Brainstorm

Structural brain abnormalities in schizophrenia and depression

The studies described in this thesis were performed at the Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center Utrecht, the Netherlands.

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Brainstorm
Structurele hersen-”afwijkingen” in schizofrenie en depressieve stoornis

(met een samenvatting in het Nederlands)

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“I may not have gone where I intended to go, but I think I have ended up where I needed to be”
Douglas Adams

“If the human brain were so simple that we could understand it, we would be so simple that we couldn’t “
Emerson M. Pugh

Contents

1 Introduction

Brainstorm

This main title may need some clarification for the reader, while for me it covers it all. Probably the first association you have with this word will be that of a session to generate new ideas. In a way, that is exactly what this thesis-trajectory is all about. Brainstorm also means a short-term "malfunctioning" of the brain or the "convolution of the brain" like a gyrus, but then in a more figurative manner. As you will see, the explanatory title "structural brain abnormalities in schizophrenia and depression" should give a more in depth picture of the content of this thesis.

Introduction

Schizophrenia and major depressive disorder (MDD) share important clinical features, i.e. depressive symptoms, anhedonia, memory deficits, and lack of motivation (Häfner et al. 2005b; Häfner et al. 2005a; Lake, 2008). Moreover, during the course of schizophrenia the prevalence of depression ranges widely, from 6% to 75% (Siris and Bench, 2003). For most patients, schizophrenia as well as MDD is a life-long disorder with multiple recurrences or relapses. The chronic and recurrent course of both disorders is a major clinical issue, often requiring long-term prophylactic treatment.

Schizophrenia

Schizophrenia is a complex and severe psychiatric illness, with a life-time risk of developing the disorder of about 1%. The diagnosis of schizophrenia involves a constellation of signs and symptoms and impairment in occupational and social functioning (for diagnosis criteria see Table 1). These manifestations characteristically appear in the late second and third decades of life as a heterogeneous framework of three classes of clinical features. Positive symptoms include delusions (false beliefs), hallucinations (false perceptions), and thought disorganization. Negative symptoms refer to the loss of motivation and emotional vibrancy. Disturbances in basic cognitive functions, such as attention, executive functions, and specific forms of memory (particularly working memory), are also consistently observed in patients and are now thought to be central to the behavioural disturbances and functional disability of schizophrenia. In addition, many patients have concomitant mood symptoms including depression and anxiety (Häfner et al. 2005a; Rector et al. 2005).

Emil Kraepelin (1856–1926) was the first to differentiate schizophrenia, which he referred to as "dementia praecox" (dementia of the young), from manic depressive psychosis (Kraepelin, 1913). The term schizophrenia was introduced at the beginning of the twentieth century by Eugen Bleuler

(Bleuler, 1923). The word is derived from two Greek words: "schizo," which means to tear or to split, and "phren," which means "the intellect" or "the mind," and was sometimes used to refer to emotional functions. Thus, the word schizophrenia means the splitting or tearing of the mind and emotional stability of the patient. Bleuler classified the symptoms of schizophrenia into fundamental and accessory symptoms (Bleuler, 1923). According to Bleuler, the fundamental symptoms are ambivalence, disturbance of association, disturbance of affect, and a preference for fantasy over reality. He postulated that these symptoms are present in all patients, at all stages of the illness, and are diagnostic of schizophrenia. Bleuler's accessory symptoms of schizophrenia included delusions, hallucinations, movement disturbances, somatic symptoms, and manic and melancholic states. He believed that these symptoms often occurred in other illnesses and were not present in all schizophrenia patients. It is also noteworthy that Bleuler's reconceptualization of dementia praecox as "the group of schizophrenias" is reflected in the contemporary view that schizophrenia is a heterogeneous group of disorders with varied aetiologies, but similar clinical presentations.

Since then, numerous attempts have been made at formalizing the definition of schizophrenia and hence to distinguish it from other disorders as well as the attempts at various internal subdivisions (e.g., acute -chronic or poor outcome - good outcome subtypes). In fact, since the introduction of the concept, psychiatry has produced not less than 40 definitions of schizophrenia (Jansson and Parnas, 2007). These diagnostic classification attempts have resulted in two major classification systems, the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and International Classification of Diseases, 10th Revision (ICD-10). The DSM is now the most widely used system for diagnosing schizophrenia and other mental disorders. According to DSM-IV, schizophrenia is a chronic disorder that is characterized by psychotic episodes and a marked decline in social and occupational functioning (see Table 1). However, with the forthcoming edition DSM-V this definition may be revised.

Aetiology & Risk factors

What causes schizophrenia is a question that has occupied the mind of every psychiatric researcher for over a hundred years. So far we know that the onset of schizophrenia usually occurs in late adolescence or early adulthood and that men have an increased risk relative to women to develop the illness (Aleman et al. 2003). Certain factors have been associated with a propensity toward the illness. First, there is clear evidence for genetic predisposition. Genetic studies utilizing twin, adoption, and family history methods have

Table 1 Diagnostic Criteria for Schizophrenia according to DSM-IV

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1 month period (or less if successfully treated):

1. delusions
2. hallucinations
3. disorganized speech (e.g., frequent derailment or incoherence)
4. grossly disorganized or catatonic behavior
5. negative symptoms, i.e., affective flattening, alogia, or avolition

B. Social/occupational dysfunction: For a significant portion of time since the onset of the disturbance, one or more major areas of functioning, such as work, interpersonal relations, or self-care, are markedly below the level achieved before the onset. (Or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in criterion A present in attenuated form (i.e., odd beliefs, unusual perceptual experiences).

D. Schizoaffective and mood disorder exclusion: Schizoaffective disorder and mood disorder with psychotic features have been ruled out because either 1) no major depressive, manic, or mixed episodes have occurred concurrently with the active-phase symptoms; or 2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiologic effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. Relationship to a pervasive developmental disorder: If there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

(Adapted from Diagnostic and Statistical Manual of Mental Disorders, 4th ed.)

yielded evidence that the risk for schizophrenia is elevated in individuals who have an affected family member; the closer the level of genetic relatedness, the greater the likelihood the relative will also suffer from schizophrenia (Gottesman, 1991; McGuffin et al. 1995). A healthy twin with an affected monozygotic co-twin has the highest risk to develop schizophrenia (50%), followed by offspring of two schizophrenic parents (45%) (Cardno and Gottesman, 2000; Gottesman, 1991; McGuffin et al. 1995). However, schizophrenia is not a disorder in the Mendelian way like Huntington in which a single gene is the cause of the illness. The role of environmental factors in the development of schizophrenia is evident from the fact that monozygotic twins have less than 100% concordance rates of schizophrenia. Moreover, both gene-gene and gene-environment interactions are involved in the onset and endurance of the disease. It is possible that the genetic liability for schizophrenia sometimes results from a mutation that occurs in only the affected member of discordant monozygotic twin pairs. However, findings from studies of discordant MZ twins indicate that the rate of schizophrenia is elevated in the offspring of non-affected co-twins (Gottesman and Bertelsen, 1989; Kringlen and Cramer, 1989), which suggests that some individuals possess a genetic vulnerability for schizophrenia that they pass on to their offspring despite the fact that they are never diagnosed with the illness, i.e. they are called obligate carriers.

