, including the striatum, thalamus, pallidum, and dorsal cortical areas, and therefore it has been suggested that the medial PFC may play an important role in moderating visceral processes related to emotions (Price & Drevets, 2010). Also, the nucleus accumbens, a part of the ventral striatum, showed lower connectivity with the salience network. This region receives projections from the amygdala, and further projects to the medial PFC and thalamus (Price & Drevets, 2010), and has been repeatedly associated with reward processing. Moreover, a failure to sustain activation is this region during upregulation of positive emotional states has been linked to anhedonia in MDD patients (Heller et al., 2009). This finding corroborates the present observation of diminished connectivity of the striatum with the salience network that was associated with positive word evaluation and suggests that decreased fronto-striatal coupling with the salience network may be specific to lacking a positive mood state. However, we were not able to investigate whether para(limbic)–cortical connectivity varied as a function of hedonic capacity or current mood state, as we did not include a measure specifically probing current mood state and anhedonia.

The present results are in concordance with the low-frequency seed-based correlation analysis of Anand et al. (2005), who showed decreased limbic-cortical connectivity of the pallido-striatum with the ACC during positive, negative, and neutral picture viewing in MDD. However, in this seed-based analysis, time series of only four seeds were included in the analysis and therefore connectivity of the striatal regions with other prefrontal regions could not be studied. Our results indicate that the decreased connectivity of the striatal regions during positive, negative, and neutral word classification extends to the dorsolateral PFC, inferior frontal gyrus, and dorsomedial PFC. Similar results have been observed in a small adolescent MDD sample during resting-state imaging, in which decreased connectivity of the subgenual ACC and a network including the rostral ACC, inferior frontal gyrus, medial frontal pole, and dorsolateral cortical areas was demonstrated, independent of illness severity (Cullen et al., 2009).

In the present study we could not replicate findings of decreased connectivity

of the amygdala with hippocampal regions, or with orbitofrontal or medial PFC regions that have been observed in both task- and resting-state connectivity analyses (Johnstone et al., 2007; Hamilton & Gotlib, 2008; Matthews, Strigo, Simmons, Yang, & Paulus, 2008; Almeida et al., 2009; Lui et al., 2009; Veer et al., 2010). This discrepancy in findings could be the result of the template ICN method used. The independently derived ICNs used in this study did not include limbic regions and therefore detecting connectivity differences between (para)limbic and prefrontal regions is less likely than studies that use a limbic seed region. However, synchronicity between posterior visual regions and the amygdala was observed in the mean spatial maps of the lateral visual component across participants from both groups, but no differences were observed in this component, consistent with previous reports (Anand et al., 2005; Cullen et al., 2009).

In the present study, we used a new approach to study functional connectivity during the execution of an emotional word classification task. We used template ICNs to create subject specific component maps, based on the times series that best fit the weighted templates using the dual regression approach (Beckmann et al., 2009). This template-based dual regression approach has the advantage that templates are acquired independently of the data under study (Kriegeskorte et al., 2009). These template components have been found to be highly consistent and have been independently detected in a number of populations (e.g., Alzheimer's disease (Rombouts et al., 2009), MDD (Veer et al., 2010), healthy controls (Beckmann et al., 2005)). Overall, use of the template ICNs and dual regression approach resulted in highly consistent components across groups, even though the number of acquired brain volumes differed between participants. Indeed, a strength of this approach is that it is not limited to standard fMRI task paradigms with associated restrictions on the timing and number of trials, but can be used to study sustained engagement of brain networks over the duration of a task. Another advantage is that the template-ICN dual regression method allows for the study of connectivity across groups and tasks in a standardized and unbiased way, which is crucial for studies involving comparisons between clinical and non-clinical samples. Also, using the template ICNs should increase reproducibility of effects, due to the independence of the ICN templates in reference to the data under study. Taken together, we believe that this template-based dual regression method is a potent approach to study differences in functional connectivity during both rest and task paradigms, particularly when comparing across patient groups (or scan sessions in treatment studies) when an unbiased and reliable comparison is crucial.

Using an unbiased whole-brain functional connectivity approach, we showed decreased connectivity of medial prefromtal regions and ventral subcortical and paralimbic regions in MDD during the execution of an emotional word evaluation task with a distributed the salience network. Future studies should test the specificity and generalizability of our results to different MDD

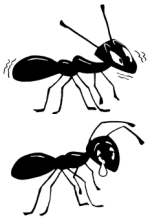
sub-samples and different tasks. For example, medial PFC connectivity with the salience network was unrelated to illness severity but was related to the number of words as 'positive'. This finding might suggest that decreased fronto-striatal coupling within the salience network may be specific to lacking a positive mood state. Given the power of this technique to compare distributed patterns of connectivity in an unbiased way, future studies should also examine treatment-related changes of connectivity with the salience network.

ACKNOWLEDGEMENT

The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Quality of Healthcare (IQ healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute). S.A.R.B. Rombouts is the recipient of a (VIDI) grant from The Netherlands Organization for Scientific Research (NWO). The Erasmus staff exchange programme is acknowledged for making the collaboration between M.J. van Tol, Leiden University Medical Center, and T. Johnstone (University of Reading) possible. M.J. van Tol had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PARTICIPANTS

Participants were recruited from NESDA (Netherlands Study of Depression and Anxiety), a large-scale multi-site longitudinal observational cohort study. The rationale, methods, and recruitment have been described in detail elsewhere. (Penninx et al., 2008) In short, NESDA has been designed to be representative of those with depressive and anxiety disorders in different health care settings and stages of the developmental history. Therefore, the sample is stratified for setting (community, primary care, and specialized mental health) and set up to include a range of psychopathology. Out of the 2981 NESDA respondents (main sample, T1), participants aged between 18 and 57 years were asked to participate in the NESDA neuroimaging study (T2; N=301)) if they met DSM-IV criteria for a half-year diagnosis of MDD and/or anxiety disorder (Panic Disorder, Social Anxiety Disorder, or Generalized Anxiety Disorder), or no lifetime DSM-IV diagnosis (i.e. healthy controls [HC]). Personality disorders were not obtained/screened for and so were not used in the inclusion/exclusion criteria, although persons with known personality disorders (either reported by psychiatrist or participant) were not included in the NESDA sample. The total MRI sample is described in detail elsewhere (van Tol et al., 2010).



CHAPTER 6

REDUCED MEDIAL PREFRONTAL CORTEX VOLUME IN ADULTS REPORTING CHILDHOOD EMOTIONAL MALTREATMENT

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BIOLOGICAL PSYCHIATRY, 2010; 68(9): 832-838. Published as *van Harmelen, van Tol et al. (2010)*

SUMMARY

Background: Childhood emotional maltreatment (CEM) has been associated with a profound and enduring negative impact on behavioral and emotional functioning. Animal models have shown that adverse rearing conditions, such as maternal separation, can induce a cascade of long-term structural alterations in the brain, particularly in the hippocampus, amygdala, and prefrontal cortex. However, in humans, the neurobiological correlates of CEM are unknown.

Methods: Using high-resolution T1-weighted 3T magnetic resonance imaging, anatomical scans and a whole-brain optimized voxel-based morphometry approach, we examined whether healthy control subjects and unmedicated patients with depression and/or anxiety disorders reporting CEM before age 16 (n= 84; age: mean= 38.7) displayed structural brain changes compared with control subjects and patients who reported no childhood abuse (n= 97; age: mean= 36.6).

Results: We found that self-reported CEM is associated with a significant reduction in predominantly left dorsal medial prefrontal cortex volume, even in the absence of physical or sexual abuse during childhood. In addition, reduced dorsal medial prefrontal cortex in individuals reporting CEM is present in males and females, independent of concomitant psychopathology.

Conclusions: In this study, we show that CEM is associated with profound reductions of medial prefrontal cortex volume, suggesting that sustained inhibition of growth or structural damage can occur after exposure to CEM. Given the important role of the dorsal medial prefrontal cortex in the regulation of emotional behavior, our finding might provide an important link in understanding the increased emotional sensitivity in individuals reporting CEM.

INTRODUCTION

Every year, approximately one in ten children growing up in Western societies experiences childhood emotional maltreatment (CEM) (Gilbert et al., 2009). Emotional maltreatment encompasses any act of commission (i.e., verbal abuse) or omission (i.e., emotional neglect) that is (potentially) harmful, or insensitive to the child's emotional development and has been associated with a profound and enduring negative impact on behavioral, emotional, and social functioning (Egeland, 2009; Gilbert et al.,2009). For instance, CEM is associated with maladaptive emotional functioning in adulthood (Teicher, Samson, Polcari, & McGreenery, 2006), which in turn is a key vulnerability factor for the development of psychiatric disorders when faced with stressors in later life (Beck, 2008). In line with this, CEM is an important predictor for the development of depressive and anxiety disorders in adulthood (Gibb, Chelminski, & Zimmerman, 2007; Spinhoven et al., 2010). However, the neurobiological correlates underlying the emotional sensitivity in individuals reporting CEM remain unknown.

In animals, adverse rearing environments such as maternal separation, loss, or isolation rearing induce a cascade of long-term alterations on the level of cognitive functioning, hypothalamic-pituitary-adrenal axis functioning, (immediate) gene expression, and brain morphology (Sanchez, Ladd, & Plotsky, 2001). Structural alterations in the brain include reduced dendrite length, dendritic branching, spine density, and suppression of neurogenesis and have predominantly been observed in limbic structures (amygdala, hippocampus) and prefrontal cortex (PFC) (Arnsten, 2009; Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 2008; Sanchez et al., 2001). In line with this, human studies examining the neuroanatomical correlates of childhood maltreatment in adults found decreased gray matter volume in the hippocampus (Kitayama, Vaccarino, Kutner, Weiss, & Bremner, 2005; Vythilingam et al., 2002) and medial PFC (Andersen et al., 2008; Cohen et al., 2006; Frodl, Reinhold, Koutsouleris, Reiser, & Meisenzahl, 2010; Tomoda et al., 2009; Treadway et al., 2009). However, these studies focused mainly on the impact of sexual (Andersen et al., 2008; Kitayama et al., 2005; Vythilingam et al., 2002) and/or physical abuse (Tomoda et al., 2009) or did not exclude co-occurence of different types of abuse (Cohen et al., 2006; Frodl et al., 2010; Treadway et al., 2009).

One way through which chronic stress may lead to structural changes is through enhanced activation of neuroendocrine systems (McEwen, 2008). During chronic stress, increased secretion of glucocorticoids (i.e., the stress hormone cortisol in humans) interferes with the transcriptional mechanisms that control the expression of brain-derived neurotrophic factor (BDNF), a growth factor that has been linked to neuronal proliferation and plasticity (McEwen, 2008; Nestler et al., 2002). In this way, chronic stress may inhibit cytogenesis and increase vulnerability to attrition within the hippocampus, amygdala, or PFC (Lupien et al., 2009; McEwen, 2008). In line with these findings, childhood maltreatment has been linked to enhanced cortisol reactivity to psychosocial

stress in patients with depression and anxiety disorders (Elzinga, Spinhoven, Berretty, de Jong, & Roelofs, 2010; Heim et al., 2000; Heim et al., 2002) and to blunted cortisol reactivity in healthy subjects (Carpenter et al., 2007; Elzinga et al., 2008). Additionally, altered patterns of cortisol reactivity during stress have been found in individuals reporting CEM (Carpenter et al., 2009). Furthermore, white matter tract abnormalities were found in a small sample of young adults reporting CEM (n =16) (Carpenter et al., 2009; Choi, Jeong, Rohan, Polcari, & Teicher, 2009). However, it is unknown whether CEM is similarly associated with structural gray matter abnormalities in adulthood. Given the important role of the limbic brain regions (hippocampus and amygdala) and the medial PFC in the perception and regulation of emotional behavior and stress response (Arnsten, 2009; Cardinal, Parkinson, Hall, & Everitt, 2002; Lupien et al., 2009; McEwen, 2008; Sanchez et al., 2001), gray matter abnormalities in (one of) these regions might underlie the maladaptive emotional functioning associated with CEM.

Therefore, we sought to investigate the effect of CEM on adult brain morphology in unmedicated patients currently diagnosed with depression and/or anxiety disorder controls. We used high-resolution magnetic resonance imaging (MRI) and a whole-brain optimized voxel-based morphometry (VMB) analysis approach, specifying the amygdala, hippocampus, and medial PFC (including the anterior cingulate gyrus) as regions of interest. We examined whether adult patients and controls reporting multiple incidents of CEM before age 16 (n= 84) displayed structural brain changes in compared with patients and controls who did not report a history of childhood abuse (n= 97). In addition, to examine whether these structural brain changes are related to the development of psychopathology, we investigated whether these brain alterations were more apparent in individuals with a depression or anxiety disorder compared with individuals who never developed a depression or anxiety disorder.

METHODS

PARTICIPANTS

Participants were drawn from the Netherlands Study of Depression and Anxiety (NESDA), (n= 2981), a large cohort study (Penninx et al., 2008). A subset of the NESDA participants (both patients and controls) was selected to undergo MRI scanning for the NESDA MRI study. Inclusion criteria for patients in the NESDA-MRI study were current major depressive disorder (MDD) and/or anxiety disorder (ANX; panic disorder and/or social anxiety disorder and/or generalized anxiety disorder in the past six months according to DSM-IV criteria). Diagnoses were established using the structured Composite International Diagnostic Interview (Robins et al., 1988), administered by a trained interviewer. Exclusion criteria were the presence of Axis I disorders other than MDD, panic disorder, social anxiety disorder, or generalized anxiety disorder; any use of psychotropic medication other than a stable use of selective serotonin reuptake inhibitors or infrequent benzodiazepine use (three times two tablets weekly or within 48 hours before scanning). Additional exclusion criteria for both patients and

controls were the presence or history of major internal orneurological disorder; dependency or recent abuse (past year) of alcohol or drugs; hypertension (>180/130 mmHg); heavy smoker (>5 cigarettes/day); and general MRI contraindications. The healthy control participants had no lifetime depressive or anxiety disorders and were not taking any psychotropic drugs. Thus, 301 native Dutch-speaking participants (233 patients and 68 controls) were included and underwent MRI at one of the three participating centers (i.e., Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen). The ethical review boards of each participating center approved this study. All participants provided written informed consent.

CLINICAL ASSESSMENTS

In the NESDA study, childhood maltreatment was assessed with the Nemesis Trauma Interview (De, Bijl, Smit, Vollebergh, & Spijker, 2002). In this interview, respondents were asked whether they had experienced emotional neglect, psychological abuse, physical abuse, and/or sexual abuse before age 16; how often this had occurred (i.e., never, once, sometimes, regularly, often, or very often); and what their relationship with the perpetrator was. Emotional neglect was described as "people at home didn't listen to you, your problems were ignored, and you felt unable to find any attention or support from the people in your house". Psychological abuse was described as "you were cursed at, unjustly punished, your brothers and sisters were favored—but no bodily harm was done". CEM was defined as multiple incidents (more than once) of emotional neglect and/or emotional abuse before age 16 years, because we assumed that only multiple incidents of emotional abuse and/or emotional neglect might be associated with neuroanatomical changes. Overall CEM frequency was defined as the most frequent occurrence as reported (e.g., if psychological abuse occurred often and emotional neglect sometimes, overall CEM score is often). Negative life events were assessed using the List of Threatening Events Questionnaire (LTE-Q) (Brugha, Bebbington, Tennant, & Hurry, 1985). In addition, at the day of scanning, depression and anxiety severity was measured using the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery & Åsberg, 1979) and the Beck Anxiety Inventory (BAI) (Beck et al., 1988).

ADDITIONAL EXCLUSION CRITERIA

High-resolution anatomical images were obtained from 291 participants (imaging data from 10 participants were excluded because of poor image quality). Additionally, two healthy control subjects were excluded from the NESDA-MRI study because of MADRS scores that indicated possible depressive symptomatology on the day of scanning (MADRS>8) (Muller et al., 2000). For this study, individuals using selective serotonin reuptake inhibitors were excluded (n= 79) given their potential effect on neuronal plasticity (Dranovsky & Hen, 2006). Additionally, individuals reporting physical or sexual abuse but

no CEM were also excluded (n= 5). Finally, individuals reporting only a single incident of CEM (n= 24) were excluded. Our final sample (n= 181) consisted of 84 participants re- porting CEM and 97 participants who reported no abuse (the No Abuse group).

THE CEM AND NO ABUSE GROUPS

The CEM group consisted of participants who reported emotional neglect and/or psychological abuse during childhood that had occurred sometimes, regularly, often, or very often (n= 84: i.e., MDD, n= 20; ANX, n= 21; comorbid depression and anxiety disorder [CDA], n= 30), and controls, n= 13; 36 of these 84 participants also reported childhood physical and/or sexual abuse, Table 1). The No Abuse group consisted of individuals who did not report CEM, physical abuse, or sexual abuse (n= 97: i.e., MDD, n= 22; ANX, n= 22; CDA, n= 13; and controls, n= 40). In the CEM group, 96.4% (n= 81) of the participants reported having been emotionally neglected, whereas 57.1% (n= 48) reported having been psychologically abused, and 54% (n= 45) reported both emotional neglect and psychological abuse. In addition, 97.6% reported that the individual's biological parents were the perpetrators of CEM (n= 82).

MAGNETIC RESONANCE IMAGING ACQUISITION

Imaging data were acquired using Philips 3-tesla MR systems (Philips, Best, The Netherlands) located at the participating centers, equipped with a SENSE-8 (Leiden University Medical Center and University Medical Center Groningen) and a SENSE-6 (Academic Medical Center) channel head coil. For each subject, an anatomical image was obtained using a sagittal 3-dimensional gradient-echo T1-weighted sequence (repetition time: 9 msec; echo time: 3.5 msec; matrix 256x256; voxel size: 1x1x1mm; 170 slices). Each scanning session also included several functional MRI runs, both "resting state" and task related. These results, as well as those of VBM comparisons between diagnostic groups (irrespective of childhood maltreatment), have been reported elsewhere (van Tol et al., 2010).