The neurodevelopmental hypothesis of schizophrenia suggests that a disruption of brain development underlies the later emergence of psychosis during adulthood. Events that adversely affect fetal development are now considered to be potential environmental triggers of genetic vulnerability. It is also plausible that they are sufficient, on their own, to produce vulnerability to schizophrenia. There is extensive evidence that obstetrical complications (OC's) have an adverse impact on the developing fetal brain, and numerous studies have shown that schizophrenia patients are more likely to have a history of OC's (for a review see (Cannon et al. 2002)). Included among these are pregnancy problems, such as toxemia and preeclampsia, as well as labour and delivery complications.

A number of other environmental factors have been associated with the illness. First, stimulant and cannabis (ab)use can induce psychosis and use during adolescence and increases the risk for psychosis in adulthood (Semple et al. 2005). To date, the association between cannabis use and psychosis is well established, but it remains unclear whether cannabis use precipitates schizophrenia or whether cannabis use is a form of self-medication (Hall and Degenhardt, 2000). In addition, patient with schizophrenia who use cannabis show more pronounced brain volume reductions over time relative to patients

with schizophrenia who do not use cannabis (Rais et al. 2008). Second, there is substantial evidence indicating that childhood physical, sexual and emotional abuse are causal factors for schizophrenia (Read et al. 2005). Third, social factors such as (parental) socioeconomic status may play a role in the aetiology of schizophrenia (Cantor-Graae, 2007). However, a recent meta-analysis supports the role of migration in schizophrenia in which it is suggested that the role for social factors in the development of schizophrenia arises primarily from studies of migrants. Finally, (psycho)social stressors and increased vulnerability to stress have been related to the onset of schizophrenia and to relapse rates (Phillips et al. 2006; Walker and Diforio, 1997). It is important to note that despite an association with the abovementioned environmental (risk) factors, most individuals who experience these adversities do not develop the schizophrenia.

Major depressive disorder

The word depression is widely used in general speech to describe those emotional states characterized by a lowering of the spirits, dejection or sadness. As such, depression is the normal human reaction to the loss of something valued. In contrast, major depression refers to a clinical state that consists of a group of symptoms forming a recognizable pattern with more or less complete recovery between episodes. These symptoms include emotional (e.g. feelings of dysphoria, sadness), somatic (e.g. sleep disturbances, loss of energy, weight changes), behavioural (e.g. psychomotor agitation or retardation) and cognitive (e.g. feelings of worthlessness or inappropriate guilt, recurrent thoughts of death or suicide, difficulty thinking or concentrating) manifestations (American Psychiatric Association, 1994). In DSM-IV, nine symptoms are listed as qualifying for major depression (see Table 2), with a requirement that at least five be present, including at least one of two core symptoms. The core symptoms of depression are depressed mood, and loss of interest or pleasure. These core symptoms reflect the view that depressive disorder is essentially a disorder of mood or affect. With an estimated lifetime risk of at least 10% (Kessler et al. 2003; Weissman et al. 1996) major depressive disorder (MDD) is one of the most common psychiatric illnesses. Moreover, depressive symptoms are highly prevalent among other psychiatric disorders such as schizophrenia (Häfner et al. 2005b) alcohol and drug abuse (Kessler et al. 1996; Regier et al. 1990), and post-traumatic stress disorder (Kilpatrick et al. 2003).

The concept of depression as a disease goes back a long way. Hippocrates described melancholia (black bile) as a condition in which patients had fears and despondencies for a long time. Manic as well as depressive moods were

Table 2 Diagnostic Criteria for Major Depressive Disorder to DSM-IV

A. Characteristic symptoms: A minimum of five symptoms from the following list have been present during the same 2-week period and represent a change from previous functioning. One of the symptoms must be #1 or #2, as listed below:

1. Depressed mood most of the day, nearly every day, as indicated either by subjective report (e.g. feels sad or empty) or observation made by others (e.g. appears tearful)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day, as indicated either by subjective account or observation made by others. Do not include symptoms that are clearly due to general medical condition or mood-incongruent delusions or hallucinations
3. Significant weight loss when not dieting or weight gain (e.g. a change of more than 5% of body weight in a month) or decrease or increase in appetite nearly every day
4. Insomnia or hypersomnia nearly every day
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or specific plan for committing suicide

B. The symptoms do not meet the criteria for a mixed episode

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning

D. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism)

E. The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation

(Adapted from Diagnostic and Statistical Manual of Mental Disorders, 4th ed.)

attributed to melancholy (Jackson, 1986). Hundred years B.C. Soranus of Ephesus thought of disease as due to organ pathology rather than "ill" humours. According to Soranus, mania and melancholy were different illnesses; mania was a mental disorder of reason without fever, and melancholy was defined as sadness without fever. Most psychiatric terms have changed meaning over time. Melancholia later became more clearly associated with the more modern idea of melancholy or despair. In the last 200 years many concepts have been introduced into the classification of depression, including manic-depressive disorder/insanity, bipolar disorder, and depression. Although melancholia remained the dominant diagnostic term, depression gained increasing currency in medical treatises and was a synonym by the end of the twentieth century. It was again Emil Kraepelin who may have been the first to use "depression" as the overarching term, referring to different kinds of melancholia as depressive states. Henry Maudsley proposed an overarching category of affective disorder (Lewis, 1934). However, it lasted to the 1960s when the modern separation into unipolar and bipolar disorder was introduced (Angst, 1966; Perris, 1966). With the introduction of the diagnostic classifications of DSM-IV and ICD-10, depression is now specified as a clinical syndrome, defined by the presence of a number of clinical features, but not requiring a specific aetiology, and acknowledging the possibility of both psychological and biological causative factors.