IMAGE PROCESSING

An optimized VBM approach following the Diffeomorphic Anatomical Registration through Exponentiated Lie algebra (DARTEL) (Ashburner, 2007) was performed using SPM5 (Statistical Parametric Mapping software; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 7.1.0 (The MathWorks Inc., Natick, MA, USA). VBM-DARTEL preprocessing included the following steps: 1) manual reorientation of the images to the anterior commissure; 2) segmentation of the anatomical images using the standard segmentation option implemented in SPM5; 3) applying the DARTEL approach for registration, normalization, and modulation, leaving the images in DARTEL space (In this approach, a DARTEL template is created based on the deformation fields that are produced by the segmentation procedure. Next, all individual deformation

TABLE 1:
CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS REPORTING CHILDHOOD EMOTIONAL MALTREATMENT VS. NO ABUSE

Childhood Emotional Maltreatment (CEM), ANX=Anxiety Disorder(s), MDD=Major Depressive Disorder, CDA=Comorbid Depression Anxiety, A=Academic Medical Center Amsterdam, L=Leiden University Medical Center, G=University Medical Center Groningen, MADRS=Montgomery Asberg Depression Rating Scale, BAI=Beck Anxiety Inventory, U, X2=Mann Whitney U, and Chi-square test statistics of CEM vs No Abuse comparisons, SEM=Standard Error of Mean.

		No Abuse (*n* =97)	CEM (*n* =84)	*U*	X^2	*p*
Gender	% M/F	33/67	34.5/65.5		.05	.83
Age	Mean (SEM)	36.57 (1.09)	38.68 (1.09)	3612		.19
Education	Mean (SEM)	13.27 (0.29)	12.81 (0.35)	3706.5		.29
Handedness	% L/R	11/89	5/95		2.56	.11
Current diagnosis	*n* MDD	22	20		.09	.76
	n ANX	22	21		.02	.88
	n CDA	12	30		6.72	.01
	n HC	40	13		13.75	.00
Liefetime diagnosis	% MDD	43.29	77.38		25.64	.00
	% ANX	41.24	67.86		12.83	.00
MADRS	Mean (SEM)	7.10 (.94)	14.45 (1.89)	2272.5		.00
BAI	Mean (SEM)	8.79 (1.04)	12.85 (1.08)	2651.5		.00
Scan location	% A/L/G*	28.9 /41.2/29.9	38.1/38.1/23.8		1.89	.39
Concurrent abuse	*n* Physical	0	15			
	n Sexual abuse	0	8			
	n Physical and Sexual abuse	0	13			
Gray Matter	Mean (SEM)	740.40 (7.98)	721.78 (7.33)	3604		.18
White Matter	Mean (SEM)	491.33 (6.94)	494.69 (6.53)	3884		.59

fields are warped (and modulated) to match this template.), and 4) smoothing of the gray matter and white matter images using an 8-mm, full-width-at-half-maximum Gaussian kernel to increase the signal-to-noise ratio. In the resulting gray matter images, each voxel represents an absolute amount of gray matter volume, equivalent to the gray matter volume per unit before normalization.

VBM ANALYSIS

Gray matter (or white matter) segments in native space were used to calculate absolute total gray matter (white matter) volumes. Next, smoothed gray matter (white matter) density images were entered into a voxel-by-voxel analysis of variance for between-group comparisons, with age and total absolute gray matter (white matter) as covariate to correct for total brain volume. Effect of center was added by means of two dummy variables as extra regressors in all analyses. To achieve maximal sensitivity and to optimize voxel residual smoothness estimation and exclude false positives in non-gray matter (-white matter) tissue, voxelwise comparisons were masked using a comparison-specific explicit optimal threshold gray matter (white matter) mask created using the Masking toolbox (http://www.cs.ucl.ac.uk/staff/g.ridgway/masking) (Ridgway et al., 2009).

For the a priori–set regions of interest (medial PFC, anterior cingulate cortex, amygdala, and hippocampus), we set a threshold of $p<.001$, uncorrected, with a spatial extent threshold of 50 contiguous voxels for group interactions. To further protect against Type I error, small volume correction was applied by centering a sphere of 16-mm around the peak voxel. The resulting volumes of interest had to meet $p<.05$, corrected for family wise error (FWE) rate at the voxel level, to be considered significant (i.e., $Z>3.50$). For regions not specified a priori, a voxel level threshold of $p<.05$ whole brain, FWE corrected, was set. If significant group differences were observed in the VBM analysis, we exported the volume of the significant clusters per subject to SPSS. Clinical and demographic group differences were analyzed using SPSS-17 (http://www.spss.com), and in all analyses, age, total gray matter (white matter) volume, and dummy regressors for the scan centers were included as covariates.

RESULTS

THE NEUROANATOMICAL CORRELATES OF CEM

To investigate the neuroanatomical correlates of CEM, we first set up a VBM analysis to compare the gray matter density maps/images of individuals reporting CEM (n= 84) with gray matter density maps of the No Abuse group (n= 97). These analyses revealed that CEM was associated with a 5.14% reduction in the left dorsal medial PFC ([x=-11, y=23, z=40], Brodmann area 8, cluster size/number of voxels (K)= 263, Z =3.80, $p< .05$ (small volume corrected; Table 2)).No significant differences were observed in hippocampus or amygdala or in other brain regions. Only at a very low threshold was CEM associated with reduced

right posterior hippocampal volume ([x=29; y=-35, z=-6], Z= 2.06, ns). Additionally, CEM was not associated with a significant increase in regional gray matter volumes. Finally, CEM was not associated with white matter reductions in or surrounding our regions of interest or with increased regional white matter volume.

To explore possible interactions among CEM, current diagnosis, and sex, a 2 (CEM) x 4 (diagnosis: MDD, ANX, CDA, and controls) x 2 (sex) univariate analysis of covariance (ANCOVA) was performed, with local dorsal medial PFC volume (milliliters [ml]) as a dependent factor. Again, individuals from the CEM group had smaller dorsal medial PFC volumes than the No Abuse group ([CEM (M ± SEM)]: 4.80 ± .06 ml vs. no abuse: 5.06 ± .06 ml; $F_{1,161}$ = 12.36, p< .01, Cohen's d= .53). Interestingly, there was no interaction between CEM and diagnosis ($F_{3,161}$ = .45, p= .72, d= .01), and post hoc analyses indicated that the dorsal medial PFC reductions are present in all groups, even though the numbers were relatively small for such comparisons (one-sided: MDD, $F_{1,34}$ = 8.65, p< .01, d= .93; ANX, $F_{1,35}$= 2.55, p=.06, d= .50; CDA, $F_{1,35}$= 2.63, p=.06, d= 55; and controls, $F_{1,45}$= 1.85, p= .09, d=.44). In addition, there was no interaction between CEM and sex ($F_{1,161}$= 2.01, p= .99, d= .21). Taken together, these results indicate that reduced dorsal medial PFC volume was present in all CEM groups (i.e., male and female subjects with MDD, ANX, CDA, and in the control group). Moreover, similar results were obtained when depression and anxiety severity were added as covariates ($F_{1,155}$= 12.41, p< .01, d= .53)[1], indicating that our results cannot be explained by the presence of more severe depressive and/or anxious symptomatology among individuals reporting CEM.

1 Four participants were missing because of incomplete depression or anxiety data (one reported CEM).

NEUROANATOMICAL CORRELATES OF ISOLATED EMOTIONAL MALTREATMENT IN CHILDHOOD

To exclude the possibility that our results are driven by concurrent history of physical and/or sexual abuse in some of the participants, we conducted a whole-brain VBM analysis to compare the gray matter density maps of individuals reporting only CEM (n= 48, i.e. MDD, n= 13, ANX, n= 12, CDA, n= 13, and controls, n= 10; Table S-1 in the supplemental material 1) with individuals reporting No Abuse (n= 97). n this analysis, the 36 individuals who also reported childhood physical and/or sexual abuse were excluded. This analysis showed that individuals reporting only CEM had a volume reduction of 7.2% in left and right (although predominantly left) dorsal medial mPFC ([x=-11, y=21, z=40], Brodmann's area 8, K= 767, Z= 4.37, p< .05 [small volume corrected]; Table 2), extending into the anterior medial PFC and anterior cingulate gyrus (Figure 1, Table 2). Furthermore, no hippocampal, or amygdalar differences were observed, nor decreases in other brain regions.

Again, only at a very low threshold was CEM associated with reduced right posterior hippocampal volume ([x=29, y=-35, z=-6], Z= 2.45, ns). Finally, CEM was not associated with white matter reductions in or surrounding our regions

TABLE 2:
THE NEUROANATOMICAL CORRELATES OF CHILDHOOD EMOTIONAL MALTREATMENT VS. NO ABUSE

Childhood Emotional Maltreatment (CEM), R=right sided, L=left sided, BA=Brodmann Area, K=cluster size (number of voxels), **p<.05, SVC 16 mm FWE corrected.

CEM (n=84) < No Abuse (n=97)

				DARTEL-coordinate			
R/L	BA	region	k	x	y	z	z-value
L	8	Medial prefrontal gyrus	263	-11	23	40	3.80 **
L+R	8	medial prefrontal gyrus	767	-11	21	40	4.37 **
				-5	9	42	

Only CEM (n=48) < No abuse (n=97)

				DARTEL-coordinate			
R/L	BA	region	k	x	y	z	z-value
R	9/32	cingulate gyrus/medial prefrontal gyrus	87	6	18	36	
R	10/32	cingulate gyrus/medial prefrontal gyrus		9	44	16	3.53 **
				11	47	9	

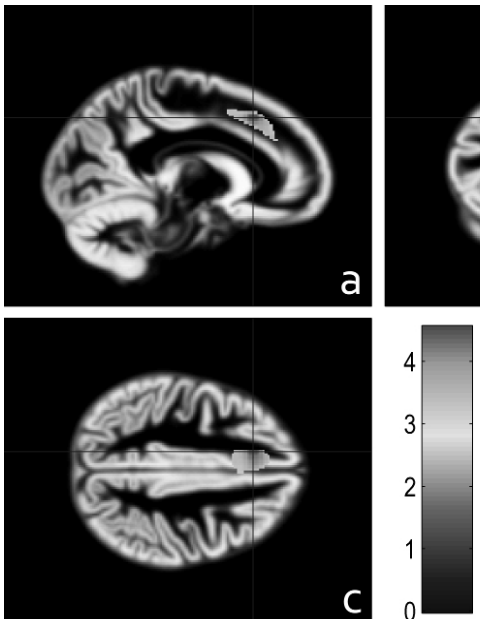


FIGURE 1 :

The medial PFC region showing 7.2 % volume reduction amongst individuals reporting only childhood emotional maltreatment displayed on a sagittal (A),coronal (B) and transversal (C) plane.

A full-color image can be found on the supplementary sheet.

of interest or with increased regional gray matter of white matter volume.

An ANCOVA confirmed the main effect of CEM (CEM: M ± SEM: 4.78 ± .07 ml; No Abuse: 5.15 ± .06 ml; $F_{1,125}$= 15.15, p< .001, d= .69). Moreover, the reduced dorsal medial PFC volume was present in all CEM groups and in both male and female individuals (i.e., no interaction was found with diagnosis; $F_{3,125}$ = .27, p=.85, d= .09), and even within these small groups, post hoc analyses revealed a (marginally) significant (one-sided) impact of CEM only on dorsal medial PFC volume (MDD, $F_{1,27}$= 7.72, p<.01, d=1; ANX, $F_{1,26}$=2.93, p<.05. d=.63; CDA, $F_{1,18}$= 3.20, p< .05, d= .73; and controls, $F_{1,42}$= 1.96, p= .08, d= .51), and CEM did not interact with sex ($F_{1,125}$=.08, p=.78, d= .05). Additionally, these results could not be explained by higher symptom severity among individuals reporting CEM because similar results were obtained when depression and anxiety severity were added as covariates ($F_{1,116}$= 15.72, p< .001, d= .70)[2].

2 History of alcoholism (abuse or dependence as measured with the Composite International Diagnostic Interview) did not affect the results (i.e., history of alcoholism [yes or no] was not a significant covariate in the analysis), F1,124= 1.76, P=.19, nor did it have an impact on the main effect of CEM on dorsal medial PFC volume, F1,124= 15.98, p< .001, d= .71).

ASSOCIATIONS BETWEEN FREQUENCY OF EMOTIONAL MALTREATMENT AND MPFC VOLUME

To investigate whether the dorsal medial PFC volume reductions were dependent on CEM frequency, we performed a 5 (frequency of CEM: No Abuse, sometimes, regularly, often, and very often) x 4 (diagnosis: MDD, ANX, CDA, and controls) ANCOVA, with local dorsal medial PFC volume (ml) as a dependent factor. The analysis revealed a main effect of frequency of CEM on dorsal medial PFC volume [$F_{4,120}$= 4.89, p< .001, d= .39][3] which did not interact with psychopathology [$F_{12,120}$= .93, p= .52, d= .17]. As is illustrated in Figure 2, dorsal medial PFC volumes were reduced in individuals who reported that CEM happened regularly or more often. Individuals reporting CEM occurred "sometimes" did not have a significantly lower dorsal medial PFC volume compared with the no abuse group; however, this group was extremely small (n= 4), and therefore caution is warranted when interpreting the findings of these individuals.

3 Exclusion of the "sometimes" group (n= 4), due to its small size, did not affect the results, including the main effect of CEM on dorsal medial PFC volume [F1,120= 15.8, p<.001, d= .73].

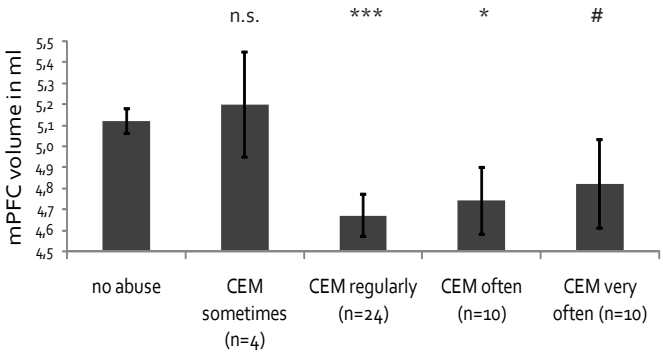


FIGURE 2:

Estimated Marginal Means and Standard Error of Mean (SEM) of medial PreFrontal Cortex (mPFC) volume amongst the different frequencies of Childhood Emotional Maltreatment (CEM), and contrast results of EM frequencies vs. the No Abuse group. ***= p <.000, * = p<.05, # = p =.056, n.s. = not significant (two-sided).

In this study, self-reported CEM was found to be associated with a significant reduction in predominantly left dorsal medial PFC gray matter volume, independent of sex, and psychiatric status, at least in individuals who reported CEM occurring regularly or more frequently. Furthermore, the dorsal medial PFC gray matter volume reduction was not due to concomitant childhood physical and/or sexual abuse, because the reductions were also found when CEM was experienced in absence of concurrent childhood physical and/or sexual abuse. These findings provide an important clinical extension of preclinical observations that the dorsal medial PFC is highly sensitive to the effects of chronic stress in childhood (Arnsten, 2009; Lupien et al., 2009; Sanchez et al., 2001). The medial PFC is one of the brain regions that undergo major developmental changes during childhood and adolescence (Lupien et al., 2009; Sanchez et al., 2001)). Exposure to emotionally abusive episodes during this developmental period may increase secretion of glucocorticoids, which may interfere with the transcriptional mechanisms that control the expression of brain-derived neurotrophic factor and may thereby inhibit cytogenesis and increase vulnerability to attrition within the medial PFC (Arnsten, 2009; Heim et al., 2000; Lupien et al., 2009; Sanchez et al., 2001). Moreover, the fact that reductions in hippocampal volume were only observed at a very low threshold and no significant changes were observed in the amygdala, concurs with findings of animal models on isolation rearing or maternal separation that indicate a specific and profound impact on the medial PFC (Levine et al., 2008; Sanchez et al., 2007), in comparison with the hippocampus and amygdala (Schubert, Porkess, Dashdorj, Fone, & Auer, 2009). For example, in animals, it has been shown that architectural changes in prefrontal dendrites can be observed after only one week of stress or even after a single stressful incident (Arnsten, 2009). In contrast, structural changes in the hippocampus only appear after several weeks of stress, which might be an indication that the medial PFC is more sensitive to the detrimental effects of stress (Arnsten, 2009).

The finding that CEM is associated with (predominantly left) dorsal medial PFC reduction is of particular interest considering that the medial PFC plays an important role in emotion regulation (Cardinal et al., 2002; Phillips et al., 2003a). Moreover, reduced activity in the left PFC in particular has been associated with negative emotional states (Demaree, Everhart, Youngstrom, & Harrison, 2005). Furthermore, the dorsal medial PFC is essential for the regulation of autonomic and neuroendocrine stress response and arousal associated with emotional states and behavior, whereas the ventral medial PFC has been implicated in generating these emotional states as well as behavior (Drevets et al., 2008; Phillips et al., 2003a). The dorsal and ventral medial PFC are reciprocally functionally related, and abnormalities in the function of either, or both, may be associated with abnormalities in emotional behavior and regulation (Phillips et al., 2003a). In line with these findings, decreased blood flow in the dorsal medial PFC has been associated with increased autonomic responsiveness, anxiety,

and sad mood (Phillips et al., 2003a) In addition, dorsal medial PFC dysfunctions have been implicated in many psychiatric disorders, including depressive disorders (Drevets et al., 2008) and anxiety disorders (Zhao et al., 2007). Taken together, these results suggest that the reduced dorsal medial PFC volume may (in part) underlie the enhanced emotional sensitivity associated with CEM. It should be noted that, contrary to our predictions, the reduced medial PFC volume associated with CEM was independent of psychopathological status, indicating that the reduced medial PFC volume was present not only in individuals with psychopathology but also in controls who never developed a depression or anxiety disorder (although the number of controls with reported CEM is relatively small, and effect sizes of medial PFC reductions were also smaller in the controls than in individuals with depression and/or anxiety). Therefore, reduced medial PFC volume does not seem to be directly linked to the development of depressive or anxiety disorders in individuals reporting CEM. This finding is more consistent with the idea that additional risk factors, such as genetic makeup (Frodl et al., 2010; Gatt et al., 2009; Joffe et al., 2009) alone or in interaction with exposure to stressful life events during adulthood may additionally determine who will subsequently develop a depressive and/ or anxiety disorder (Beck,2 008; Caspi & Moffitt, 2006). In line with this suggestion, individuals with current depressive and/or anxiety disorder reporting CEM (n= 65) indeed reported more negative life events (mean ± SEM: 5.96 ± .55) than controls reporting CEM (n= 13; 4.62 ± .24, t_{76}=-2.26, p< .05).