Aetiology & Risk factors

Depression is known to be a multifactorial disease by origin. Different biological, genetical and psychosocial factors are known to be behind the psychopathology of depressive disorders. The aetiological (risk) factors presented here are considered to be the most relevant ones in the context of this thesis. First, several lines of evidence - family, twin and adoption studies - suggest that MDD is a familial disorder. It has been estimated that among first degree relatives (parents, siblings or children) of depressive pro-bands the relative risk to develop MDD is two to three times higher compared with the general population (Weissman et al. 1996). Moreover, the risk for MDD is substantially increased in those with relatives also affected with MDD and that most, or all, of this risk is genetically transmitted (Sullivan et al. 2000). Affected individuals at high familial risk for MDD tend to have recurrent episodes, high levels of episode-related impairment, and perhaps an early age at onset (Kendler et al. 1999). In addition, in a large twin study, genetic factors have been shown to play a greater role in the aetiology of major depression among females than in males (Kendler et al. 2001a). This may explain why the risk to develop MDD is twice as high in women relative to men.

The concept of psychosocial/environmental factors behind the aetiology of depression is based on the fact that the onset of depression is often preceded by stressful life events and/or crises of a person's life-cycle. The overall sensitivity to the depressogenic effects of stressful life events seems to be equal for both genders, even though the psychosocial stressors themselves, preceding major depression in adulthood, differ between genders (Kendler et al. 2001b). Recently a functional polymorphism in the promoter region of the serotonin transporter (5-HTTLPR) gene was found to predict depression in interaction with major stressors (Caspi et al. 2003). That is, presence of this genetic characteristic moderated the likelihood of reacting to a major stressor with depression, suggesting that an etiological pathway related to serotonergic neurotransmission exists.

A wide range of environmental adversities such as job loss, marital difficulties, major health problems, and loss of close personal relationships are associated with a substantial increase in risk for the onset of MDD (Kendler et al. 2001b). A range of difficulties in childhood including physical and sexual abuse, poor parent-child relationships, and parental discord and divorce almost certainly increase the risk for MDD later in life. Certain kinds of personality traits appear to predispose to MDD, with the best evidence available for the trait termed "Neuroticism." Neuroticism, first proposed by the British psychologist Eysenck, is a stable personality trait that reflects the predisposition to develop emotional upset under stress. A range of other risk factors has been proposed for MDD, although in general the evidence for the existence of a causal association is weaker. These would include low social class, urban residence, separated or divorced marital status, low levels of social support.

Brain imaging

Magnetic resonance imaging (MRI) has been useful in revealing subtle structural brain abnormalities in patients relative to healthy controls. One of the main advantages of MRI is that brain scans are acquired in vivo without exposure to radiation. Furthermore, with MRI it is possible to quantify the gray and white matter of brain. Most studies have employed a region of interest (ROI) measurement of brain structures by manually delineating a priori defined regions or structures. Although the anatomical validity is high in these measurements, this type of analysis is very time consuming. In addition, this method does not easily allow for comparison of many brain regions or large subject groups. To overcome this issue, investigators have begun to employ voxel-based morphometry (VBM; (Ashburner and Friston, 2000)), a fully automated whole-brain measurement technique, to examine structural MR images of the brain. By surveying the whole brain, VBM

provides a non-biased measure of highly localized regions that may not be investigated in hypothesis-based studies that employ more labour-intensive ROI measurement techniques. Defined by Ashburner and Friston (Ashburner and Friston, 2000) as “a voxel-wise comparison of the local concentration of gray matter between two groups of subjects”, VBM tests for residual tissue concentration differences that remain after all subjects MRI scans are spatially normalized into the same standardized stereotaxic space. Gray matter (GM) is segmented out and then smoothed using convolution with a Gaussian kernel. Due to the nature of the normalization procedure, VBM analyses are less sensitive to shape differences and thus may enjoy high reliability potentially at the expense of validity. They must therefore be employed with the caveat that errors in normalization may confound results. It is also important to distinguish between the relative within voxel concentrations of gray matter (i.e. differences in the proportion of GM contained within a given voxel) as calculated by VBM and the absolute volumes revealed in ROI analyses.

Of interest is also the relatively new MRI application of cortical thickness, i.e. a measure to determine the thickness of the gray matter of the human cerebral cortex (Davatzikos and Bryan, 1996; Fischl and Dale, 2000; Kabani et al. 2001; Thompson and Toga, 1996). Cortical thickness is determined by the size, density, and arrangement of neurons, neuroglia, and nerve fibers. Cortical thinning is frequently regionally specific and can therefore provide important additional information for characterizing disease-specific neuroanatomical changes. Although cellular characteristics cannot be quantified directly in neuroimaging data, cortical thickness may more closely reflect cytoarchitectural abnormalities than cortical volume does (Fischl and Dale, 2000; Kabani et al. 2001).

Structural neuroimaging findings

Schizophrenia

A range of structural brain abnormalities have been reported in schizophrenia. In chronic schizophrenia results of several meta-analyses reflect smaller cerebral volumes and greater total ventricular volumes in patients relative to healthy control subjects. Other volumes that are found smaller were the hippocampus, the parahippocampus, the amygdala, the frontal lobes, and the temporal lobes (Ellison-Wright et al. 2008; Honea et al. 2005; Wright et al. 2000). In addition, findings from first episode schizophrenia studies suggest similar brain volume reductions, except for temporal lobe and amygdala volumes (Steen et al. 2006; Vita et al. 2006).

Accumulating evidence indicates that various brain regions experience progressive tissue loss after the first psychotic onset of schizophrenia (reviewed

by (Pantelis et al. 2005) and (Hulshoff Pol and Kahn, 2008)). However, the timing and regional pattern of these changes remain unclear, and it is unknown whether these changes are caused by the effect of long-term illness or the influence of antipsychotic medication or outcome. Nevertheless, it is clear that schizophrenic patients show aberrant trajectories of brain volume change during adolescence (Rapoport et al. 1999) and adulthood (van Haren et al. 2008b).

Major depressive disorder

Although brain abnormalities have been identified in MDD, the number of neuroimaging studies in patients with this illness pale in comparison to those performed in schizophrenia (for meta-analyses see: (Boos et al. 2007; Honea et al. 2008; Steen et al. 2006; Wright et al. 2000)). Despite an incomplete understanding of the neural circuitry underlying MDD, there is growing consensus that several brain areas are involved in depression (Beyer and Krishnan, 2002; Campbell and MacQueen, 2006; Drevets, 2000; Sheline, 2003; Videbech, 1997). Although reviews have appeared summarizing the results of neuroimaging studies of the hippocampus (Campbell et al. 2004; Videbech and Ravnkilde, 2004) and anterior cingulate cortex (Hajek et al. 2008), there have been no attempts to integrate all volumetric neuroimaging studies in depression to date. Suggesting that longitudinal volumetric neuroimaging studies in depression are sparse is an understatement. To date, only one research group has investigated brain volume changes in MDD. In this three year longitudinal VBM study patients showed higher volume decline in the anterior cingulum, left amygdala, and right dorsal medial prefrontal cortex and bilaterally in the hippocampus, compared with controls (Frodl et al. 2008b). However, when hippocampal and amygdalar volumes were segmented manually, no progressive volume decreases were found after one (Frodl et al. 2004) or three (Frodl et al. 2008a) years scan-interval.

Outline

In this thesis, we describe several studies concerning brain volume abnormalities in schizophrenia as well as in major depressive disorder.

Genetics

Twin studies

Twin studies are particularly informative to examine the relative contribution of genetic and environmental risk factors in the brain, especially when discordant twins are used. Monozygotic (MZ) twin pairs discordant for schizophrenia have an advantage over dizygotic (DZ) twin pairs discordant for schizophrenia, because the genetic predisposition to develop schizophrenia is the same in the MZ twin pairs. A genetic role is suggested when MZ patients and their co-twins differ from healthy MZ twins, but do not differ from each other. The latter being more distinct in discordant MZ than in discordant DZ twin pairs (Baaré et al. 2001b). Genetic factors have been shown to be involved in the decreases in whole brain volume with additional tissue loss reflecting disease-related (possibly non-genetic) influences (Baaré et al. 2001b). In addition, genetic factors were found to be involved in the decreases in the hippocampus (Baaré et al. 2001a) although influences of environmental factors have also been reported in relation to smaller hippocampus volumes in schizophrenia (Rijsdijk et al. 2005; Suddath et al. 1990; van Erp et al. 2004). Moreover, progressive brain volume loss found in whole brain, temporal and frontal regions in patients with schizophrenia and their unaffected co-twins was found at least partly attributable to genetic factors related to the illness (Brans et al. 2008).

In **chapter 2** we set out to investigate hypothalamic volumes in MZ and DZ twin pairs discordant for schizophrenia and those of closely matched healthy MZ and DZ twin pairs. Within-twin pair similarities were used to investigate whether genetic or disease related factors can explain the variation in volume of the hypothalamus. The hypothalamus plays a central role in the Hypothalamus-Pituitary-Adrenal (HPA) axis, one of the primary biological systems that mediate the psychological experience of stress. There is evidence supporting a role for stress in triggering or exacerbating psychotic episodes in schizophrenia as well as in other psychosis (Phillips et al. 2006). Several other lines of evidence suggest a link between HPA-activity and psychosis: First, illnesses associated with elevated cortisol levels (e.g., Cushing's syndrome) and the administration of corticosteroids have the potential to induce psychotic symptoms. Second, patients with schizophrenia and other psychotic disorders manifest HPA dysregulation as well as pronounced reductions in volume of the hippocampus (Nelson et al. 1998), a brain region that plays a

role in dampening HPA-activity. Third, there is a synergistic relation between activation of the HPA-axis and activation of dopaminergic circuits that have been implicated in psychosis. Finally, factors implicated in the aetiology of schizophrenia, particularly prenatal factors, can contribute to HPA dysregulation (Walker, 2008). Therefore, it is of interest to investigate whether those functional abnormalities are also characterized by structural abnormalities in one of the important structures in the HPA-axis.

Candidate genes

Findings from behavioural genetic studies of schizophrenia lead to the conclusion that the disorder involves multiple genes, rather than a single gene (Gottesman, 1991). This conclusion is based on several observations, most notably the fact that the pattern of familial transmission does not conform to what would be expected from a single genetic locus, or even a small number of genes. Rather, the genetic liability seems to involve multiple genes acting in concert, or numerous single susceptibility genes acting independently. Consistent with this assumption, attempts to identify a genetic locus that accounts for a significant proportion of cases of schizophrenia have not met with success. Instead, researchers using various molecular genetic techniques have identified a number of genes that seem to account for a very small proportion of cases or of variance in liability. The two major strategies used to localize genetic variation for schizophrenia are candidate gene association studies and (genomewide) linkage studies. The conceptual basis of a candidate gene study is straightforward and entails the comparison between the genotype frequencies for a particular genetic marker in cases with schizophrenia and appropriately matched controls. Candidate gene selection is usually based on knowledge of the etiopathology and, for schizophrenia, most association studies have focused on the genes encoding proteins involved in some way with the dopamine pathways (and more recently also on glutamate and myelin-related genes). There have been at least 1421 studies of 770 candidate genes and almost 7000 polymorphisms in schizophrenia (SZgene database accessed March 2009; (Allen et al. 2008)). Despite the tremendous amount of work, the candidate gene approach has not yielded replicable associations with schizophrenia that meet a high standard of proof. A few established candidate genes have been found, such as dysbindin, neuregulin, DISC1, but these findings stem from positional (linkage) rather than functional hypotheses (e.g. (Williams et al. 2009)). The conceptual basis of geno-mewide linkage studies is more complex. Nonparametric linkage is based on the regression of phenotype (schizophrenia) on genotype in families, usually containing multiple affected individuals, such as affected sibling pairs. Genetic markers

for genomewide linkage studies (around 400 polymorphic markers in the past but now totalling 10,000 single nucleotide polymorphisms (SNPs) or more) are selected to be relatively evenly spaced across the genome. These studies are not based on prior pathophysiological knowledge but rather can discover new knowledge about the locations of genes that might predispose to or protect against the development of schizophrenia.