Although our results are compelling, several potential limitations must be taken into account. The use of the DARTEL VBM approaches is not without its limitations (Ridgway et al., 2008), although recent studies (McLaren, Kosmatka, Kastman, Bendlin, & Johnson, 2010; Yassa & Stark, 2009) demonstrated that it is an improvement to standard voxel-based approaches. In addition, the sensitivity of the DARTEL approach for detecting hippocampal atrophy has been demonstrated in MDD patients (Bergouignan et al., 2009). Nevertheless, manual tracing or shape-based analyses techniques, as have been used in most previous studies on hippocampal structural abnormalities, might be more sensitive in detecting deformations compared with an automated segmentation approach. Furthermore, although a clinically diagnosed posttraumatic stress disorder diagnosis was an exclusion criterion for NESDA, unidentified current or lifetime posttraumatic stress disorder symptoms may still have been present, which may have influenced our findings. However, CEM-related medial PFC gray matter reductions were also present among controls who had never developed a depressive or anxiety disorder; therefore, it is unlikely that current or lifetime posttraumatic stress disorder may have confounded our results. In addition, history of childhood maltreatment was retrospectively assessed by means of an interview, and it is important to acknowledge the inherent subjectivity of self-reported CEM. For instance, the retrospective assessment of CEM may be subject to recollection bias, so that individuals with current psychopathology may over-report, whereas controls

may underreport, a history of childhood maltreatment. However, in the NESDA sample, current affective state did not moderate the association between CEM and lifetime affective disorder, indicating that recall of CEM in the current sample was not critically affected by current mood state (Spinhoven et al., 2010). Finally, our findings are based on a cross-sectional study. Whereas the idea that CEM is associated with dorsal medial PFC gray matter volume reductions fits well with numerous preclinical studies, the possibility of reversed causality cannot be excluded. For instance, individuals with reduced dorsal medial PFC volume might report more CEM as a result of impaired emotion regulation. Another explanation may be that the reduced dorsal medial PFC volume was preexistent and that inadequate emotion regulation associated with reduced dorsal medial PFC volume might increase children's risk for exposure to CEM. Following this line of thought, one would expect that reports of presence or absence of life stressors later in life would also be related to dorsal medial PFC volume. Nevertheless, presence of life stressors (yes or no) was not associated with medial PFC volume ($F_{1,109}$= .09, p= .76, d= .05], and the impact of CEM on medial PFC volume remained unchanged when adding presence of life events into the analysis ($F_{1,109}$= 9.08, p<.01, d= .54]. Theoretically, longitudinal studies examining neuroanatomical developmental changes over time among individuals reporting CEM are needed to shed more light on the aetiology of our findings. To the best of our knowledge, such studies have not yet been conducted, and from an ethical point of view, it would be highly problematic to prospectively follow children who are known to be exposed to CEM without interfering in their situation.

In summary, we found in a large sample of unmedicated adults that self-reported CEM is associated with a substantial reduction in dorsal medial PFC gray matter volume. In line with an accumulating number of animal studies (Caspi & Moffitt, 2006; Levine et al., 2008; Sanchez et al., 2007; Sanchez et al., 2001), our finding suggest that sustained inhibition of growth, or even structural damage, can occur after exposure to emotional maltreatment in childhood. In addition, previous studies have shown that CEM is associated with altered hypothalamic-pituitary-adrenal axis reactivity to stress (Carpenter et al., 2009; Elzinga et al., 2008) and that CEM is an important predictor for the development of depressive and anxiety disorders in adulthood (Gibb et al., 2007; Spinhoven et al., 2010). Given the important role of the mPFC in the perception and regulation of emotional behavior and stress responses (Arnsten, 2009; Cardinal et al., 2002; Lupien et al., 2009; McEwen, 2008; Radley, Williams, & Sawchenko, 2008; Sanchez et al., 2001), our finding might provide an important link in understanding the increased emotional sensitivity in individuals reporting CEM.

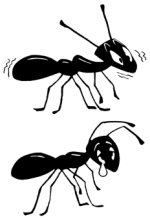
ACKNOWLEDGEMENT

The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (ZonMw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Health and Care Research (IQ Healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos). The principal investigator B.M.E was funded by a VIDI grant (grant number 016•085•353) awarded by NWO. All Authors declare no conflicts of interest.

TABLE S-1.
CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS REPORTING ONLY CHILDHOOD EMOTIONAL MALTREATMENT VS. NO ABUSE.

Childhood Emotional Maltreatment (CEM), ANX= Anxiety Disorder, MDD= Major Depressive Disorder, CDA= comorbid major depressive disorder and anxiety disorder, S=sometimes, R=regularly, O=often, V= very Often, A= Amsterdam Medical Center, L= Leiden University Medical Center, G= University Medical Center Groningen, MADRS= Montgomery Åsberg Depression Rating Scale, BAI= Beck anxiety inventory, F, U, X^2 =F ratio, Mann Whitney U, and Chi-square test statistics, SEM= Standard Error of Mean.

		No abuse (n=97)	only CEM (n=48)	U	X^2	*p*
Gender	% M/F	33/67	42/58		1.05	.30
Age	Mean (SEM)	36.57 (1.09)	37.6 (1.60)	2204.5		.60
Education	Mean (SEM)	13.27 (0.29)	13.56 (0.41)	2229		.67
Handedness	% L/R	11/89	6/94		.95	.33
Current diagnosis	*n* MDD	22	12		2.31	.13
	n ANX	22	12		2.94	.09
	n CDA	12	13		.00	1
	n HC	40	10		18.00	.00
Liefetime diagnosis	% MDD	43.29	72.92		11.31	.00
	% ANX	41.24	60.42		4.74	.00
MADRS	Mean (SEM)	7.10 (.94)	12.30 (0.16)	1428		.00
BAI	Mean (SEM)	8.79 (1.04)	11.91 (1.41)	1603		.01
Scan location	% A/L/G*	28.9 /41.2/29.9	37.5/39.6/22.9		1.34	.51
Gray Matter	Mean (SEM)	740.40 (7.98)	739.73 (8.79)	2260		.76
White Matter	Mean (SEM)	491.33 (6.94)	499 (9.57)	2145		.44



CHAPTER 7

AFFECTIVE PERSONALITY TRAITS ARE LINKED TO VOLUME OF THE ORBITOFRONTAL CORTEX AND AMYGDALA

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SUBMITTED FOR PUBLICATION

SUMMARY

Background: Neuroticism and extraversion are personality factors associated with the development of affective disorders, possibly through differential impact on brain structures implicated in emotional processing. To date, studies on brain morphology mainly focussed on neuroticism, yielding conflicting findings concerning the association with personality, partially due to methodological issues and samples under study.

Methods: In the present study, high resolution structural T1-weighted MR images of 65 healthy adults were processed using an optimized Voxel Based Morphometry approach. Multiple regression analyses were performed to test for associations of neuroticism and extraversion with prefrontal and subcortical volumes.

Results: Orbitofrontal and right amygdala volume were both positively related to extraversion. Neuroticism scores not significantly correlated with these brain regions.

Conclusion: The present results indicate that the reduced likelihood on developing affective disorders associated with high extraversion may be related to modulation of emotion processing through the orbitofrontal cortex and the amygdala

Neuroticism and extraversion are personality factors that have been directly linked to emotional states: neuroticism has been associated with susceptibility to negative emotional states, whereas extraversion has been linked to susceptibility to positive emotional states (Larsen & Ketelaar, 1991). Also, neuroticism has been shown to correlate positively, whereas extraversion correlated negatively, with (subsyndromal) symptoms of depression and anxiety in the general population (Jylha & Isometsa, 2006). Other personality traits, such as agreeableness, openness to experiences, and conscientiousness have been proposed to play a more indirect role in influencing affective states (Larsen & Ketelaar, 1991). Consequently, neuroticism and extraversion may respectively put one at risk for (Bienvenu et al., 2004), or protect one against (Clark, Watson, & Mineka, 1994) development of affective disorders.

More insight in the relation between personality and brain regions associated with emotion processing and regulation may help to understand the influence of personality on the manifestation of subclinical anxiety and depression symptoms. On a functional level, it has previously been shown that neuroticism modulates neural activity in prefrontal and subcortical brain regions related to affective processing (Canli, 2004; Cremers et al., 2010). Neuroticism has been related to more amygdala activation in response to distracting negative facial expressions during a cognitive task (Haas, Omura, Constable, & Canli, 2007) and to less anterior cingulate cortex–amygdala connectivity during processing of negative emotional facial expressions (Cremers et al., 2010). Extraversion on the other hand, was found to be positively associated with amygdala activation in response to happy faces (Canli, 2004). Together, these findings indicate that core brain structures involved in emotion processing, such as the amygdala and the ventral part of the prefrontal cortex, including the orbitofrontal cortex, may play a role in the relation between personality and affective behaviour.

Structural variation might underlie these functional relations of personality and regions related to emotion perception and appraisal. In an adolescent sample, an association between extraversion, but not neuroticism, and prefrontal volume (Blankstein, Chen, Mincic, McGrath, & Davis, 2009) has been observed, which has also been demonstrated in adults (DeYoung et al., 2010), although not consistently (Wright et al., 2006). Therefore, the relation between brain volume and affect related personality traits remains unclear. Inconsistencies in reported findings may be due to methodological and technical issues, such as the use of different segmentation and normalization strategy (e.g. analysis based on modulated vs unmodulated images (Omura, Todd Constable, & Canli, 2005)), not accounting for total brain volume (Gardini, Cloninger, & Venneri, 2009), or to sample characteristics and processes of brain maturation (e.g. by including only elderly (Jackson, Balota, & Head, 2009; Wright, Feczko, Dickerson, & Williams, 2007) or only adolescents (Blankstein et al., 2009). Aging is an important predictor of regional brain volume, and the process of aging (including brain maturation) has been shown to interact with

personality (Jackson et al., 2009). It is therefore necessary to further elucidate the complex relation between affect related personality traits and regional brain volume in an adult sample, controlling for these important factors. In the present study, we used an optimized voxel based morphometry approach to investigate the relationship between neuroticism, extraversion, and regional brain volume in a large sample of healthy adults. We focussed on brain regions involved in the initial processing of emotional information (amygdala) and on regions related to the appraisal and decision making influence of emotional information, (anterior cingulate cortex and orbitofrontal cortex) (Drevets et al., 2008). As such, we aimed to identify neuro-anatomical substrates associated with affect-related personality traits.

METHODS

ETHICS STATEMENT

The study was carried out in accordance with the Declaration of Helsinki. Also, the Ethical Review Boards of the Leiden University Medical Center, Academic medical Center Amsterdam, and University Medical Center Groningen approved this study. All participants provided written informed consent after complete description of the study.

PARTICIPANTS

Sixty-five healthy control participants were selected from the NESDA (Netherlands Study of Depression and Anxiety) neuroimaging study (Penninx et al., 2008). The MRI main sample is described in detail elsewhere (van Tol et al.,2010). Exclusion criteria for the current sample were: 1) a history of or current DSM IV axis I pathology, 2) taking any psychoactive drugs, 3) the presence or history of major internal or neurological disorder, 4) dependency or recent abuse (past year) of alcohol and/or drugs, 5) hypertension 6) general MR-contraindications.

PERSONALITY QUESTIONNAIRE

To asses personality traits, all participants completed the NEO Five Factor Inventory (Costa & McGrae, 1992).

IMAGE ACQUISITION

Imaging data were acquired using Philips 3-tesla MR-systems (Best, The Netherlands) located at the Leiden University Medical Center (LUMC), Academical Medical Center (AMC) University of Amsterdam, and University Medical Center Groningen (UMCG), equipped with a SENSE-8 (LUMC and UMCG) and a SENSE-6 (AMC) channel head coil, respectively. For each subject, anatomical images were obtained using a sagittal 3D gradient-echo T1-weighted sequence (TR=9 ms, TE=3.5 ms; matrix 256x256; voxel size: 1x1x1mm; 170 slices).

DATA ANALYSIS

Voxel Based Morphometry (VBM) following the Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra software (Ashburner, 2007) implemented in Matlab 7.1.0 (The MathWorks Inc., Natick, MA, USA) was used. The preprocessing and masking procedure is described in detail elsewhere (van Tol et al., 2010). Briefly, after segmentation, data were registered, normalized, and modulated using the DARTEL pipeline. Gray matter (GM) images were normalized to Montreal Neurological Institute (MNI) space and smoothed at 8 mm full width at half maximum (FWHM). In the resulting images, each voxel represents an absolute amount of brain volume, equivalent to the brain volume per unit prior to normalization.

Next, we performed a regression analysis with neuroticism and extraversion as independent variables. Age, gender, scan center, and gray matter total volumes were entered as nuisance variables. Based on the literature on emotion processing in affective disorders (Drevets et al.,2008), we set the following regions of interest (ROI): amygdala, orbitofrontal cortex (OFC), anterior cingulate cortex (ACC). Results are reported at $p<.05$ Family wise error (FWE) corrected for the spatial extent of the ROIs (small volume corrections). The ROIs were defined by the Automated Anatomical Labeling (AAL) templates implemented in the Wake Forest University (WFU) pickatlas (Maldjian et al., 2003). For regions other than the ROI's, a voxel level threshold of $p<.05$ whole brain FWE corrected was set a priori. Demographic and clinical data were analyzed with SPSS 16.0 (SPSS Inc., Chicago, Il, USA) and significance was set at $p<.05$.

RESULTS

SAMPLE CHARACTERISTICS AND PERSONALITY SCORES

The age range of the 65 participants (42 females) was: 21-56, Mean (M) =40.5, standard deviation (sd) =9.7; years of education: M=14.3, sd=2.9). Neuroticism (range= 13-36; M=24.2, sd=4.9) correlated negatively to extraversion (range 27-56; M=43.7, sd=6.2) (r =-0.4, $p<.05$) and total gray matter volume (r =-0.3, $p<.05$). Extraversion was positively correlated to total gray matter volume (r =0.3, p<.05). No correlation was observed between neuroticism or extraversion and age, years of education, and sex (all $p>.13$).

PERSONALITY AND REGIONAL BRAIN VOLUME

The right orbitofrontal cortex (BA 11; MNI coordinates: [x=13 y=16 z=-14], Z=3.77, clustersize= 329) and right amygdala (MNI coordinates: [x=21 y=-10 z=-21], Z=3.34, clustersize= 31)) showed a positive relation with extraversion. Neuroticism did not show a significant relation with regional brain volume in any of our regions of interest. Adding the other three personality variables (openness, agreeableness, and conscientiousness) from the NEO_FFI did not significantly change the results (orbitofrontal cortex: Z=3.28; amygdala, Z=3.50). Results are showed in figure 1.

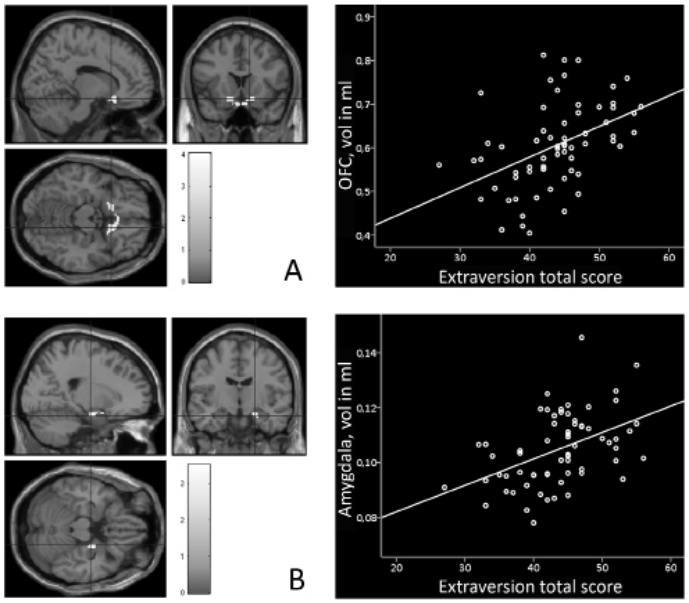


FIGURE 1: CORRELATIONS OF NEUROTICISM AND EXTRAVERSION IN THE AMYGDALA AND OFC

A) Positive correlation of extraversion and volume of the right orbital frontal gyrus

B) Positive correlation of extraversion and right amygdala volume.

Correlations of extraversion with volume of the orbitofrontal cortex and amygdala are overlayed on the mean high-resolution T1-weighted template in MNI space at $p<.005$ for display purposes.

DISCUSSION

In the present report, we used an optimized VBM approach to test for personality related variations in regional brain volume, while controlling for age and gender, and demonstrated a positive correlation of extraversion and OFC and amygdalar volume in an adult sample. This result confirms the role of the OFC in personality, a region that was already associated with personality changes in the case report of Phineas Gage (Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994). We also found a positive correlation between extraversion and volume of the amygdala. However, we did not find strong structural correlates of neuroticism.

The observation that extraversion correlated positively with volume of both the OFC and amygdala is of interest because of the role these regions play in affective processing (Rolls, 2004; Rolls & Grabenhorst, 2008). The OFC has often been associated with controlling reward and punishment related behavior, emotion regulation, and decision making (Kringelbach & Rolls, 2004). The amygdala has a well-documented role in emotion processing and has bi-directional connections with the OFC (Price, 2003). Interestingly, a positive correlation of OFC thickness with extraversion and fear extinction has been described in a small study (Rauch et al., 2005). In another study, humor-driven activation, reflecting hedonic capacity, was found to be positively correlated

ACKNOWLEDGEMENT

Henk Cremers and Karin Roelofs are supported by the Netherlands Organization of Scientific Research (NWO), VIDI grant #452-07-008. The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Scientific Institute for Quality of Healthcare (IQ healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute).

with extraversion in the right OFC (Mobbs, Hagan, Azim, Menon, & Reiss, 2005). In the amygdala, activation during happy face viewing was found to be positively related to extraversion (Canli, 2004). Hence, the increased volumes of OFC and amygdala may play a role in the increased sensitiveness to positive, pleasant information and (social) reward, and the propensity to experience positive affect which characterizes extraversion (Clark et al., 1994). The nature of this role, however, awaits further elucidation, as increased volumes of GM in brain areas may be reflective of processes involving amongst others glia cells, inhibiting or excitatory interneurons, and neurons. However, these processes can not be assessed directly in vivo.

The present result is in line with findings of Blankenstein and colleagues (2009) who used a similar methodology (i.e. an optimized VBM approach) in an adolescent sample. The present results are also in line with results of previous studies that have shown a relation between personality and orbitofrontal cortical thickness (Wright et al., 2006) and lateral PFC volume (Wright et al., 2007) in smaller samples of young adults and elderly. However, Wright and colleagues (2006) reported that neuroticism was negatively related to OFC volume and extraversion was positively related to lateral PFC volume in a young adults. Accordingly, in the present study, we expected neuroticism to account for a substantial portion of the volumetric variation in regions associated with emotional perception and regulation. However, no such relation was observed. Instead, extraversion was found to be the main predictor of regional brain volume in affective brain regions. Discrepancy in findings might be due to methodological issues such as outlined before; in this study we used an optimized VBM method in a relatively large adult subject sample.

In the present study we examined structural correlates of extraversion and neuroticism in a cross-sectional design. Therefore, it is possible that the found correlates are not primary, but secondary to individual personality characteristics. For instance, high extraversion is known to be associated with different lifetime experiences than low extraversion. As of yet unpublished finding from the same NESDA study indicate that extraversion and negative life events mediate the course of depressive symptoms (Spinhoven et al. 2011). Extraversion and lifetime experiences may also lead to a differential effect on brain structures involved in social interaction or hedonia.

Given the augmenting evidence for a significant relation between extraversion and OFC and amygdala volumes, it will be important to further elucidate this relation in studies addressing structural and functional connectivity of the OFC and the amygdala. This would also provide more insight the trajectory from health to psychopathology, and as such identify neuroanatomical markers for the risk of affective disorders.

CHAPTER 8

SUMMARY
GENERAL DISCUSSION
FUTURE PERSPECTIVES

Brain structure and brain function in patients with Major Depressive Disorder (MDD) and prevalent anxiety disorders were studied in this thesis. The first aim of this study was to identify disorder specific and shared neuroanatomical and functional abnormalities, with a focus on emotional information processing and executive functions. Structural and functional MRI patterns of patients with both MDD and anxiety disorders (comorbid depression-anxiety) were directly compared with those of patients with only MDD or anxiety disorders. The second aim of this study was to study the relation of common risk factors of mood- and anxiety disorders, namely neuroticism and childhood emotional abuse, and regional brain volume. Giving insight into the shared and distinct neurobiological underpinnings of MDD and anxiety disorders is important to understand their high comorbidity, for the classification of MDD and anxiety disorders, and subsequently for the treatment and course of these highly prevalent psychiatric disorders.

Both brain morphometry and brain function related to cognitive and emotional processes were investigated with the use of magnetic resonance imaging (MRI). The anxiety disorders included in this study were social anxiety disorder, panic disorder, and generalized anxiety disorder. The studies described in this thesis are based on data acquired in the context of the Netherlands Study of Depression and Anxiety (NESDA), a large scale, multi-site, observational cohort study. In this final chapter, we will summarize and discuss the main results of the studies presented (Chapter 2-7). Finally, implications and recommendations for future research are provided.

SUMMARY OF RESULTS

The question whether MDD, comorbid depression-anxiety disorder, and anxiety disorders were associated with volumetric abnormalities in regions linked to emotion perception and regulation was adressed first. In **Chapter 2**, we showed that reduced volume of the rostral and dorsal anterior cingulate cortex (ACC) is a generic effect in MDD and anxiety disorders. This ACC reduction was observed independent of comorbidity and was unrelated to variations in illness severity, medication use, and sex. This generic effect supports the notion of a shared aetiology in MDD and anxiety disorders and may reflect a common symptom dimension related to altered emotion processing. Decreased regional volume of the inferior frontal cortex in MDD and lateral temporal cortex in anxiety disorders without comorbid MDD, on the other hand, may reflect disorder-specific symptom clusters. In addition, early onset of depression was found to be associated with reduced volume of the subgenual ACC, extending into the orbitofrontal gyrus, compared with healthy controls. This additional volume reduction in the affective subdivision of the ACC further suggests that vulnerability for MDD is related to volume of the ventral ACC.

The neural (functional MRI) correlates of emotional word memory in MDD and anxiety disorders were investigated in the next chapter. Studying neural

correlates of emotional memory processes is of interest to MDD and anxiety disorders, because abnormalities in the encoding and remembering of emotional information might reinforce negative mood and could contribute to the prolonged course of the disease (Elliott et al., 2002). In **Chapter 3**, we demonstrated that decreased activation of the hippocampus during positive word encoding is a common phenomenon in depression and anxiety disorders that may constitute a common vulnerability for biased information processing in MDD and anxiety disorders. This effect was observed independent of illness severity, medication use, and regional volume. Moderately and severely depressed MDD patients were additionally characterized by increased activation of the insula, amygdala, and by trend-wise increased activation of the striatum and prefrontal cortex during encoding of negative words. These findings may mark an increased sensitivity for negative stimuli. Furthermore, illness severity dependent increased activation of fronto-polar regions in comorbid depression-anxiety, irrespective of valence was observed. These results emphasize the current distinction between MDD and anxiety disorders (with and without comorbid MDD) with respect to processing of mood-congruent information (i.e., negative words), although a hippocampal hypo-response to positive words may mark a general insensitiveness to positive information.

The involvement of dorsal prefrontal regions such as the dorsal ACC, dorsolateral PFC, and dorsomedial PFC during the non-emotional Tower of London visuospatial planning task was studied in **Chapter 4.** Here, we demonstrated that only moderately to severely depressed MDD patients are characterized by increased left dorsolateral prefrontal activity as a function of executive task load together with subtle performance differences. Mildly depressed and remitted MDD patients, as well as anxiety patients showed normal task performance and no activation differences were observed. Combined these results indicate that prefrontal hyperactivation during high planning demands is a state (i.e., depressive episode dependent) characteristic and not a trait (i.e., depressive episode independent) characteristic of MDD. We conclude that executive dysfunction during a non-emotional visuospatial planning task is not a feature of anxiety disorders, supporting the current diagnostic distinction between anxiety disorders and depression.

In **Chapter 5**, we studied whole-brain connectivity during an emotional word classification task using template independent component networks. We aimed to directly investigate the connectivity of subcortical and (para)limbic regions associated with emotion perception and generation of emotional states on the one hand, and dorsal cortical regions associated with emotional regulation on the other hand. We demonstrated that patients with MDD who were life-time free of anxiety disorders showed decreased connectivity of the medial PFC and ventral-striatal regions within the salience network (Seeley et al., 2007) during the classification of negative, neutral, and positive words, unrelated to illness severity. Furthermore, connectivity of the medial PFC with the task-executive

network was positively related to the number of words classified as positive in the MDD group. This decreased connectivity of fronto-striatal regions within the salience compared with controls confirms the hypothesis of a relative ventral-dorsal decoupling that may serve to explain the compromised emotional regulation in MDD.

In **Chapter 6** we focused on the long-term anatomical correlates of childhood emotional maltreatment. We demonstrated that childhood emotional maltreatment is associated with lower volume of the dorsal medial PFC, independent of current psychopathological status and independent of concomitant physical or sexual abuse. This finding suggests that sustained inhibition of growth or structural damage can occur after exposure to childhood emotional maltreatment. Given the important role of the dorsal medial prefrontal cortex in the regulation of emotional behavior, our results may provide an important link in understanding the increased emotional sensitivity in individuals reporting childhood emotional maltreatment.

The correlations of the personality traits neuroticism and extraversion was studied in **Chapter 7**. We showed that orbitofrontal and subgenual ACC volume in healthy control participants was positively related to extraversion. No relation with neuroticism was observed. In addition, extraversion was positively correlated to right amygdalar volume. These findings indicate that extraversion is a potent factor in predicting regional brain volume in structures related to emotional processing. High levels of extraversion may modulate emotion processing through the orbitofrontal cortex, thereby affecting the likelihood of developing affective disorders.

Taken together, these findings suggest that MDD and common anxiety disorders share neuroanatomical and neurophysiological abnormalities, but at the same time are characterized by unique neuroanatomical abnormalities and activation patterns during cognitive and emotional processes. Next, we showed that besides current psychopathology, personality factors and emotional traumatic experiences during childhood are associated with variations in volume of structures that are important for mood regulation. Therefore, these effects could mediate the vulnerability for developing MDD and anxiety disorders. Overall, no effect of SSRI use on functional and structural measures was observed.

This is the first series of neuroimaging studies that studied MDD, prevalent anxiety disorders, and comorbid depression-anxiety together, while at the same time controlled for important factors such as medication use and illness severity.

In this section, the shared neuroanatomical- and functional MRI correlates of MDD and anxiety disorders will be discussed, followed by a discussion of the unique neurophysiological correlates of MDD and anxiety disorders. Next, the question of whether to regard comorbid depression-anxiety as MDD plus anxiety or as a different diagnostic category will be discussed. Subsequently, risk factors of mood and anxiety disorders will be discussed. Also, the result of relatively normal cognitive functioning in our sample will receive attention. Furthermore, the stability of effects and the question of causality will be addressed. Finally, the present results will be reflected on in light of the frequently used neuroanatomical models of mood regulation and depressive pathology. Considerations and implications of the results presented in this thesis will be discussed.

MDD = ANXIETY DISORDERS

This study demonstrated for the first time that MDD and anxiety disorders share morphologic and activation abnormalities. Both MDD patients and patients with anxiety disorders showed reduced volume of the dorsal and rostral ACC. This volumetric abnormality was also observed in patients with both MDD and anxiety disorders. ACC reductions have been previously observed in MDD and panic disorder, but groups had not been compared directly. Also, studies in MDD and anxiety disorders performed to date were often confounded by the inclusion of comorbid anxiety or MDD, respectively, and the specificity of these findings remained therefore unclear. Evidence is now provided that suggests that MDD and anxiety disorders share a common neuroanatomical characteristic that may explain, at least in part, the emotional dysregulation that is associated with both anxiety disorders and MDD. Interestingly, this abnormality is observed in the rostral ACC, a region that has been considered the emotion-cognition integration component in the Mayberg model (1997) (See **chapter 1**). A common abnormality in this region in both depression and anxiety indicates that regulation between dorsal and lateral cortical regulatory and subcortical and (para)limbic appraisal regions may be compromised owing to this structural abnormality. This rostral ACC abnormality may lead to sub optimal frontal-subcortical regulation, resulting in a variety of symptoms associated with both mood and anxiety disorders.

Next, a common abnormality in MDD and anxiety disorders was observed during the encoding of mood-incongruent (i.e., positive) words. MDD and anxiety patients showed less activation of the right hippocampus, also when a diagnosis of both depression and anxiety was present. This suggests that abnormal processing of positive information might be considered a trait phenomenon of both depression and anxiety disorders, because the effect was observed independent of illness severity. This suggestion is further supported by the word classification pattern that was observed in both MDD and anxiety

disorders: fewer words were classified as positive compared with controls, whereas no difference in classification of negative words was observed. In the study of MDD and anxiety disorders, the focus has been predominantly on processing of mood congruent or disorder specific information. The shared abnormality as demonstrated in this study may be linked to the increased (negative) self-focus that is associated with both depression and anxiety (Borden, Lowenbraun, Wolff, & Jones, 1993; Spurr & Stopa, 2002; Watkins & Teasdale, 2004). Processing of self-referential material, for example, has been associated with increased memory success (Symons & Johnson, 1997). In a sad emotional state, negative words may be more relevant for the self than positive words, and therefore self-relevant information is more likely to affect the negative mood state. As a result, positive words may be considered less salient and as a consequence are processed in a less efficient manner. This less efficient processing of positive information may be reflected in the words classification behavior, prolonged response times and the reduced hippocampal activation during positive encoding. Consequently, positive information may be less potent in affecting the current mood state, thereby influencing the course of the disease in a negative way as well. However, the influence of a negative self-focus on processing of external positive information and the role of the hippocampus therein needs to be further investigated.

These results underline the importance of abnormalities in processing positive information in MDD and anxiety disorders and further emphasize that next to the traditional focus on mood-congruent processing abnormalities, mood-incongruent processing abnormalities should also be considered. Not only in the study of MDD, but also in the study of anxiety disorders. Recently, evidence was provided that patients with MDD have difficulties in engaging in positive mood states for prolonged periods of times, as was reflected in a failure to sustain activity in the nucleus accumbens (Heller et al., 2009), which is an important structure implicated in the reward system. Whether such a mechanism generalizes to patients with (comorbid) anxiety disorders, remains to be elucidated.

MDD ≠ ANXIETY DISORDERS

Besides the shared characteristics, distinct neuroanatomical and neurophysiological abnormalities were observed in MDD and anxiety. These unique characteristics could help explain the specific symptomatology of the disorders.

MDD patients, but not patients with anxiety disorders with or without MDD, showed lower volume of the right inferior frontal gyrus compared with controls. The inferior frontal gyrus region has been associated with processes of selective attention, cognitive conflict resolution, goal maintenance, and emotional inhibition (Forstmann et al., 2008; Lieberman et al., 2004; Matthews

et al., 2009; Ochsner et al., 2009; Phillips et al., 2003a). Interestingly, in our study, prefrontal abnormalities during executive processes were only observed in moderately and severely depressed patients and not in patients with remitted or mildly depressed states, nor in patients with current anxiety disorders. Although connectivity was not investigated in patients with anxiety disorders or comorbid depression-anxiety disorders, our results of decreased connectivity of the ventral striatum and the ventral medial PFC within the salience network during the execution of an emotional word classification task further suggests that top-down control of prefrontal regions over ventral emotion generating regions is compromised in MDD. Importantly, the specificity of this finding for MDD needs to be tested. In this study, we did not test for the directionality of cortical-paralimbic connectivity. However, dorsal prefrontal regions have been uniquely associated with top-down emotional regulation (Ochsner et al., 2009).

Moreover, moderately and severely depressed MDD patients showed increased responsiveness of the amygdala, striatum, and PFC during encoding of negative words. This may indicate an increased sensitivity for negative information in general that is specific to MDD in the 'active' depressed state, because it was not observed in remitted and mildly depressed MDD patients nor in patients with anxiety disorders. Also, MDD patients showed depressive state dependent ACC hyperactivation during encoding of positive words and state-independent increased activation of the left insula during encoding of negative words. Together these results indicate that the increased 'sensitivity' of limbic and prefrontal regions for negative information and increased attentional control during positive encoding is primarily associated with depressive pathology. Furthermore, these observations are in line with the suggestion that MDD and MDD with comorbid anxiety disorders should be considered different diagnostic categories.

In summary, MDD is uniquely characterized by inferior frontal volume reduction, state dependent dorsolateral PFC activation abnormalities as a function of planning load, increased sensitivity for encoding negative information, and by an increased demand for attentional control during encoding of positive words as reflected in increased ACC activation. The latter phenomenon may be reflective of a conflict resolution between the personal mood state and the emotional valence of the words presented during the task. Together these unique characteristics indicate more deficient involvement of dorsal frontal regions in MDD than in anxiety disorders, together with increased sensitivity for general negative information.

Structural abnormalities specific for anxiety disorders were observed as well. Patients with a current diagnosis of anxiety, but not those with a concurrent diagnosis of MDD, showed decreased volume of the superior temporal gyrus volume. This may mark a neuroanatomical vulnerability related to impaired evaluation of interoceptive information (Uchida et al., 2008) and altered threat processing (Phillips et al., 1998). These processes may be more specific for anxiety disorders than for MDD. During recognition of positive words, patients

with anxiety disorders showed increased activation in the left inferior frontal gyrus. No subcortical functional abnormalities were observed in patients with anxiety disorders in this study, which may be related to the more general tasks that were administered to investigate emotional processes: no specific (social) threat stimuli were included in the functional imaging paradigms. The use of social or situational threat paradigms may better highlight anxiety specific abnormalities, as the symptomatology of anxiety disorders is often more situational bound that in MDD. However, fearful faces were presented in the emotional faces paradigm, a task that was included in the NESDA neuroimaging study but not subject of this thesis. Analysis did not reveal functional abnormalities during viewing of fearful faces in patients with anxiety disorders, nor in the other patient groups (Demenescu et al., 2010). To conclude, our results suggest that outpatients with prevalent anxiety disorders are not functionally affected during executive task execution and processing of general negative information.

COMORBID DEPRESSION-ANXIETY VS. MDD AND ANXIETY

Contrary to our expectations, patients with comorbid depression-anxiety showed structural and functional abnormalities similar to patients with only MDD or anxiety disorders. This is surprising, considering the higher symptom severity in comorbid depression-anxiety on both measures of depression and anxiety than in 'singular' (i.e., without comorbidity) MDD and anxiety. Because comorbid depression-anxiety is associated with worse outcome, we expected to see this reflected in the neuroanatomical and functional patterns. It is possible that the currently used paradigms in combination with the current analysis approaches were not sensitive enough to detect differentiating characteristics in comorbid depression-anxiety. Nevertheless, patients with comorbid depression-anxiety were not characterized by comorbid depression-anxiety specific abnormalities, but by abnormalities in rostral ACC volume and right hippocampal reactivity during positive word encoding similar to patients with singular MDD and anxiety disorders. In the moderately and severely depressed state, patients with comorbid depression-anxiety were characterized by increased fronto-polar recruitment when recognizing previously encoded emotional words, independent of valence. As a function of visuospatial planning only trend-wise increased dorsal PFC activation was observed. Together these results might suggest that comorbid depression-anxiety patients are characterized by increased frontal recruitment during attentional demanding tasks only in the presence of emotional distraction or content, and only in the severely depressed and anxious state. However, at the moment, this suggestion is tentative and needs further investigation.

Volume reductions in the inferior frontal gyrus were observed in patients

with MDD only, and not in patients with comorbid depression-anxiety. This difference in result could be the result of a difference in aetiology in MDD and comorbid depression-anxiety: in most comorbid depression-anxiety patients, the anxiety disorder was the first to manifest. Possibly, anxiety related pathology is the primary pathology, and the occurrence of the comorbid depression later on only minimally affects the primary neuropathology. Interestingly, this explanation does not clarify why the superior temporal gyrus volume abnormality was only observed in anxiety without MDD and not in comorbid depression-anxiety. However, these findings indicate that anxiety and anxiety with comorbid MDD are not characterized by an identical neurobiological profile. These differences might explain the syndrome specific characteristics.