Candidate gene analyses and whole genome scans have provided some evidence for the involvement of several specific genes, such as Disrupted in schizophrenia1 (DISC1), the dopamine DRD2 and DRD4 receptor genes, Neuregulin1 (NRG1) and several chromosomal regions (i.e., regions on chromosomes 6, 8, 13, and 22)(Allen et al. 2008; Badner and Gershon, 2002). Subsequently, studies have focused on testing specific genetic markers in known candidate genes for association with endophenotypes, such as brain morphometry. In this approach an association is tested between measured variation in a gene and variation in brain structure and/or function (Glahn et al. 2007). Several candidate genes for schizophrenia including NRG1, RGS4, COMT, GRM3, G72, DISC1, and BDNF (Lawrie et al. 2008; van Haren et al. 2008a), have been associated with brain morphology.

In **chapter 3**, we describe an imaging genetics study relating a variant in the BDNF (brain-derived neurotrophic factor) gene to hippocampal volume change over time in patients with schizophrenia and healthy controls. The BDNF gene is a member of the growth factor family and plays an important role in neuronal differentiation during development as well as in synaptic plasticity, and neuronal survival in the adult brain which may be impaired in schizophrenia (Angelucci et al. 2005; Binder and Scharfman, 2004). A single nucleotide polymorphism (SNP) in the BDNF gene that produces an amino acid substitution (Valine to Methionine) at codon 66 (Val66Met) has been reported to affect the activity-dependent secretion of BDNF in neuronal cell cultures (Chen et al. 2004; Egan et al. 2003), human hippocampal function (Egan et al. 2003; Hariri et al. 2003) and episodic memory (Dempster et al. 2005; Egan et al. 2003; Tan et al. 2005). The rationale for this study is based on the fact that hippocampal volume is reduced in schizophrenia (Nelson et al. 1998; Wright et al. 2000) and BDNF affects hippocampal volume and structure. However, little is known about the association between progressive brain volume change in schizophrenia and BDNF genotype. The aim of this study was to investigate the relationship between hippocampal volume change in patients with schizophrenia and healthy control subjects and BDNF genotype.

Confounding factors in neuroimaging & psychiatry

Confounding describes an association between two variables of interest (e.g. an exposure and an illness) that appears to be direct and causal, but in fact exists because of an unconsidered variable(s). This leads to a spurious interpretation of the relationship between the two variables. It is a type of error that results from the failure to recognize (i.e. identify, measure, and account for) a factor that contributes to an effect, leading to the erroneous conclusion that an observed association represents a cause and effect relationship. As confounding obscures the real effect, in other words the etiological importance of a variable, it needs to be prevented or removed as much as possible. There are several ways of addressing confounding such as restriction or by matching. Restriction would, at least partially, take away a confounding factor, but it hampers extrapolation of the study results to other groups. By means of matching, confounding factors are matched between groups. In case-control studies this means that for a possible confounder like age, for each patient with a certain age a control subject is selected with the same age or group-wise matching.

In psychiatry and neuroimaging research typical confounders include age, sex, social economic status, IQ and race. For these variables it is relatively easy to control for by matching on a group or individual level. However, there may also be specific illness-related confounders for which it is impossible to match with healthy control subjects. Moreover, for some confounders there is no need to control for, because these factors are of separate interest. In the following section (possible) confounding factors of interest, namely age, medication use and smoking will be described in relation to brain abnormalities in schizophrenia and/or depression.

Age

Aging spares no organ or system, and in due course affects everything, from cell to thought. However, the pace of aging varies among individual organisms, organs and (brain)systems. The normal brain undergoes considerable morphological changes with aging starting at young age until late life. Studying these changes is paramount to differentiate normal age-related brain variations from the effects of diseases affecting brain structure over time in psychiatric patients.

The onset of schizophrenia is typically in late adolescence or early adulthood. The neurodevelopmental model of schizophrenia posits subtle disease processes that affect critical circuits in the brain early in development that then manifest in disease around the time of puberty or shortly thereafter. Abnormal brain development at one stage may hinder normal maturation of later

developing structures and their functions.(Murray and Lewis, 1987; Weinberger, 1987). Therefore it is interesting and also important, to investigate age-related changes in patients with schizophrenia. In childhood-onset schizophrenia the progressive gray matter loss has been explained by a disruption in normal brain development (Rapoport et al. 1999). Recently, Van Haren and co-workers reported that the trajectories of cerebral gray and white matter volume change over time differed between patients with schizophrenia and healthy individuals (van Haren et al. 2008b). In these studies, only global brain volume change was examined. There is an ongoing discussion whether the hippocampus is already affected during the period of transition or after illness onset (Pantelis et al. 2007). Despite the volume differences reported in cross-sectional studies, longitudinal brain imaging studies have failed to find hippocampal volume change over time in patients with schizophrenia as compared to healthy controls.(DeLisi et al. 1997; Whitworth et al. 2005; Wood et al. 2001). However, these studies examined first episode patients and chronic patients with schizophrenia within a limited age range [20-35 yrs], making it difficult to disentangle the influence of age-related and illness-related changes on hippocampal volume. In **chapter 4**, we therefore chose to examine age-related hippocampal volume change in a large sample of patients with schizophrenia and healthy control subjects with a less restricted age range than previous studies.

Of major importance is understanding the distinction, if any, between the effects of aging and long-term illness in late life of individuals with an early or late onset depression. These distinctions may be quite important because there are reasons to believe that the symptoms, level of impairment, and prognosis of these conditions are quite different in people with a later-life onset as compared with those who have experienced the illness over the course of their lifetime. Reduced orbitofrontal cortex volumes are frequently found in early onset depression in late life relative to healthy elderly subjects (Ballmaier et al. 2004; Lavretsky et al. 2004; Lavretsky et al. 2007; Lee et al. 2003). However, one of the most consistent findings in MDD, a smaller hippocampus (Campbell et al. 2004; Videbech and Ravnkilde, 2004), seems to be more prominent in late onset compared with early onset depression when examined in late life. So far, almost all studies used a region of interest approach to examine patients with early onset depression in late life. In **chapter 5** we therefore chose to use cortical thickness measurements and voxel-based morphometry (VBM) to examine elderly females with early onset depression with age matched control women. By surveying the whole brain, cortical thickness measurements and VBM provide a non-biased measure of highly

localized regions that may not be investigated in hypothesis-based studies that employ more labour-intensive ROI measurement techniques. In addition, cortical thinning is frequently regionally specific and can therefore provide important additional information for characterizing disease-specific neuroanatomical changes. Moreover, neither cortical thickness measurements nor the combination with VBM has been done before in major depressive disorder; therefore our findings may contribute to a better understanding of brain abnormalities associated with MDD.