In sum, no evidence was provided for the suggestion that comorbid depression-anxiety is associated with more severe structural and functional abnormalities than patients with singular MDD or anxiety disorders on the measures included in this study. Although not subject of this thesis, fMRI was also applied during an emotional face viewing task data in the NESDA neuroimaging study. Analyses also failed to demonstrate differences between patients with comorbid depression-anxiety and patients with singular MDD and/or anxiety disorders in amygdalar response to negative emotional faces (Demenescu et al., 2010). Nevertheless, on measures presented in this thesis, comorbid depression-anxiety showed overlap with the functional and structural pattern observed in both anxiety disorders and MDD, but no overlap with MDD specific abnormalities was observed. Analysis of symptom course trajectories of the NESDA sample suggests that patients with anxiety disorders and comorbid depression-anxiety disorder are characterized by lower recovery rates and a higher proportion of patients with a chronic course than MDD patients without comorbid anxiety disorders (Penninx et al., 2010). The present neuroimaging results further suggest that patients with comorbid depression-anxiety disorders are not primarily characterized by MDD related pathology. Whether comorbid depression-anxiety is better characterized by anxiety related pathology on a neurobiological level should be studied further.

STATE VS. TRAIT

In this study, all patients fulfilled criteria for MDD, panic disorder, social anxiety disorder or generalized anxiety disorder in the last six months (plus scan interval). Remission as discussed in this thesis should therefore be considered 'recent remission' and caution should be taken when discussing our observations in light of proper state vs. trait related phenomenon. In this study, rostral ACC, inferior frontal gyrus, and superior temporal gyrus volume was observed independent of illness severity. Whether normalization of regional brain volume occurs in the absence of symptoms for a longer period

of time could not be tested in this cross-sectional study. Longitudinal analyses should confirm the hypothesis that reduced volume in these regions is a trait phenomenon of depression and anxiety disorders and therefore most likely constitutes a common vulnerability for a new anxiety- or depressive episode. The only longitudinal volumetric study performed in MDD to date indicates that remitted patients show less volume decline than non-remitted patients after a 3 year stable remission period (Frodl et al., 2004). Importantly, no volume 'normalization' was reported in remitted patients (Frodl et al., 2004).

Depression and anxiety severity related abnormalities were observed during tasks as measured with functional MRI. For example, in MDD prefrontal over-recruitment during planning, increased amygdalar, striatal and prefrontal cortex activation during the encoding of negative words, and increased dorsal ACC activation during the encoding of positive words, was found to be specific for the moderately and severely depressed state. Trait-like effects were observed during the encoding of positive and negative words in MDD, but not as a function of planning load. Hippocampal blunting during positive encoding and the insular hyperactivation in MDD during negative encoding were observed independent of current symptom severity. In anxiety disorders, hippocampal hypoactivation during encoding of positive words and inferior frontal hyperactivation during recognition of positive words were observed, although the latter was trend-wise associated with severity of anxiety. Relative decoupling of the ventral striatum and medial prefrontal cortex with a task executive network in MDD during classification of emotional words was observed independent of depression severity as well. Whether these trait-like effects persist after six month of full and stable remission should be studied using a longitudinal analysis approach.

Taken together, our results indicate that MDD, comorbid depression-anxiety, and anxiety disorders without MDD are characterized by trait like abnormalities that may constitute a vulnerability factor in patients who recovered from an anxious or depressive episode. However, the stability of effects need to be confirmed in longitudinal analyses, for example with the use of the longitudinal imaging data that are obtained in the context of the NESDA neuroimaging study. These data are currently available and analyzed. State related abnormalities, on the other hand, may reflect the episode specific symptoms, such as increased negative focus on the self and increased attentional problems in MDD.

THE ROLE OF RISK FACTORS IN NEUROANATOMY OF MDD AND ANXIETY

Early life experiences and personality characteristics were related to volume in regions associated with emotion perception and regulation, next to abnormalities related to current psychopathological status. Remarkably, the

experience of two or more incidents of psychological trauma or emotional neglect was associated with lower volume of the dorsal medial prefrontal cortex compared with participants who reported no incidence of any type of trauma in their lives. This volumetric abnormality was observed independent of current psychopathological state and is most likely associated with an early disruption of the human stress system in the developmental phase. These results indicate that vulnerability for developing mood- and anxiety disorders after exposure to early life trauma may be mediated by a neuroanatomical abnormality in regions associated with regulation of stress response. Next to regulation of stress responses, the dorsomedial PFC has been associated with the regulation of autonomic responses and regulation of arousal associated with emotional states and behavior (Phillips et al., 2003a; Urry et al., 2009). Abnormal morphometry of this region could therefore affect normal affective regulation, and could enhance sensitiveness for mood- and anxiety disorders.

In addition to these abuse related effects, we found that extraversion was associated with volume of the orbitofrontal cortex and orbitofrontal cortex/subgenual ACC. These regions have been associated with the gating of information in- and outflux from the amygdala (Price, 2003) and with control over amygdalar responsiveness (Milad & Rauch, 2007). The orbitofrontal cortex (including the subgenual ACC) could therefore be considered an visceral regulatory component, in line with the Mayberg model (1997). Furthermore, extraversion was positively associated with volume of the amygdala, a key area in salience detection and processing of emotional cues. Even though we only studied variation of the personality factors neuroticism and extraversion and only in healthy control participants, these results underline that personality factors are associated with morphometry of areas important for emotional perception and regulation. High levels of neuroticism have been associated with an increased risk for develop mood and anxiety disorders (Bienvenu et al., 2004), whereas high levels of extraversion have been found to act as a protective factor for developing mood and anxiety disorders (Clark et al., 1994). In this context, the present results suggest that this vulnerability may be mediated by personality dependent variations in orbitofrontal volume. In a functional connectivity study, also based on NESDA neuroimaging data, neuroticism has been shown to influence connectivity of the ACC and amygdala during negative face viewing (Cremers et al., 2010). Although the authors controlled for variations in extraversion, they did not test for the direct effect of extraversion on amygdala connectivity.

Alternatively, the present results could be interpreted as being reflective of variations in depression and anxiety related symptomatology in healthy subjects. Neuroticism has been shown to correlate positively with symptoms of depression and anxiety, even in the general population, whereas extraversion was found to be negatively correlated with symptoms of depression and anxiety in the general population (Jylha & Isometsa, 2006).Neuroticism and extraversion could therefore be regarded important personality dimensions

reflecting depression and anxiety related symptomatology. In this context, our results indicate that volume of subgenual regions vary as a function of this personality dimension (i.e., extraversion), even in healthy participants, and may reflect subsyndromal or normal variations in depressive and anxious symptomatology.

Interestingly, we also observed lower gray matter volume of the subgenual and orbitofrontal volume in patients with early age at onset of the first depressive episode in addition to the rostral ACC reduction that was observed in all patients (**Chapter 2**). Although we did not test for the correlation of extraversion in this region in patients with onset of MDD before the age of 18, these volumetric abnormalities suggest that the orbitofrontal region is an important region associated with vulnerability for developing depression and anxiety disorders. Given the numerous connections of this subgenual ACC/OFC regions with other important regulatory regions, this may also explain the heightened vulnerability for developing mood and anxiety in patients at high risk and the worse course of patients with early onset of depression.

In summary, risk factors associated with MDD and anxiety disorders were associated with volumetric variation in structures that have been linked to emotional perception and emotional regulation. Therefore, these factors are potent to influence the likelihood of developing mood and anxiety disorders. However, the exact causal mechanism from risk factor to affective disorder should be further investigated. Possibly, these factors set the homeostasis of the emotional regulation circuitry different, thereby making the brain circuitry associated with emotional and stress regulation more susceptible for dysregulation under stressful events, such as negative life events. The fact that childhood maltreated patients with MDD and/or anxiety disorders were characterized by the experience of more negative life events supports this suggestion (**Chapter 6**).

CAUSE OR EFFECT?

In this study, we investigated the relation between current psychopathology, emotional childhood abuse, and personality factors on one hand, and brain function and structure on the other hand using a cross-sectional design. Although early life factors such as early onset of depression and the experience of childhood emotional maltreatment appear to have a profound influence on brain morphometry, no conclusions with respect to cause and results can be drawn from the studies presented in this thesis. It is plausible though, that childhood emotional trauma would lead to abnormalities in the 'settings' of the human stress system, thereby affecting neurogenesis of regions that are rich in glucocorticoid receptors and are important for emotional control. However, this statement on causality can't be derived from the current study design. A current diagnosis of depression and/or anxiety disorder was associated with

volumetric and activation abnormalities as well. Whether these abnormalities are causal to the symptoms associated with MDD and anxiety disorders remains unclear. In our voxel based morphometry study, no relation between the number of episodes and volume of the rostral ACC or volume of the hippocampus was observed, suggesting that multiple episodes of depression do not aggravate the neuropathology. Again, however, the cross-sectional study design does not allow to infer on cause and result relationships and to study long-term effects of disease factors within patients. Longitudinal analyses should give insight into the causality of the relationship. To date, longitudinal imaging studies in MDD and anxiety disorders are virtually non-existent, with the exception of the volumetric studies of Frodl et al. (2004). Moreover, no longitudinal analyses in at risk populations have been performed that could answer the cause or effect questions.

COGNITIVE PERFORMANCE IN MDD AND ANXIETY

Contrary to our expectations, patients with MDD, comorbid depression-anxiety, and anxiety disorders showed no gross abnormalities in task performance during the visuospatial planning task and during the emotional word memory task. Only two cognitive tasks were administered, and therefore only little can be concluded about the status of neuropsychological functioning in MDD and anxiety disorders. The present results of relatively normal cognitive executive performance and absence of mood-congruent memory biases could be explained by the methods used to assess cognitive functions and by the moderate symptom severity of the outpatient sample included. Both options and implications of our findings will be discussed.

Behavioral results of the Tower of London task indicated only subtle slowing in the most depressed MDD patients during execution of the most difficult trials, in the context of normal planning accuracy. It could be argued that this computerized version was not sensitive enough to detect differences between groups, possibly owing to the two-answer option provided (as opposed to open-end questions/trials). However, in other psychiatric groups, this version of the Tower of London was sensitive enough to detect abnormalities in task performance (van den Heuvel et al., 2005a). Additionally, no effect of diagnosis on overall memory performance was observed in the present study. Nevertheless, performance differences were observed with the use of the same task in participants at high risk for developing depression or anxiety (Wolfensberger et al., 2008), suggesting the task is sensitive enough to detect performance differences. In this study, the retention interval was relatively short. Lengthening the retention interval or including a free-recall session could increase the sensitiveness of the test to detect subtle cognitive biases or deficits. Also, no effect of diagnosis on memory performance of mood-congruent or mood-incongruent words was observed. Possibly, words presented during the encoding phase were not salient enough to influence memory formation. Also,

presented words were not disorder-specific and therefore may not have been potent to affect memory formation. No depression or anxiety specific words were presented: instead words had a genera;;u negative, neutral, or positive connotation.

Because cognitive performance was also unaffected in the most severely anxious and depressed patients in the present sample, an alternative explanation is that cognitive functions are relatively unaffected in outpatients with MDD and/or anxiety disorders. Our results of normal cognitive performance in MDD and anxiety disorders, although sparingly assessed, are in line with a number of neuropsychological studies in outpatient samples that reported normal neuropsychological functioning in MDD and anxiety (Castaneda et al., 2008; Castaneda et al., 2010; Gladsjo et al., 1998; van Wingen et al., 2009). Our results of relatively unaffected planning and memory performance support the suggestion that cognitive capacities are not diminished in MDD and anxiety. Our results of slightly longer response times and increased dorsolateral PFC activation in the most difficult trials may be reflective of the narrowed attentional focus hypothesis instead (in: Hartlage et al., 1993): extra frontal resources were recruited to focus on external task demands that were not depression-relevant. Moreover, emotional words did not affect memory performance in a mood-congruent of mood-incongruent manner. Nevertheless, abnormal activation of limbic areas was observed during encoding of emotional information. Combined with the observation of slower encoding of positive words, our results suggest that positive information is to a lesser extent linked to (less) available positive material. Thereby, positive information could be less potent in affecting mood in a positive manner.

Discrepancies in results with studies that reported cognitive deficits in MDD and anxiety disorders (Airaksinen, Larsson, & Forsell, 2005; Basso et al., 2007; Paelecke-Habermann, Pohl, & Leplow, 2005; Porter et al., 2003; Purcell et al., 1997; Rose & Ebmeier, 2006; Tavares et al., 2007) may lie in differences in clinical characteristics. In this study, all patients were recruited in the outpatient setting, were never hospitalized for their psychiatric disorder, were relatively young (all aged under 57), did not use any antidepressant medication other than SSRIs at the time of the scanning session, were moderately depressed on average, and were free of a diagnosis of alcohol abuse. Our findings are therefore unlikely affected by negative side effects of, for example, sedative drugs on visuospatial capabilities and concentration (Golombok, Moodley, & Lader, 1988). Instead, our findings may be representative of the on average mildly depressed outpatient sample.

Interestingly, cognitive dysfunction could contribute to the functional impairment experienced by the patients and could therefore constitute a predictor or reason for hospitalization (Elliott, 1998; Jaeger et al., 2006; Withall et al., 2009). Neurocognitive findings obtained in the inpatient setting may therefore not necessarily generalize to the outpatient population. Elliott et al. (1996) demonstrated that inpatient performed worse on several

neuropsychological test in a direct comparisons of MDD in- and outpatients. Importantly, the in- and outpatients did not differ on any clinical variable, including illness severity. In addition, Purcell et al. (1997) reported that in a group of young MDD outpatients, those with a history of hospitalization performed worse on executive tasks. Together, these findings suggest that cognitive dysfunction is not merely a symptom of the disease, but could be a predictor of clinical care utilization.

IMPLICATIONS FOR NEUROANATOMICAL MODELS OF MDD AND ANXIETY

ANTERIOR CINGULATE CIRCUITRY

Our results emphasize the importance of the rostral ACC for both MDD and anxiety disorders. The rostral ACC has been implicated in the cognition-emotion integration hub, also including the medial PFC and orbitofrontal gyrus (Mayberg, 1997). This structural abnormality is likely to affect brain function, although the relation between structural and functional abnormalities in psychiatric disorders is barely studied. However, the importance of the rostral (or pregenual) ACC region for adaptive emotion regulation has been emphasized in the study of both MDD (Mayberg, 1997; Seminowicz et al., 2004) and anxiety disorders (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006). Our results support the suggestion of a shared neuropathology in MDD and anxiety disorders, and indicate that this rostral or pregenual ACC subdivision should be considered for a general neuroanatomical model of psychopathology.

Functionally, the rostral ACC has been less frequently associated with depressed or anxious psychopathology than the subgenual ACC, a more ventral ACC locus. However, abnormal BOLD responses in the pregenual ACC have been observed and linked to altered glutaminergic metabolism in MDD (Walter et al., 2009). Deep brain stimulation of the subgenual ACC has been associated with immediate symptom improvement on symptoms such as restlessness, mood, interests, and motivation, even in treatment resistant MDD (Lozano et al., 2008). Also, this subgenual locus has been associated with treatment response in social anxiety disorder (Furmark et al., 2002). This may indicate that the subgenual ACC is a target area for successful treatment, but could also be considered an important starting point to understand the neuropathology of MDD and anxiety disorders.

A diffusion tensor imaging study that applied probabilistic tractography, a technique that calculates the direction of white matter tracts from any chosen seed, demonstrated that the subgenual and pregenual ACC are distinct regions in terms of structural connectivity (Johansen-Berg et al., 2008). Although both ACC subregions were found to project on the dorsal ACC, frontal poles, hypothalamus, and nucleus accumbens in healthy participants, the pregenual ACC was shown to have the strongest connections with the medial PFC and

dorsal ACC. The subgenual PFC on the other hand was most strongly connected to the amygdala, anterior hippocampus, nucleus accumbens, hypothalamus, and orbitofrontal cortex. Therefore, stimulating the subgenual ACC most likely influences the subcortical and (para)limbic emotion appraisal and generation regions, and could explain the immediate improvements in mood and motivation. In fact, deep-brain stimulation of the subgenual PFC decreased metabolism in the orbitofrontal cortex and medial PFC, also after six month follow up in treatment responders (Lozano et al., 2008).

Importantly, the pregenual ACC is anatomically connected to the dorsal ACC (Johansen-Berg et al., 2008). Functionally, the dorsal ACC has been found to influence amygdala activity negatively, both direct and via the posterior cingulate gyrus (Stein et al., 2007). In this thesis, we also described lower volume in the posterior cingulate gyrus in MDD and anxiety disorders, although below threshold. This finding suggests that connectivity of the dorsal ACC with the amygdala may be compromised in two ways: as a result of lower volume of the rostral and dorsal ACC, and as a result of lower posterior cingulate volume. Moreover, the pregenual ACC was found to inhibit amygdala activation during an emotional task in healthy controls, a process that was associated with emotional conflict resolution (Etkin et al., 2006). In treatment studies of MDD, the pregenual ACC was found to differentially influence metabolism in the subgenual ACC, orbitofrontal cortex, and dorsolateral PFC, depending on successful or unsuccessful treatment (Seminowicz et al., 2004). Finally, the pregenual ACC has been found to show abnormal connectivity with the anterior insula in MDD, dependent on illness severity (Horn et al., 2010). To sum up, the pregenual ACC appears an important region that is interconnected with a large number of other important regions implicated in neuroanatomical models of affect regulation. As it appears, the pregenual and subgenual ACC regions should both be considered important visceral regulation areas and should be considered at the center of an 'emotion regulation circuitry' affected in MDD and anxiety disorders.

In this context, our finding of reduced dorsal and rostral ACC gray matter (extending into the subgenual regions and adjacent white matter volume) in MDD indicates that communication with the dorsal ACC, subgenual ACC, orbitofrontal cortex, and medial PFC may be compromised in outpatients with MDD and/or anxiety disorders, leading to a range of symptoms. This suggestion is further supported by our findings of decreased connectivity of the medial PFC and ventral striatum with a salience network involving dorsal prefrontal regions, including the dorsal ACC, dorsomedial PFC, ventrolateral PFC, and dorsolateral PFC, although unrelated to volume of the rostral ACC. However, we did not investigate the connectivity within this task executive network in patients with anxiety disorders or comorbid depression-anxiety, and therefore the generalizability of this result to anxiety and comorbid depression-anxiety remains unclear. Previous studies have emphasized the importance of the medial PFC in both mood regulation through cognitive appraisal (Johnstone

et al., 2007; Ochsner et al., 2009), self-referential processing (Lemogne et al., 2009), and affective conflict processing (Ochsner et al., 2009). In summary, abnormal mood and anxiety regulation in MDD and anxiety disorders may be the result of compromised communication between rostral ACC - medial PFC, rostral ACC – dorsal ACC, dorsal ACC – amygdala, dorsal ACC – posterior cingulate gyrus – amygdala, amygdala – hippocampus, amygdala – subgenual ACC – orbitofrontal gyrus, originating from a common volumetric abnormality of the rostral ACC. Functional connectivity and structural connectivity studies should test whether deficient connectivity between these regions is linked to lower volume of the rostral ACC.

LATERAL FRONTAL AND TEMPORAL CIRCUITRY

In addition to the proposed common neuropathology, inferior frontal gyrus and the lateral temporal regions may moderate the emotional circuitry in a disorder specific manner. For example, Brodmann 45 in the inferior frontal gyrus, or ventrolateral PFC, projects on the dorsolateral PFC, medial PFC, orbitofrontal cortex (Price & Drevets, 2010). It has been suggested that the ventrolateral PFC could be regarded a multi-modal cortex which connects to circuits responsible for emotions as well (Price & Drevets, 2010). In the context of mood regulation in MDD, the ventrolateral PFC has been associated with increased activation when reappraising negative stimuli and abnormal engagement in a ventrolateral PFC - ventromedial PFC – amygdalar circuitry (Johnstone et al., 2007). This circuitry is associated with effort to down-regulate negative emotions. Therefore, abnormal volume of the right inferior frontal gyrus in MDD could contribute to this abnormal circuitry and affect the capability to regulate negative emotions. Additionally, increased left dorsolateral PFC activation was observed as a function of increasing planning load in moderately and severely depressed MDD patients, suggesting that even in the context of emotional non-demanding task demands, extra frontal resources are called upon to focus on external task demands. This finding may indicate that the dorsolateral PFC in the active depressed state is less potent in controlling dorsolateral PFC dependent emotion regulation. The superior temporal gyrus on the other hand, projects to the medial prefrontal circuitry as well (Price & Drevets, 2010), and thereby could affect medial PFC linked emotional regulation as well.

MEDIAL TEMPORAL AND LIMBIC CIRCUITRY

Although frequently associated with abnormal processing of emotional stimuli in MDD and anxiety disorders, only modest abnormalities in amygdalar function were observed. No volumetric differences were found in the amygdala. Abnormal activation was demonstrated in moderately to severely depressed MDD patients only during the encoding of negative words. Analysis of the emotional faces data acquired in the context of the NESDA neuroimaging study, although not subject of this thesis, failed to provide evidence for the suggestion that abnormal amygdalar and subcortical signaling in response to

sad and fearful faces is at the base of the disorders (Demenescu et al., 2010). However, the fact that no gross between group effect was observed in the amygdala in anxiety and comorbid depression-anxiety, does not rule out the amygdala as an important structure in the pathophysiology of depression and anxiety. For example, in an emotional conflict resolution task, the rostral ACC was found to inhibit amygdalar response in healthy controls (Etkin et al., 2006), whereas this inhibitory effect was absent in patients with generalized anxiety disorder (Etkin et al., 2010).

In the NESDA study, connectivity during 'resting state' was studied in first episode MDD patients. Veer et al. (2010) reported decreased connectivity of the amygdala and the frontal poles with an affective network including the anterior insulae, dorsal ACC, ventromedial PFC, temporal poles, and amygdalae. Also, using NESDA neuroimaging data, Cremers et al. (2010), demonstrated that connectivity of the right amygdala and the medial PFC during negative face viewing is positively correlated with neuroticism scores, whereas connectivity of the left amygdala and the dorsal ACC was found to correlate negatively with neuroticism in healthy controls. As mood and anxiety disorders are associated with high levels of neuroticism, it is likely that amygdalar-dorsal PFC connectivity maybe be altered in patients with MDD and/or anxiety disorders compared with controls. Finally, the orbitofrontal and subgenual anterior cingulate cortex may also affect amygdalar – medial PFC/ACC connectivity, as neuroticism was also found to be positively correlated with orbitofrontal and subgenual ACC volume (**Chapter 7**). Given the direct links between the orbitofrontal cortex and subgenual ACC on one hand and the amygdala on the other hand, neuroticism might further modulate amygdala – ACC connectivity via the orbitofrontal cortex. In conclusion, it is possible that amygdalar abnormalities become more evident and meaningful when studied in relation to activation of other regions.

In this, the hippocampus was only found to be functionally affected in MDD and anxiety disorders: hippocampal blunting was observed during positive word encoding. This result suggest that positive processing is altered in MDD, and most likely reflects diminished hedonic capacity. This suggestion was further supported by our finding of decreased connectivity of ventral striatal, including the nucleus accumbens and medial PFC areas within a salience network, related to classifying positive words. However, we could not test the effect of hedonic capacity in this study, as no measure of state anhedonia was administered on the day of scanning. Nevertheless, neural circuitry in MDD and anxiety could differ along a dimension of affective state, such as sad mood or lack of positive affect. This suggestion is supported by recent work, where an inability to maintain nucleus accumbens activation over time during positive affect regulation was related to individual differences in anhedonia in MDD patients (Heller et al., 2009). The nucleus accumbens is an important region in the human reward circuitry and these results suggest that the reward circuitry fails during positive affect regulation in MDD. Whether inter-individual variations in other depression or anxiety specific dimensions, such as sad mood or anxious

arousal, are related to a specific neural circuitry as well should be studied more extensively.

In summary, implications for models of emotional regulation in MDD and anxiety based on results presented in this thesis are 1) the rostral ACC should be implicated in a general model of affective disorders, as it is potent to influence a wide variety of regions implicated in normal emotional regulation; 2) the inferior frontal gyrus, dorsolateral PFC, and superior temporal gyrus could affect the emotional circuitry in a disorder specific way; 3) variations in regional brain volume related to risk factors could contribute to the understanding of the complex neuropathology of depression and anxiety disorders; 4) current mood state could influence the dynamics of the mood circuitry, as valence specific functional abnormalities were observed.

SAMPLE CHARACTERISTICS

The NESDA neuroimaging study is the first structural and functional MRI study implemented in a large observational and longitudinal framework. The NESDA study is unique in that it includes both measurements of phenomenological as well as biological factors and aims to integrate psychosocial and biological models in order to examine predictors of the long-term course and consequences of MDD and anxiety disorders. The major advantage of large-scale observational cohort studies is the possibility to include a large and well-characterized sample of participants with a large variety of symptoms, comorbidity, personal history, and other 'real life' characteristics. This allows epidemiologists to identify patterns of psychopathology related to biological factors, aetiology, treatment response, and outcome. Nevertheless, some issues should be considered.

First, since the epidemiological NESDA cohort was recruited through general practitioners and outpatient clinics, we may not have been fully able to capture the most severe end of the depressive and anxious spectrum. Also, patients that first participated in an intensive interview session and subsequently were willing to participate in a two-hour MRI- and interview session, may not be most representative of the MDD sub-sample with large motivational problems.

Furthermore, because in NESDA the focus is on MDD, dysthymia, social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder, other modules of the Composite International Diagnostic Interview (CIDI) were not included in the interview such as those of posttraumatic stress disorder, obsessive-compulsive disorder, eating disorders, bipolar disorder, and attention hyperactivity disorder. Although, participants with a formal diagnosis of a psychiatric disorder not subject of NESDA were not included in the NESDA sample, it is possible that a percentage of participants included in NESDA neuroimaging sample formally fulfilled diagnosis for a psychiatric disorder that is associated with specific brain abnormalities.

Related to this issue, the healthy controls included in this study were also

obtained from a 'real life' sample. All were life time free of a diagnosis or subthreshold diagnosis of MDD or anxiety disorders, but a portion of the healthy controls were recruited from an at-risk population, namely from the Adolescents at Risk for Anxiety and Depression (ARIADNE) cohort study. The ARIADNE study focuses on the offsprings of patients with mood- and anxiety disorders. Also, for healthy controls recruited through the general practitioners, having a first-degree relative with a psychiatric disorder was not an exclusion criterion. Several neuroimaging studies focusing on cognitive and emotional processes in healthy subject, but also in psychiatric samples, included 'super' healthy control participants without a first degree relative with mood- and anxiety disorders or history of childhood trauma. The inclusion of including 'real life' healthy controls in NESDA had the major advantage of allowing us to study the effects of risk factors for developing mood- and anxiety disorders across patients and healthy controls, but may constitutes a source of discrepancy between our study and some published neuroimaging studies. A second advantage of this recruitment strategy is that our healthy controls were also recruited from NESDA, underwent the same screening as patients, and were scan-naïve. Therefore, in this study, familiarity with researchers and/or experimental procedures could be ruled out as a potential confound. In contrast, earlier studies included a control group consisting of, for example, staff members (Elliott et al., 1997; Fitzgerald et al., 2007; Okada et al., 2003). In these studies, it remains therefore unclear whether functional brain abnormalities are related to the psychopathology under study, or are related to the differences in state anxiety due to the novelty of the situation.

Finally, in this thesis, panic disorder, social anxiety disorder, and generalized anxiety disorder were studied in concert, as if these disorders are not characterized by syndrome specific abnormalities. However, panic disorder, social anxiety disorder, and generalized anxiety disorder have been associated with unique activation patterns in imaging studies and a unique neurobiology (Blair et al., 2008; Mathew, Coplan, & Gorman, 2001). Nonetheless, we did not aim to study anxiety disorder specific features, but concentrated on the shared neurophysiological features of prevalent anxiety disorders that frequently co-occur with MDD.

MRI

In the present thesis, magnetic resonance imaging was administered for measures of brain volumetry and brain activation related to executive and emotional processes. MRI could be used for other measurements as well, including. but not limited to, white matter tract integrity measurements and measurements of metabolites in brain tissue (e.g., glutamate). Other imaging techniques available have their specific advantages and disadvantages with respect to spatial and temporal resolution. In this section, considerations with respect to the currently administered imaging techniques will be discussed.

Although a wealth of information on brain tissue properties and oxygenation

differences is available in these scans, the techniques have their limitations. The MRI techniques used in this study are able to measure brain morphometry and activation at a spatial resolution of one or several millimeters and are capable to estimate volume and activation at this spatial resolution. The temporal resolution of fMRI in our study was 2.3 seconds, meaning brain tissue was imaged once in 2.3 seconds. Together with the spatial resolution of our fMRI sequence of 3 mm, observations in one 3D volume unit of 3 x 3 x 3 millimeter (1 voxel), this means that no microscopic observations can be made and that single cell firing or the net result of firing of groups of neurons at a resolution of milliseconds could not be recorded. In the case of BOLD-fMRI, we measured the net result in heamodynamic changes of a large number of neurons, related to neural firing (Logothetis,2002). Subsequently, we compared activation in one region during a certain condition to activation in that region to another condition, and therefore calculated a relative activation measure. In **Chapter 7**, we calculated the correlation of low frequency BOLD fluctuations as a measure of intrinsic connectivity. However, BOLD imaging can not distinguish between inhibitory and excitatory neural processes, and comparisons of absolute activity between two or more brain regions can not be made (Logothetis, 2002). Also, with the use of the currently used imaging techniques, no information on neurotransmitter concentration or activity was obtained. Disregulated neurotransmission is thought to play a major role in depression and anxiety disorders and likely affects regional neuronal activity or connectivity. Studying binding of pharmacological agents and neurotransmitter metabolism across the brain would be highly interesting and would provide important additional information on the neuropathology of MDD and anxiety disorders. However, such measurements could not be obtained with the techniques used in this study. In addition, the temporal resolution of functional MRI is poorer than for electroencephalography, and therefore does not allow to record very rapid neuronal firing. Nevertheless, MRI is one of the preferable techniques to study the brain, as it has the advantage of allowing studying both activation and structure in the same session. Moreover, fMRI allows studying activation deep in the brain, such as limbic activity, in a non-invasive way without the need to ingest a radioactive tracer.

CONSIDERATIONS FOR FUTURE OBSERVATIONAL MRI STUDIES

Structural MRI serves as an excellent tool to study the brain's morphometry in an observational set-up. Structural imaging techniques are minimally affected by day to day variations in symptomatology, medication use, time of scanning and site of scanning when similar MRI machines are used. Using functional MRI in an observational study is more problematic due to the large number of factors that is potent to influence the BOLD pattern. BOLD patterns are more likely to be influenced by factors such as time of day, quality of sleep the night before the scanning session, and subjective tension level during the scanning

session. Therefore, cross-sectional analysis could be insufficiently able to detect variability in BOLD patterns related to a current diagnosis owing to other structural noise factors.

For future observational functional MRI studies, a design that allows for an intra-subject challenge of subcortical/(para)limbic-cortical activity is recommended. For example, performing the Tower of London planning task before and after a positive or depressed mood induction session, or a pharmacological challenge, allows to study within-subject variability in response to a fixed experimental factor. Such a design would allow to study the flexibility or adaptability of the mood regulation circuitry within patients, a design that would be less sensitive to between subject factors such as quality of sleep, time of day etc. Monitoring within scanner variability over time and between-scanner variability is also highly recommended in order to get an estimate of the variability in the data due to scanner variability.

Finally, using the data obtained from the same sample for several publications is defensible considering the costs and effort taken by both researchers and participants. However, before setting up a large study, the maximal number of publications resulting from (sub) data sets should be determined, in order to set an appropriate alpha for considering significance for each report in order to sufficiently and appropriately control for multiple comparisons.

IMPLICATIONS

Our results suggest that MDD and anxiety disorders do not constitute distinct entities when considering their neuroanatomical and functional MRI profile, but instead share characteristics that may explain the maladaptive emotion regulation. In the case of comorbid depression-anxiety, researchers, and possibly clinicians as well, should keep in mind that the comorbid depression-anxiety is not simple MDD plus anxiety when considering structural and functional abnormalities related to emotional regulation. Instead, comorbid depression-anxiety might be better characterized by anxiety neuropathology as the primary neuropathology. For example, comorbid depression-anxiety disorder does not resemble MDD with respect to executive abnormalities and encoding of negative information. Therefore, in the study of regional volume or activation, this comorbidity of anxiety disorders may constitutes an important predictor instead of a confounder.

MDD and anxiety disorders appear to share functional and structural abnormalities, while at the same time, the disorders appear to be characterized by syndrome specific abnormalities. This pattern of both shared and unique characteristics seems to parallel the symptomatology of MDD and anxiety: large overlap in symptoms exists, but disorder specific symptoms are present as well. A more dimensional approach in characterizing both phenomenology and neurobiology of depression and anxiety disorders could aid to identify the shared neuropathology related to shared symptom dimensions. Such

an approach might also better capture the variability in neural abnormalities related to illness severity.

We demonstrated that childhood emotional maltreatment and variations in personality factors are also related to variations in volume of regions associated with emotional regulation. Although the anatomical correlates of emotional maltreatment was observed independent of psychopathological status, this aetiological factor could influence the neural circuitry normally responsible for adaptive emotion regulation. Variation in personality may also affect the neurocircuitry: orbitofrontal and subgenual ACC volume variations may affect normal emotional regulation. It is therefore recommended that future imaging studies do not consider early advert life events including childhood emotional maltreatment and variations in personality structure as confounds, but instead considered these factors as potentially important predictors of neurobiological variation in MDD and anxiety disorders.

Finally, symptom severity does not influence every aspect of brain structure and function as measured in the NESDA neuroimaging study. We observed shared structural and functional abnormalities in both MDD and anxiety disorders, even in the remitted state. This may further underline the importance of regular check-ups and 'maintenance' in patients with a (recent) history of depression and/or anxiety disorders. Training in focusing on positive events, for example in the context of mindfulness-based therapy may protect patients from relapsing into a new episode of depression and anxiety. However, the effects of such training on brain circuitry in remitted patients need to be further investigated.

SUGGESTIONS AND CHALLENGES FOR FUTURE STUDIES

SUGGESTIONS FOR FUTURE IMAGING STUDIES

In future neuroimaging studies that aim to get insight in the shared neuropathology of MDD and anxiety, connectivity of the prefrontal cortex and ventral regions should be studied in patients with anxiety disorders and directly compared to connectivity patterns in patients with MDD. In that way, the specificity of our finding of a decreased coupling of ventral striatal and medial PFC regions with a task executive network during execution of an emotional task could be tested. Related to this suggestion, studying subcortical-cortical connectivity during manipulation of mood or anxiety status, for example with the use of autobiographical scripts or pharmacological challenges, would provide important information. In this way the connectivity and the flexibility of the neural circuitry in different mood states could be tested. Such manipulations would tell us about the dynamics of the emotion generation and regulation system for several emotional states. Also, the brain circuitry involved in emotional regulation could be experimentally manipulated, for example with the use of neurostimulation techniques, thereby giving further insight in the dynamics on the neural circuitry and emotions experienced by the patient.

Furthermore, providing a challenge when studying cognitive processes in cross-sectional studies is important. Such a challenge could be a mood or stress induction session before a specific task. Also, challenges could be integrated in the task. For example, a mixed design including alternating blocks of executive trials without emotional distraction and executive trials with emotional distraction could be administered, thereby testing the limits of executive control in MDD and anxiety disorders. Such a design could be more sensitive for detecting emotional relevant disturbances in attentional control, as suggested by Wang et al (2008). Furthermore, inclusion of a mood and anxiety regulation task would be essential in the study of depression and anxiety.

Next, characterizing patients along dimensions of depression and anxiety related symptom dimensions, as proposed by dimensional models of psychopathology, could perhaps better capture the structural and functional correlates of current mood and anxiety status than a categorical characterization. In this study, we observed that inter-subject variability in factors such as personality dimensions was associated with regional brain volume in healthy controls. It is possible that using the between subject variation within patients in an more optimal way, by for example describing their symptomatology in a multi-dimensional space could help us further unravel the complex story of brain involvement in mood and anxiety disorders.