Medication

The development of antipsychotics represents one of the most important successes of applied neuroscience. In most schizophrenia patients, antipsychotic drugs bring a significant improvement in psychotic symptoms and better health and quality of life. However, while antipsychotic drugs provide a basic therapeutic tool for the treatment of schizophrenia and other psychotic conditions, their effectiveness is associated with a series of unresolved questions.

Compelling evidence summarized in several reviews (Harrison, 1999; Horacek et al. 2006; Konradi and Heckers, 2001) indicates that antipsychotic medication can induce anatomical changes at the level of regional brain volume, synapse morphology, and synapse number. In neuroimaging research, the first study that reported antipsychotic-associated structural brain change was published in 1994 (Chakos et al. 1994). Since then, several studies have investigated the influence of antipsychotic medication intake with brain volumes (for reviews see (Navari and Dazzan, 2009; Scherk and Falkai, 2006; Vita and De Peri, 2007). Since it is becoming generally accepted that schizophrenia is characterized by progressive volume changes (Hulshoff Pol and Kahn, 2008), it is of interest to examine the influence of antipsychotic medication intake in a longitudinal design. As described in the ***Age*** section, we examined age-related hippocampal volume change in patients with schizophrenia and healthy controls. In **chapter 4**, we also set out to investigate the relationship between cumulative dose of antipsychotic medication during the scan-interval and hippocampal volume change in patients with schizophrenia. As mentioned earlier, patients with schizophrenia show reductions in hippocampal volume relative to healthy subjects. In addition, decreased hippocampal volumes have been associated with decreased memory and poorer executive function (Antonova et al. 2004) and aberrant cognitive function is one of the key features of schizophrenia (Barch, 2005). Furthermore, evidence from animal studies indicates that atypical antipsychotics such as quetiapine and olanzapine increase neurogenesis in the

hippocampus (Kodama et al. 2004; Wakade et al. 2002; Xu et al. 2006; but see (Schmitt et al. 2004)). Moreover, olanzapine and quetiapine have been associated with increased hippocampal cell proliferation and prevention of brain-derived neurotrophic factor (BDNF) decrease compared to typical antipsychotics such as haloperidol (Parikh et al. 2004; Park et al. 2006; Xu et al. 2002).

Smoking

Patients with schizophrenia smoke more frequently and heavily than the general population. Patients with a mental illness are about twice as likely to smoke as is common in the general population (Lasser et al. 2000) with schizophrenia patients (and patients with substance abuse disorder) displaying the highest prevalence (66% (Poirier et al. 2002)). This observation in view of the well established role of nicotinic, cholinergic neurotransmission in cognition led to the hypothesis that people with schizophrenia may use nicotine as a self-medication to ameliorate cognitive symptoms associated with their disease. The effects of nicotine on cognition may be most pertinent to the problem of schizophrenia, but schizophrenia patients may also smoke to regulate mood and reduce stress (Kumari and Postma, 2005). Despite the well-known health hazards of cigarette smoking, its effect on the brain has hardly been studied. Cross-sectional studies show that subjects who smoke cigarettes have smaller gray matter volumes and/or densities in the prefrontal, anterior cingulate, occipital, and temporal cortices (including parahippocampal gyrus), thalamus, substantia nigra and cerebellum compared to non-smokers (Brody et al. 2004; Gallinat et al. 2006). Moreover a negative association has been reported between the total number of cigaretes and volume of frontal and temporal lobes and cerebellum (Gallinat et al. 2006). Indirect evidence that excessive volume loss might occur is provided by a recent study showing that during a 5-year follow-up the decline in global cognitive function, memory function, and cognitive flexibility was greater among smokers than never smokers (Nooyens et al. 2008). What remains unknown is whether change in volume is influenced by smoking cigarettes and more importantly whether the excessive change as is seen in schizophrenia patients is confounded by smoking behaviour. Earlier we reported on excessive brain volume change in patients with schizophrenia relative to healthy individuals (van Haren et al. 2008b). As part of this study we investigated the effect of tobacco smoking on brain volume change in controls and patients with schizophrenia in **chapter 6**.

Meta-analysis

Meta-analysis has been defined as 'the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings' (Glass GV, 1976). Although there has always been some controversy about its validity, meta-analysis has become increasingly popular as the number of studies with similar protocols has grown. By systematically combining studies, one attempts to overcome limits of size or scope in individual studies to obtain more reliable information about for example brain abnormalities or treatment effects. In the field of neuroimaging in major depressive disorder, a large amount of literature reviews have been published (just to name a few: (Beyer and Krishnan, 2002; Campbell and MacQueen, 2006; Drevets, 2000; Drevets, 2001; Drevets, 2003; Drevets et al. 2008; Sheline, 2003; Soares and Mann, 1997; Videbech, 1997)). However, a meta-analysis goes beyond a literature review, in which the results of the various studies are discussed, compared and perhaps tabulated, since it synthesizes the results of the individual studies into a new result. The key to a meta-analysis is defining an effect size statistic capable of representing the quantitative findings of a set of research studies in a standardized form that permits meaningful comparison and analyses across the studies (Lipsey and Wilson, 2001). Therefore, the rationale for the study described in **chapter 7** is straightforward and based on the following premises. First, although reviews have appeared summarizing the results of neuroimaging studies there have been no attempts to integrate all volumetric neuroimaging studies in depression to date. Second, such an effort could clarify the role of specific brain areas in the pathogenesis of MDD and give future directions on which topics and brain areas should receive more and detailed attention.

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2 Hypothalamus volume in twin pairs discordant for schizophrenia

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Abstract

Monozygotic and same-sex dizygotic twin pairs discordant for schizophrenia were compared with matched control twin pairs in order to disentangle genetic and environmental contributions to variation in hypothalamus volume. A decrease in hypothalamus volume was found in patients or discordant twin pairs compared to healthy controls which could be attributed to the decrease in total brain volume. Higher within-twin pair similarities in monozygotic compared to dizygotic twin pairs, suggests that hypothalamus volume might be partly genetically controlled.

Brain abnormalities in schizophrenia have consistently been demonstrated in structural magnetic resonance imaging studies. The most replicated findings are increases in the ventricular system. Also, reductions in total brain, hippocampus and amygdala volumes have been reported (Wright et al. 2000). Twin studies indicate that there is a genetic component to the aetiology of schizophrenia. Prior twin studies in schizophrenia have also found pronounced genetic contributions to total brain volume decrements (Baaré et al. 2001b), and environmental factors contributing to increased lateral ventricle volume (Rijsdijk et al. 2005).