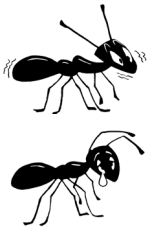
Finally, multivariate and multi-model approaches could help us find differentiating patterns of abnormal volume or activity in MDD and anxiety disorders. So far, univariate approaches have been predominantly used in the study of MDD and anxiety disorders, where a univariate test is performed per smoothed voxel. These methods may be less sensitive in detecting differentiating patterns in the highly integrated network of limbic-cortical regions. Multivariate analyses approaches are sensitive to spatially distributed information, whereas using univariate approaches test for between group differences per voxel. Using multivariate approaches could lead to the detection of highly differentiating features that could also be used in disease state prediction. Such multivariate approaches would both increase the sensitivity ánd specificity of functional and structural imaging findings. In addition, integrating imaging data acquired using different modalities, could help understanding the complex interactions of different regions. Combining information of regional volume, regional brain activation, metabolism, and functional and structural connectivity would be essential to understand the complex dynamics of adaptive and maladaptive mood regulation.

CHALLENGES: TREATMENT RESPONSE AND COURSE PREDICTION

A major challenge for future neuroimaging studies in MDD and anxiety disorders is to identify a set of features that could predict vulnerability for

developing MDD and anxiety disorders, illness course and treatment response. Currently, treatment response is unsatisfactory: up to 60% does not reach a good response (Ressler & Mayberg, 2007) and only a small percentage responds to mono-therapy with an SSRI. Being able to predict who will respond to mono-therapy with an SSRI or cognitive behavior therapy, or a combination of the two, would be very useful in clinical practice. Longitudinal studies will be very important for identifying such predictive features. Long-term outcome and treatment response could then be predicted from functional and structural MRI patterns, in combination with detailed information on symptomatology, occurrence of life events, medical and psychiatric comorbidity, family status of depression and anxiety, personality, psychological measures including dysfunctional belief systems, and occurrence of childhood maltreatment. So far, studies have indicated cognitive, structural, and functional features that were associated with treatment responses (Canli et al., 2005; Chen et al., 2007; Langenecker et al., 2007; Li et al., 2010). Symptom improvement in MDD have been associated with amygdalar response (Canli et al.,2005), ACC and insular volume (Chen et al., 2007), dorsolateral PFC volume (Li et al., 2010), and ACC activity (Chen et al., 2007). Including genetic variations could be very useful as well. Not only have genetic variations in, for example, the serotonin transporter gene been associated with the risk of developing mood- and anxiety disorders in the context of adverse life events (Caspi et al., 2003). Furthermore, the serotonin transporter gene has been associated with variations in amygdala response (Bertolino et al., 2005; Hariri et al., 2005; Lau et al., 2009), amygdala – ACC connectivity (Pezawas et al., 2005), and brain volume in (para)limbic regions (Frodl et al., 2008a) in healthy controls. Moreover, genetic variations have been related to treatment response, associated with responsiveness of the amygdala and ACC during emotional processing (Baune et al., 2010). Such multi-factorial models, including amongst others clinical, genetic, and neuroimaging information could then be used to provide an optimal treatment strategy for patients. As NESDA obtained information on all these measures, a first step in setting up such a model will be taken in the nearby future.

CHAPTER 9



APPENDIX: DSM-IV CRITERIA
REFERENCE LIST
LIST OF ABBREVIATIONS
DUTCH SUMMARY/NL SAMENVATTING
LIST OF PUBLICATIONS
CURRICULUM VITAE

MAJOR DEPRESSIVE DISORDER

A Major Depressive Episode (MDE) is characterized by 1) depressed/low mood and/or 2) loss of interest or pleasure in daily activities during most of the day, nearly every day, for at least two weeks. Next to these core symptoms of depressed mood or loss of the capacity to experience pleasure (also know as 'anhedonia'), an MDE is characterized by at least three (or four when only one of the first two criteria are met) of the following symptoms that are present nearly every day:

- weight loss or weight gain when not dieting;
- insomnia or hypersomnia (loss of sleep or excessive sleeping);
- psychomotor agitation or retardation (restlessness or slowing of daily activity);
- fatigue or loss of energy;
- feelings of worthlessness or excessive or inappropriate guilt;
- attentional problems or indicisiveness;
- recurrents thoughts of death or recurrent suicidal ideation without a specific plan or a suicide attempt or plans for committing suicide.

Symptoms are not better accounted for by bereavement or a diagnosis of mania or personality disorder. Social, occupational, educational or other important functioning should be impaired to fullfil the criteria for a diagnosis of MDD.

SOCIAL ANXIETY DISORDER

Social Anxiety Disorder (SAD), also know as 'social phobia' is characterized by a persistent fear of one or more social or performance situation in which the person is exposed to unfamiliar people or to possible scrutiny by others and the individual fears that he or she will act in a way (or show anxiety symptoms) that will be embarrassing and humiliating. A diagnosis is made when:

- exposure to the feared situation almost invariably provokes anxiety, which may take the form of a situationally bound or situationally pre-disposed panic attack (see Panic Disorder);
- the person recognizes that this fear is unreasonable or excessive;
- the feared situations are avoided or else are endured with intense anxiety and distress;

The avoidance, anxious anticipation, or distress in the feared social or performance situation(s) interferes significantly with the person's normal routine, occupational/educational functioning, or social activities or relationships, or there is marked distress about having the phobia. Also, the

fear or avoidance is not due to direct physiological effects of a substance (e.g., drugs, medications) or a general medical condition and is not better accounted for by another mental disorder.

PANIC DISORDER

Panic Disorder (PD) is characterized by recurrent unexpected Panic Attacks (see below), and at least one of the attacks has been followed by 1 month (or more) of one (or more) of the following:

- persistent concern about having additional attacks;
- worry about the implications of the attack or its consequences (e.g., losing control, having a heart attack, "going crazy");
- a significant change in behavior related to the attacks;

The Panic Attacks are not due to the direct physiological effects of a substance (e.g., drugs, medications) or a general medical condition (e.g., hyperthyroidism) and are not better accounted for by another mental disorder.

PANIC ATTACK:
A panic attack is a discrete period of intense fear or discomfort, in which four (or more) of the following symptoms developed abruptly and reached a peak within 10 minutes:

- palpitations, pounding heart, or accelerated heart rate;
- sweating;
- trembling or shaking;
- sensations of shortness of breath or smothering;
- feeling of choking;
- chest pain or discomfort;
- nausea or abdominal distress;
- feeling dizzy, unsteady, lightheaded, or faint;
- derealization (feelings of unreality) or depersonalization (being detached from oneself);
- fear of losing control or going crazy;
- fear of dying;
- paresthesias (i.e, numbness or tingling sensations);
- chills or hot flushes.

AGORAPHOBIA
A diagnosis of PD is further specified as PD with agoraphobia or without agoraphobia. Agoraphobia is the anxiety about being in places or situations from which escape might be difficult (or embarrassing) or in which help may

not be available in the event of having an unexpected or situationally predisposed Panic Attack or panic-like symptoms. Agoraphobic fears typically involve characteristic clusters of situations that include being outside the home alone; being in a crowd, or standing in a line; being on a bridge; and traveling in a bus, train, or automobile. As a result, the situations are avoided (e.g., travel is restricted) or else are endured with marked distress or with anxiety about having a Panic Attack or panic-like symptoms, or require the presence of a companion.

GENERALIZED ANXIETY DISORDER

Criteria for the diagnosis of a Generalized Anxiety Disorder (GAD) are met when a person experiences at least six months of excessive anxiety and worry about a variety of events and situations and there is significant difficulty in controlling the anxiety and worry. Also, three or more of the following symptoms should be present for most days over the previous six months:

- feeling wound-up, tense, or restless;
- easily becoming fatigued or worn-out;
- concentration problems;
- irritability;
- significant tension in muscles;
- difficulty with sleep.

The symptoms cause significant distress or problems in daily life functioning, but are not due to the direct physiological effects of a substance (e.g., a drugs, medications) or a general medical condition and are not better accounted for by another mental disorder.

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LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ANX	Anxiety disorders (PD, SAD, GAD)
BA	Brodmann Area
BOLD	Blood Oxygenated Level Dependency
CEM	Childhood Emotional Maltreatment
CDA	Comorbid Depression-Anxiety
dACC	Dorsal Anterior Cingulate Cortex
DLPFC	Dorso-Lateral PreFrontal Cortex
dmPFC	Dorso-Medial PreFrontal Cortex
EF	Executive Functions
FMRI	Functional Magnetic Resonance Imaging
GAD	Generalized Anxiety Disorder
GM	Gray Matter
HPA-axis	Hypothalamus-Pituitary-Adrenal axis
IFG	Inferior Frontal Gyrus
MDD	Major Depressive Disorder
mPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
OFC	Orbitofrontal Cortex
PCC	Posterior Cingulate Cortex
PD	Panic Disorder
PET	Positron Emission Tomography
rACC	Rostral Anterior Cingulate Cortex
SAD	Social Anxiety Disorder
sgACC	Subgenual Anterior Cingulate Cortex
SPECT	Single Photon Emission Computed Tomography
SSRI	Selective Serotonin Reuptake Inhibitor
TE	Echo Time
ToL	Tower of London
TR	Time to Repetition or Repetition Time
WM	White Matter

Depressie en angststoornissen zijn veel voorkomende psychiatrische stoornissen die gepaard gaan met een hoge ziektelast voor de persoon en zijn omgeving. Ongeveer 30% van de mensen die aan een depressieve stoornis lijdt, lijdt ook aan een angststoornis, terwijl ongeveer 50% van de mensen die aan een angststoornis lijdt ook ooit een depressieve episode door zal maken. Wanneer depressie en angst samen voorkomen, wordt gezegd dat de stoornissen 'comorbide' voorkomen. Deze comorbide stoornis van angst en depressie is in verband gebracht met een langere ziekteduur, meer verlies van arbeidsproductiviteit, en ernstigere symptomen of klachten. Doordat depressie en angst zo vaak samen voorkomen, maar ook door de overlap in klachten en symptomen die patiënten rapporteren, is voorgesteld dat depressieve- en angststoornissen ook een gedeelde overlap hebben in biologische factoren die kunnen bijdragen aan het ontstaan van de stoornissen.

Beeldvormend onderzoek bij patiënten met depressie heeft uitgewezen dat een aantal structuren een ander volume of activiteitsniveau hebben dan in mensen zonder deze stoornissen. De afwijkingen zijn voornamelijk gevonden in gebieden die in eerdere studies geassocieerd zijn met het waarnemen en reguleren van emoties. Een aantal van deze afwijkingen is ook gevonden in mensen met angststoornissen, maar de patiëntengroepen zijn nog nooit samen onderzocht. Het is dus onduidelijk of er gedeelde abnormaliteiten in de structuur en het functioneren van een aantal hersengebieden in angst en depressie zijn. Ook hebben eerdere studies geen rekening gehouden met de comorbiditeit van depressie en angst.

Het doel van de studies opgenomen in dit proefschrift is om te onderzoeken of depressie en angst afwijkingen in de structuur en activatie van een aantal hersengebieden delen, of juist worden gekenmerkt door unieke afwijkingen. Deze gedeelde afwijkingen zouden kunnen verklaren waarom angst en depressie zo vaak samen voorkomen en kunnen helpen de complexe pathologie van depressie en angst te begrijpen. In dit proefschrift ligt het focus op hersengebieden die in eerdere studies geassocieerd bleken te zijn met emotionele verwerking. Zowel de hersenfunctie als de hersenstructuur zijn onderzocht, en er is rekening gehouden met het gebruik van medicatie. Alle studies beschreven in dit proefschrift maken uit van de neuroimaging deelstudie van de Nederlandse Studie naar Depressie en Angst (NESDA: www.nesda.nl).

In **hoofdstuk 1** wordt het klinisch beeld van depressie en angststoornissen besproken en wordt de probleemstelling uiteengezet. Depressie is een zeer veel voorkomende psychiatrische stoornis die gekenmerkt wordt door een sombere stemming en/of een verminderd vermogen om plezier te beleven aan activiteiten of situaties waaraan in normale toestand wel plezier wordt beleefd. Om aan de diagnose 'depressie' te voldoen moet deze sombere stemming of het gebrek aan plezier of interesse minstens 2 weken aanhouden en het dagelijks functioneren beïnvloeden. Naast deze 'kernsymptomen' (stemming en verminderd plezier/interesse) lijden mensen met een depressie volgens de

criteria nog aan minstens drie van de volgende symptomen: verstoord slapen (veel meer of veel minder slapen), verstoorde eetlust (meer of minder eetlust), verminderd concentratievermogen, verstoord bewegingspatroon (rusteloos of juist veel minder beweeglijk), suïcidale gedachten, en gevoelens van waardeloosheid of extreme schuld. De angststoornissen waarover in dit proefschrift gesproken wordt, zijn: de paniekstoornis, de sociale angststoornis (ook wel sociale fobie genoemd) en de gegeneraliseerde angststoornis. Kortweg worden deze stoornissen gekenmerkt door een extreme angst voor het krijgen van een paniekaanval (paniekstoornis), voor sociale omgang of sociale prestatie (sociale angststoornis), of door algemene extreem zorgelijke gedachten (gegeneraliseerde angststoornis). We spreken van stoornissen wanneer de klachten in belangrijke mate het normale sociaal en beroepsmatig functioneren beïnvloeden.

Zoals eerder besproken, komen depressie en angststoornissen veel voor. Ongeveer een derde van de westerse bevolking zal eens in zijn of haar leven een depressieve stoornis doormaken, en voor angst zijn die cijfers ongeveer gelijk. Daarbij zal ongeveer de helft van de mensen die ooit een angststoornis krijgt, ook een depressie doormaken, terwijl ongeveer 30% van de mensen met een depressieve stoornis ooit ook aan een angststoornis zal lijden. In de meeste gevallen is de angststoornis de eerste stoornis die zich in het leven van een patiënt manifesteert, en het samen voorkomen van angst en depressie is geassocieerd met een slechter ziektebeloop (langer ziek), ernstigere symptomen, en meer uitval op het werk. Het lijkt er dus op dat mensen met depressie én angststoornissen slechter af zijn dan mensen met alleen een depressie (of angststoornis). Om deze reden is voorgesteld dat de depressie mét en de depressie zonder tegelijk optredende angststoornis als aparte stoornissen gezien zouden moeten worden. Tegelijkertijd wordt aangenomen dat de stoornissen neurobiologische afwijkingen delen, omdat zowel angststoornissen als depressies redelijk effectief behandeld kunnen worden met eenzelfde soort behandeling (antidepressiva of (cognitieve) gedragstherapie).

Een aantal studies heeft laten zien dat de activiteit of het volume van bepaalde hersenengebieden bij mensen met angst en depressie verschillen van die bij mensen zonder deze stoornissen. Hersenactiviteit en volume kunnen gemeten worden met behulp van een MRI scanner. MRI staat voor Magnetische Resonantie Imaging (vertaling: Beeldvorming). MRI scanners kunnen met verschillende instellingen een groot aantal soorten weefsel en processen afbeelden. In het geval van functionele MRI wordt met behulp van magneetvelden en radiogolven gemeten wat de verhouding is tussen zuurstofarm en zuurstofrijk bloed in het hele brein. Zuurstof hecht zich aan hemoglobine, een molecuul dat ijzerhoudend is en magnetische eigenschappen bezit. De binding van zuurstof aan hemoglobine beïnvloedt de magnetische eigenschappen van hemoglobine en op deze manier kan indirect activiteit afgeleid worden. Hierbij wordt aangenomen dat meer zuurstof

gebruik neuronale activiteit weerspiegelt, omdat neuronen in hersengebieden die actief zijn meer zuurstof gebruiken als ze actief zijn en minder zuurstof gebruiken als ze juist minder actief zijn. Het maken van een serie scans achter elkaar maakt dan de activiteit van de hersenen zichtbaar als een functie van tijd. Het geeft ook de mogelijkheid te berekenen hoe actief bepaalde hersengebieden zijn tijdens het uitvoeren van specifieke opdrachten.

De gebieden die zich anders lijken te gedragen bij depressie en angststoornissen zijn gebieden die in verband zijn gebracht met de primaire of vroege waarneming van emoties, zoals de amygdala (amandelkernen), de insulaire schors, en het onderste deel van de cingulaire schors. Deze gebieden liggen vrij centraal en diep in de hersenen. Andere, vaak iets hoger in het brein (richting schedel) gelegen hersengebieden, zijn ook in verband gebracht met angst en depressie. Gebieden in de 'frontale schors' (in het voorhoofd) zijn betrokken bij het aanpassen van de allereerste respons (bijvoorbeeld die in de amandelkernen) en dus met het reguleren van emoties. In een voorbeeld: Bij het zien van een foto van een huilend kind zullen de amandelkernen meer actief worden, en zal de ervaren emotie waarschijnlijk verdriet of medelijden zijn. Als je dan bedenkt dat het kind moet huilen omdat het blij is met een cadeau, zal het gevoel over die foto wellicht veranderen. Het is dan waarschijnlijk dat de frontale schors meer actief wordt, waardoor mogelijk ook de activiteit van de amandelkernen gedempt wordt. Aangenomen wordt dat dit mechanisme bij depressie verstoord is. Mensen met depressie lijken meer amygdala (amandelkern) activiteit te vertonen wanneer er negatieve informatie verwerkt wordt. Tegelijkertijd lijkt het erop dat de frontale schors minder actief is,en ook minder in staat is om de amandelkernen te dempen. Hierdoor kan een ontregeling van de emoties ontstaan waarbij meer negatieve emoties ervaren worden. Hoe dit 'circuit' van onder andere de amandelkernen en de frontale schors verstoord is in mensen met een paniekstoornis, sociale angststoornis, en/of gegeneraliseerde angststoornis is minder goed onderzocht dan in mensen met een depressieve stoornis. Ook is onduidelijk of in de ziekere groep van mensen met depressie én angst deze verstoring ook ernstiger is.

Naast een verstoorde activiteit is bij mensen met een depressie ook in een aantal gebieden in de hersenen een verminderd volume gevonden. Zo is bijvoorbeeld een verminderd volume gevonden in de hippocampus, een gebied dat belangrijk is voor stressregulatie en voor geheugenvorming, in de frontale schors, en in de cingulaire schors. Deze laatste schors wordt gezien als een gebied dat belangrijk is voor de integratie van primaire emotionele informatie en van cognitieve processen, die samen leiden tot een passende emotie. De cingulaire schors kan in het bovengenoemde voorbeeld van de foto gezien worden als het schakelstation tussen de amandelkernen en de frontale schors. Hoewel verminderd volume van bijvoorbeeld de cingulaire schors ook gevonden is bij mensen met paniekstoornis, is onduidelijk of het om precies hetzelfde gebied gaat, omdat er geen directe vergelijking met depressieve patiënten is gemaakt. Ook is het onduidelijk hoe deze bevindingen beïnvloed

zijn door het gelijktijdig aanwezig zijn van een depressie, omdat de onderzoekers patiënten met paniekstoornis én een depressie niet buiten de studie hadden gelaten.