It is been suggested that the Hypothalamic-Pituitary-Adrenal (HPA) axis is involved in both the experience of stress and the development of psychotic symptoms (Corcoran et al. 2003). However, few imaging studies have investigated the hypothalamus, an important structure in the HPA-axis, in patients with schizophrenia (Goldstein et al. 2007; Hulshoff Pol et al. 2005).

In this study, hypothalamic volumes in monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia were compared with those of closely matched healthy MZ and DZ twin pairs. Within-twin pair similarities were used to investigate whether genetic or disease related factors can explain the variation in volume of the hypothalamus.

Methods

Subjects

Participants in the study included 11 MZ (6 male/5 female pairs) and 11 same-sex DZ (5m/6f) twin pairs discordant for schizophrenia and 11 MZ (6m/5f) and 11 same-sex DZ (5m/6f) healthy control twins. This study was approved by the medical ethics committee for research in humans (METC) of the University Medical Center Utrecht, the Netherlands. Written informed consent was obtained from all subjects. Intracranial and total brain volumes of 84 of the twins were reported in a previous paper (Baaré et al. 2001a). In addition, gray and white matter volumes of the current sample were reported previously (Hulshoff Pol et al. 2004).

All subjects underwent psychiatric assessment procedures using the Comprehensive Assessment of Symptoms and History (CASH;(Andreasen et al. 1992) and Schedule for Affective Disorder and Schizophrenia-Lifetime Version (SADS-L; (Endicott and Spitzer, 1978) assessed by two independent raters. All patients met DSM-IV diagnosis for schizophrenia. Details of recruitment and demographic characteristics have been previously published (Baaré et al. 2001a; Hulshoff Pol et al. 2004; Hulshoff Pol et al. 2006b).

Image acquisition and analysis

Images were acquired on a Philips NT scanner operating at 1.5 T in all subjects. T1-weighted three-dimensional fast field echo (3D-FFE) scans with 160–180 contiguous coronal slices (echo time [TE] = 4.6 msec, repetition time [TR] = 30 msec, flip angle = 30°, 1x1x1.2 mm3 voxels), and T2-weighted dual-echo turbo-spin-echo (DE-TSE) scans with 120 contiguous coronal slices (TE1 = 14 msec, TE2 = 80 msec, TR = 6350 msec, flip angle = 90°, 1x1x1.6 mm3 voxels) of the whole head were used for quantitative measurements.

MRI processing methods have been described previously (Hulshoff Pol et al. 2002; Schnack et al. 2001a; Schnack et al. 2001b). Processing was done on the neuroimaging computer network of the Department of Psychiatry, University Medical Center Utrecht. In short, all images were coded to ensure investigators were blind for subject identification and diagnoses; scans were put into Talairach frame (no scaling) for segmentation purposes and corrected for inhomogeneities in the magnetic field. Quantitative assessments of the intracranial, total brain, and gray and white matter of the volumes were performed based on histogram analyses and series of mathematical morphology operators to connect all voxels of interest.

Hypothalamus segmentation

Hypothalamus segmentation procedures have been published previously (Hulshoff Pol et al. 2006a). In short, the anterior border of the hypothalamus is the lamina terminalis; this is where the anterior commissure (AC) runs. The hypothalamus ends on the first coronal slice on which the mamillary bodies are visible. The dorsal border is the AC-PC (posterior commissure) plane in transversal section, which approximates the hypothalamic sulcus. Ventrally, the hypothalamus ends where optic chiasm, infundibulum and mamillary bodies begin. Laterally it is demarcated by bundles of white matter. The mamillary bodies are not included in the segment due to its distinct morphology, its many myelineated fibers and its independent position and somewhat different origin (lamina basalis) compared to the other hypothalamic nuclei (Keyser, 1979). Segmentation is performed in the coronal plane. Extraction of the third ventricle and white matter bundles emerging from the thalamus, are removed by multiplying with the total brain and white matter segmentation.

The inter- and intrarater reliability of the hypothalamus volume measurements determined by the intraclass correlation coefficient (Bartko and Carpenter, Jr., 1976) in 10 brains was 0.93 and 0.91 respectively.

Statistical Analysis

Within-twin pair similarities of brain volumes were estimated by calculating intraclass correlation coefficients (ICC's) and their 95% confidence intervals (Bartko and Carpenter, Jr., 1976) on the unstandardized residuals of the brain volumes after correcting for age, sex and intracranial volume. ICC's have the advantage to overcome chance differences between twin 1 and twin 2 and are widely used to estimate heritability in twin studies (Baaré et al. 2001b; Hulshoff Pol et al. 2004; van Erp et al. 2004; van Haren et al. 2004). Fisher r-to-z transformations were used for statistical testing of the differences in ICC's between the groups (Sham, 1998).

Hypothalamus volumes of the patients only were compared with the volumes of healthy control twins after correcting for age, sex and intracranial (IC) or total brain volume (TB).

To investigate the effect of being a member of a discordant or control twin pair, hypothalamus volume measurements of the twin pairs were analyzed using repeated measure analysis of covariance (rm-ANCOVA). The main advantage of looking at the effect of disease in a rm-ANCOVA is that this analysis strategy makes full use of the available number of degrees of freedom. Repeated-measures analysis of covariance was done for hypothalamus volumes, with TWIN (proband or control 1, co-twin or control 2) as within subjects variable, group (discordant, healthy) and ZYG (monozygotic, dizygotic) as between subjects variables, and age, sex, and IC volume (or TB volume) as covariates. Interactions for TWIN-by-GROUP, TWIN-by-ZYG, and GROUP-by-ZYG were entered into the model. To investigate the source of the significant findings in the rm-ANCOVA, the means of the unstandardized residuals of hypothalamus volumes, after controlling for IC, age and sex between GROUP and ZYG and within TWIN were compared.

Post hoc, multivariate analysis of variance (MANOVA) was done to investigate whether sex differences in hypothalamus volume (controlled for age and TB volume) are present (irrespective of zygosity and illness).

Results

For the means of the uncorrected hypothalamus volumes, see Table 1. Within-twin pair similarities as measured by the intraclass correlation coefficient (ICC) are shown in Table 2. Within-twin pair similarities of hypothalamus volume were higher in monozygotic than in dizygotic twin pairs, irrespective of schizophrenia.