In de eerste studie beschreven in dit proefschrift (**hoofdstuk 2**) wordt het volume van een aantal structuren die belangrijk zijn voor emotionele verwerking onderzocht bij mensen die lijden aan een depressie, een angststoornis of aan beide. In **hoofdstuk 2** wordt aangetoond dat de cingulaire schors, en dan specifiek het deel dat voor het corpus callosum ligt (de zogenaamde rostrale of pregenuale cingulaire schors), een verminderd volume heeft bij mensen met alleen een depressieve stoornis, alleen een angststoornis of zowel een depressieve als een angststoornis. Deze afname treedt onafhankelijk van medicatiegebruik en ziekte-ernst op. Het is voor het eerst dat deze afname aangetoond is voor zowel angst en depressie, en dat gelet is op het samen voorkomen van de stoornissen. Naast deze gedeelde abnormaliteit in hersenstructuur bij depressie en angst, werden ook stoornisspecifieke afwijkingen gezien. Zo is alleen de depressieve stoornis (depressie) geassocieerd met een verminderd volume van een gebied in de laterale frontale schors (gyrus frontalis inferior), dat ook geassocieerd is met emotieregulatie. Mensen met alleen een angststoornis vertoonden een afname van de gyrus temporalis superior, een gebied dat vaker geassocieerd is met angst en met het verwerken van lichaamssignalen. Het kan dus zijn dat deze afname weerspiegeld dat mensen die aan angststoornissen lijden, minder goed in staat zijn om de signalen die door het eigen lichaam gegeven worden correct te interpreteren. Dit is vooral in het geval van de paniekstoornis goed denkbaar. Tenslotte wordt in **hoofdstuk 1** aangetoond dat het voorkomen van van een depressieve stoornis voor het achttiende levensjaar geassocieerd is met een lager volume in het deel van de cingulaire schors dat onder het corpus callosum ligt, de zogenoemde subgenuale cingulaire schors. Deze afname kan een grotere gevoeligheid voor het ontwikkelen van depressieve en angststoornissen weerspiegelen, omdat dit gebied meermalen geassocieerd is gebleken met afwijkingen in primaire emotionele verwerking.

Vervolgens wordt in **hoofdstuk 3** bestudeerd of emotionele informatie (positief danwel negatief) anders verwerkt en onthouden wordt door mensen met depressie en angst. In het onderzoek zijn in de scanner 40 positieve, 40 negatieve en 40 neutrale woorden aan de deelnemers getoond en door hen beoordeeld op de emotionele betekenis (positief, negatief, of neutraal; bijvoorbeeld 'dakpan' [neutraal] of verlies [negatief]). Tijdens het beoordelen van de woorden zijn scans gemaakt. Na een pauze van ongeveer 20 minuten zijn dezelfde woorden weer getoond, tezamen met 120 nieuwe woorden. Weer maakten we scans, maar nu moesten de deelnemers aangeven of ze de woorden wel of niet hadden gezien tijdens het eerste deel van de taak. Het blijkt dat de geheugenprestatie van mensen met angst en depressie niet slechter is,

maar dat mensen met een depressie, de positieve woorden iets langer bekijken voordat een beslissing genomen wordt. De MRI-resultaten wijzen daarnaast uit dat zowel de depressieve stoornis als de angststoornis geassocieerd is met een verminderde activiteit van de hippocampus tijdens het verwerken van positieve woorden die later onthouden bleken te zijn. Deze hippocampale abnormaliteit trad op onafhankelijk van ziekte-ernst en onafhankelijk van medicatiegebruik en was afwezig tijdens het beoordelen van negatieve woorden. De hippocampus is een zeer complexe en belangrijke structuur voor het opslaan van informatie, maar ook voor het koppelen van nieuwe informatie aan al bestaande informatie. Dat er een abnormale activatie optreedt tijdens het verwerken van juist positieve informatie is opmerkelijk en lijkt aan te geven dat positieve informatie als minder belangrijk bestempeld wordt. Hierdoor wordt de positieve informatie mogelijk minder aan al bestaande herinneringen gekoppeld en is positieve informatie mogelijk minder in staat is om de stemming op een positieve manier te beïnvloeden. Dat deze verminderde hippocampus activiteit ook optreedt in mensen die weer hersteld zijn van hun depressie of angststoornis, lijkt aan te geven dat ook wanneer de symptomen afwezig zijn, positieve informatie anders verwerkt wordt. Mogelijk maakt dit mensen kwetsbaar maakt voor een terugval. Hoewel we verwachtten dat mensen met een depressieve stoornis negatieve woorden relatief beter zouden onthouden, konden we dit niet aantonen. Wel zagen we dat tijdens het onthouden van negatieve woorden de insulaire schors meer activiteit vertoonde, en dat bij de ernstig depressieve patiënten de amandelkernen, het striatum en de prefrontale schors meer activiteit lieten zien. Deze laatste be-vinding onderstreept de suggestie dat wanneer patiënten depressief zijn, negatieve informatie anders verwerkt wordt, wat een verklaring kan zijn voor het op de voorgrond treden van negatieve emoties.

In **hoofdstuk 4** wordt vervolgens het functioneren van de prefrontale schors tijdens het uitvoeren van een niet-emotionele cognitieve taak bestudeerd en beschreven. Het wordt veelal aangenomen dat in depressie de uitvoerende functies, ook wel executieve functies genoemd, verstoord zijn. Onder de executieve functies vallen onder meer de volgehouden en verdeelde aandacht, het actief onderdrukken van storende informatie uit de omgeving, en het maken en uitvoeren van plannen. Deze functies doen een aanspraak op de frontale schors en de gedachte is dat het functioneren van de frontale schors bij depressie abnormaal is, wat een verklaring zou kunnen zijn, of zou kunnen samenhangen, met de verminderde aandachtsfunctie en de suboptimale emotionele regulatie. Bij depressie zijn maar weinig neuroimaging studies uitgevoerd die zich richten op het complexe proces van plannen. Tijdens de Tower of London planningstaak moesten deelnemers een plan van aanpak voor een ruimtelijk probleem maken, dat plan in gedachten uitvoeren, en tijdens de uitvoering in de gaten moesten houden dat wat ze deden, nog steeds tot het juiste resultaat zou leiden. Van alle deelnemers is vervolgens de hersenactiviteit

berekend die samenhangt met het steeds moeilijker worden van de opdrachten. Deze patronen van activiteit zijn vervolgens vergeleken tussen de patiëntengroepen. De resultaten tonen aan dat mensen met een depressie de dorsolaterale prefrontale schors meer activeren om op eenzelfde prestatieniveau uit te komen dan gezonde controles.. Het verschil in activiteit werd het sterkst waargenomen bij mensen met huidige depressieve klachten, en was vrijwel normaal in mensen bij wie de depressie in remissie was gegaan. Opmerkelijk genoeg vertoonden mensen die zowel aan een depressie als aan een angststoornis leden vrijwel normale activiteit in de dorsolaterale prefrontale schors. Wel is duidelijk dat in de groep mensen met alleen een angststoornis de hersenactivatie normaal is tijdens de planning taak. Deze bevinding geeft aan dat wanneer het op de pure cognitieve functies aankomt, patiënten met een depressieve en angstige stoornis van elkaar te onderscheiden zijn en dat frontale abnormaliteit tijdens planning specifiek voor depressie specifieke lijkt en samenhangt met de ernst van de depressie.

In **hoofdstuk 5** wordt de hypothese getoetst dat de prefrontale schors abnormale verbindingen heeft met de dieper gelegen emotionele perceptiegebieden. Dit is onderzocht door de samenhang (correlatie) tussen de activiteit van alle gebieden in het brein te berekenen. Vervolgens is een vergelijking gemaakt tussen de correlatiepatronen van een medicatie-vrije groep van mensen met een depressieve stoornis (die nooit in hun leven een diagnose van een angststoornis hebben gehad) met de correlatiepatronen in een groep gezonde controles. De correlatiepatronen zijn berekend op basis van de activatie tijdens het beoordelen van emotionele woorden. We testten verschillende 'netwerken' in het brein, waarvan uit eerdere studies bekend was dat ze samenhangen met een aantal cognitieve en emotionele processen. De resultaten van onze studie wijzen uit dat mensen met een depressie minder koppeling laten zien van de mediale prefrontale schors en het dieper gelegen striatum met een netwerk dat eerder in verband gebracht is met het verwerken van emotionele informatie. Deze relatieve 'ontkoppeling' hing samen met het aantal woorden dat als positief geclassificeerd was. Deze bevindingen bevestigen de hypothese van relatieve frontale-subcorticale ontkoppeling tijdens emotionele verwerking in depressie.

In **hoofdstuk 6** en **7** worden tenslotte de effecten bestudeerd van twee bekende risicofactoren voor angst en depressie, te weten emotionele jeugdmishandeling en persoonlijkheidsfactoren, op volume van emotiegerelateerde hersen-structuren. In **hoofdstuk 6** laten we zien dat mensen die in hun kindertijd meermalen emotionele mishandeling hebben meegemaakt, minder volume in de mediale prefrontale schors hebben dan mensen die nooit emotionele of andersoortige mishandeling hebben meegemaakt. Dit effect trad op in zowel patiënten met een diagnose van angst en depressie als in gezonde controles. De dorsale mediale prefrontale cortex is ook in dierstudies heel gevoelig gebleken

voor de negatieve effecten van extreme stress. Mogelijk wordt door de stress die emotionele mishandeling met zich meebrengt de rijping van dit gebied bij jeugdigen geremd, doordat minder groeifactoren aangemaakt worden. Onze studie suggereert dat dit volumeverschil de ontbrekende schakel is in de relatie tussen jeugdmishandeling en het ontstaan van angst en depressie. De afwijking in dit hersengebied zou mensen vatbaarder kunnen maken voor de ontregelende effecten van ingrijpende levensgebeurtenissen en daardoor kwetsbaarder maken voor angst en depressie. In **hoofdstuk 7** worden de effecten van persoonlijkheid op hersenvolume van structuren die belangrijk zijn voor emotie verwerking en –regulatie onderzocht. We richtten ons op de persoonlijkheidstrekken Neuroticisme en Extraversie, omdat deze persoonlijkheidsfactoren eerder geassocieerd bleken met respectievelijk een verhoogde kans en een verlaagde kans op het ontwikkelen van psychopathologie. We onderzochten de correlatie van deze factoren met volume van het hele brein in gezonde mensen (dus zonder ooit een diagnose van depressie en angst) en vonden dat het volume van de orbitofrontale schors en van de amygdala groter was bij meer extraversie, maar niet bij meer neuroticisme. De amygdala en orbitofrontale schors zijn gebieden die geassocieerd zijn met primaire emotionele perceptie. Het beschermende effect van extraversie heeft mogelijk dus zijn grondslag in het functioneren van deze structuur, doordat het doorsluizen van emotionele informatie anders verloopt.

Het proefschrift eindigt met een samenvatting en algemene discussie (**hoofdstuk 8**) van de bevindingen van hoofdstuk 2 tot en met 7. Ook worden beperkingen van de studies besproken en worden suggesties voor vervolgonderzoek gedaan. Samenvattend suggereren de bevindingen in dit proefschrift dat depressie en angst zowel functioneel als structurele hersenafwijkigen delen, maar dat ze tegelijkertijd gekenmerkt worden door unieke afwijkingen tijdens het uitvoeren van cognitieve en emotionele taken. Hierbij kan de rostrale cingulaire schors voorgesteld worden als een sleutelgebied in het onderzoek naar abnormale emotieverwerking en -regulatie die zowel met angst als depressie wordt geassocieerd. Het abnormale functioneren van de hippocampus tijdens positieve verwerking kan een schakel zijn in het begrijpen van het optreden van terugval in een nieuwe depressieve of angstige episode. Over het algemeen zijn er geen effecten gevonden van het gebruik van antidepressiva op hersenfunctie en structuur, dat wil zeggen: mensen die wel antidepressieve medicatie gebruikten, lieten geen andere patronen van activatie en structuur zien dan mensen die geen medicatie gebruikten. Wel blijken ervaringen in de jeugd, zoals emotionele mishandeling, en persoonlijkheidsfactoren samen te hangen met structuur (volume) van hersengebieden die belangrijk zijn voor het waarnemen en verwerken van emotionele gebeurtenissen. Dit kan een verklaring bieden voor hoe deze risicofactoren tot een kwetsbaarheid voor het ontwikkelen van een angst- of depressieve stoornis leiden.
De bevindingen beschreven in dit proefschrift ondersteunen de aanname dat

depressie- en angst hersenafwijkingen delen die de hoge comorbiditeit kunnen verklaren, maar geven tegelijkertijd aanwijzingen voor de gedachte dat depressie met comorbide angst meer gekenmerkt wordt door angstgerelateerde neuropathologie en zich onderscheidt van een 'pure' depressie. Zo werden geen ernstigere afwijkingen gevonden bij mensen met zowel angst als depressie in vergelijking met de depressie groep (zonder angst), en werden de afwijkingen die in die 'pure' depressie groep werden aangetoond, niet gevonden in de comorbide groep. Dit zou verklaard kunnen worden door het feit dat mensen met depressie en angst vaak de angststoornis als eerste ontwikkelden, en dat daardoor angstgerelateerde pathologie de boventoon voert. Verder toont dit proefschrift aan dat angst met meer stabiele hersenafwijkingen gepaard gaat, terwijl de 'actieve' depressieve fase gekenmerkt wordt door hyperreactiviteit van subcorticale structuren tijdens negatieve emotionele verwerking en hyperactiviteit van de frontale schors tijdens puur cognitief executief functioneren. Of deze frontale hyperactiviteit ook optreedt tijdens negatieve emotionele taken die ook een aanspraak doen op executieve functies is nog niet onderzocht. Een dergelijke onderzoeksopzet kan de rol van de frontale schors in het reguleren van negatieve emoties verduidelijken.

Het is belangrijk om op te merken dat deze studies beperkt rekening gehouden is met inter-individuele variatie, alhoewel een belangrijke eerste aanzet is gezet naar het meer dimensioneel beschouwen van depressie en angst in neuro-imaging onderzoek. In de hier gepresenteerde studies zijn de patiënten met alleen een huidige diagnose van depressie vaak als één groep behandeld, terwijl ze door soms heel verschillende symptoomprofielen en ziektegeschiedenis worden gekenmerkt. Ook zijn de patiënten met verschillende angststoornissen als één groep behandeld en is geen onderzoek gedaan naar de specifieke angst-symptomen (paniek, sociale angst, extreme zorgelijkheid). Dit is een beperking van onze studie en we raden ook aan dat in toekomstige studies meer naar deze inter-individuele variatie gekeken wordt, mogelijk uitgebreid met een dimensionele typering. Een dimensionele benadering van depressie en angst kan mogelijk leiden tot een adequatere beschrijving van het symptoom- en het biologisch profiel, en daarmee tot een beter begrip van de complexe psychopathologie van depressie en angst.

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Marie-José van Tol werd op 25 december 1980 geboren in Amstelveen. Zij behaalde haar VWO diploma in 1999 aan het Alkwin Kollege in Uithoorn. Hierna volgde zij de opleiding Psychologie met een specialisatie in Klinische- en Gezondheidspsychologie aan de Universiteit Utrecht. Tijdens haar studie volgde zij ook een aantal specialisatievakken in de klinische neuropsychologie en forensische psychiatrie aan de Universiteit van Amsterdam en de Vrije Universiteit Amsterdam. Op de afdeling neuropsychologie van het Leids Universitair Medisch Centrum liep zij een klinische stage. Haar wetenschappelijke stage volbracht zij op de afdeling klinische neuropsychologie van de Vrije Universiteit. Zij onderzocht de effecten van een electrostimulatie-techniek op cognitie, slaap-waak-ritme en stemming bij mensen met waarschijnlijk de ziekte van Alzheimer. In 2004 studeerde zij af en werkte na haar studie als psycholoog in psychogeriatrisch verpleeghuis 'Florence, locatie Mariahoeve' in Den Haag. In september 2005 startte zij als promovenda op de afdeling psychiatrie van het Leids Universitair Medisch Centrum. Zij onderzocht de gedeelde en unieke hersenafwijkingen bij mensen met depressie en angststoornissen met dit proefschrift als resultaat. Als onderdeel van haar promotieonderzoek werkte zij ook op het VU Medisch Centrum en het Academisch Medisch Centrum Amsterdam. Tijdens haar onderzoek bezocht zij het EEG laboratorium van dr. Alison Fox aan de Universiteit van West-Australië en het cognitieve neurowetenschappen laboratorium van dr. I.T. Johnstone, Universiteit van Reading, Verenigd Koninkrijk. Sinds 1 januari 2011 is Marie-José als post-doctoraal onderzoeker werkzaam in de groep van Prof. Dr. A. Aleman, BCN Neuroimaging Center, te Groningen. Daar onderzoekt zij de rol van mediale hersenstructuren bij ziekte-inzicht en hallucinaties in psychosen.

Marie-José van Tol was born on 25 December 1980 in Amstelveen, the Netherlands. After pre-university school, she studied psychology at the University of Utrecht, with a focus on clinical- and health, and neuropsychology. She visited the department of neuropsychology of the Leiden University Medical Center for a clinical internship in neuropsychological assessment and the department of clinical neuropsychology of the Vrije Universiteit for her scientific internship. She graduated in 2004. The following year, she worked as a psychologist in a psychogeriatric nursing home in The Hague. She started working as a PhD-student at the department of psychiatry of the Leiden University Medical Center in September 2005. In her PhD years, she also worked at the Academic Medical Center Amsterdam and VU University Medical Center. She visited the EEG lab of dr. Allison Fox at the University of Western Australia and the lab of dr. Tom Johnstone at the Centre for Integrative Neuroscience and Neurodynamic, University of Reading, United Kingdom. This thesis is the product of her PhD work. At present, Marie-José works as a post-doctoral researcher at the neuropsychiatry lab of Prof. André Aleman at the BCN Neuroimaging Center, University Medical Center Groningen, where she studies the role of midline brain structures in insight in psychosis and hallucinations.