Patients showed smaller hypothalamus volumes (corrected for age, sex, and IC) relative to healthy control twins ($F_{(1,64)} = 4.577$, $p<0.05$). The rm-ANCOVA showed a significant main effect for GROUP on hypothalamus volume

corrected for age, sex and IC ($F_{(1,38)}$= 4.822, p<0.05), reflecting a decreased hypothalamus volume in discordant twin pairs as compared to the healthy twin pairs, irrespective of zygosity. However, after correction for TB, these effects were no longer significant. No significant main effects for TWIN and ZYG, or interaction effects were found.

Irrespective of schizophrenia or zygosity, males had a significant larger hypothalamic volume than females ($F_{(1,38)}$= 6.248; p=0.02). No significant main SEX-by-GROUP interaction was found.

Table 1. Raw uncorrected Hypothalamus volumes in Twin Pairs

Group	Hypothalamus volume in ml Mean (sd)
MZ Discordant	
Patient	0.96 (0.14)
Co-twin	0.98 (0.16)
MZ Healthy	
C1	1.04 (0.10)
C2	1.01 (0.14)
DZ Discordant	
Patient	0.96 (0.14)
Co-twin	0.92 (0.14)
DZ Healthy	
C1	0.97 (0.13)
C2	1.04 (0.13)

Abbreviations: MZ, monozygotic; DZ, dizygotic; C, control

Discussion

This study compared hypothalamus volume in MZ and same-sex DZ twin pairs discordant for schizophrenia with those in healthy MZ and same-sex DZ twin pairs in order to examine whether these volume alterations are due to genetic or environmental factors.

After correction for total brain volume there was no difference in hypothalamus volume between patients as well as twin pairs discordant for schizophrenia and healthy twin pairs. This suggests that the decrease of hypothalamus volume is not specific and can be attributed to a reduction in total brain volume.

In a recent study the volume of the hypothalamus, especially the paraventricular nucleus and the mamillary bodies, was found to be increased in both patients as well as their nonpsychotic relatives (Goldstein et al. 2007). The discrepancy between these findings and the findings from the current study

may partially be explained by different anatomical borders and slice thickness. Goldstein and colleagues (Goldstein et al. 2007) included the volume of the mamillary bodies which might bias the results since an increase in mamillary bodies' volume has been reported previously in schizophrenia (Briess et al. 1998).

Table 2. Within-Twin Pair Similarities

	MZ Total	DZ Total
ICC	0.58*	0.07
95 % L	0.23	-0.35
95 % H	0.80	0.47

Data are corrected for age, gender and intracranial volume. Abbreviations: MZ, monozygotic; DZ, dizygotic; Disc, discordant; ICC, intraclass coefficient; L, lower 95% confidence interval; H, higher 95% confidence interval.

* $p<0.05$

Higher within-twin pair similarities in MZ compared to DZ twin pairs, suggests that the volume of the hypothalamus might be partly genetically controlled. However, the large and overlapping confidence intervals for intraclass correlation coefficients found in the present study reflect low statistical power. Therefore, these findings should be interpreted with caution.
Post hoc analyses revealed that hypothalamus volume was significantly larger in males than in females, irrespective of schizophrenia and after correcting for total brain volume. This is in agreement with previous post-mortem studies investigating hypothalamic nuclei (Swaab and Fliers, 1985; Zhou et al. 1995) and imaging studies examining whole hypothalamic volume (Goldstein et al. 2001; Hulshoff Pol et al. 2006a) in healthy subjects.

The current study has some limitations. Small sample sizes of 11 twin pairs per group limit the statistical power of the analyses, despite matching the groups for age, sex, birth order and handedness. This is even more problematic when investigating interactions. The analysis regarding gender differences was a post-hoc analysis. The conclusions are carefully formulated and must indeed be interpreted with caution. Secondly, no information was available on life time medication intake. Since the hypothalamus contains many dopamine receptors (Ben-Jonathan and Hnasko, 2001; Kienast and Heinz, 2006) and antipsychotics exert their effect, among others, on the dopamine system, this may effect hypothalamus volume. However, the decreased hypothalamic volumes in unaffected co-twins compared with healthy control twin pairs who never had taken antipsychotic medications suggest that it is unlikely that our findings can be explained by antipsychotic medication

intake. In conclusion, we found suggestive evidence for hypothalamic volume to be under genetic control. The decrease of hypothalamus volume can be attributed to a reduction in total brain volume.

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3 Effects of brain-derived neurotrophic factor Val66Met polymorphism on hippocampal volume change in schizophrenia

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Abstract

A functional polymorphism of the brain-derived neurotrophic factor (BDNF) gene (Val66Met) has been associated with the risk for schizophrenia and volume differences in the hippocampus. However, little is known about the association between progressive brain volume change in schizophrenia and BDNF genotype. The aim of the present study was to investigate the relationship between hippocampal volume change in patients with schizophrenia and healthy control subjects and BDNF genotype. Two structural Magnetic Resonance Imaging brain scans were acquired of 68 patients with schizophrenia and 83 healthy subjects with an interval of approximately five years. Hippocampal volume change was measured and related to BDNF genotype in patients and healthy controls. BDNF genotype was not associated with hippocampal volume change over time in patients or healthy controls, nor could we replicate earlier findings on smaller hippocampal volume in Met-carriers. However, we did find a genotype-by-diagnosis interaction at baseline demonstrating smaller hippocampal volumes in patients homozygous for the Val-allele relative to healthy Val-homozygotes. In addition, irrespective of genotype, patients showed smaller hippocampal volumes compared with healthy controls at baseline. In summary, our results suggest that the BDNF Val66Met polymorphism is not associated with hippocampal volume change over time. Nevertheless, our findings may support the possibility that BDNF affects brain morphology differently in schizophrenia patients and healthy subjects.

Schizophrenia is a severe and common psychiatric disorder characterized by reduced limbic and prefrontal cortex volumes and increased ventricular volumes (Ellison-Wright et al. 2008; Glahn et al. 2008; Honea et al. 2005; Wright et al. 2000). Evidence from family, twin, and adoption studies suggest that genetic factors play an important role (~80%) in the pathogenesis of schizophrenia (Baaré et al. 2001; Brans et al. 2008; Cardno et al. 1999). Many studies have focused on testing specific genetic markers in known candidate genes for association with the disease. However, results have been disappointing, which has been attributed in part to the difficulty relating genes to a phenotype as complex as schizophrenia. Consequently, it has been suggested that linking genes to more straightforward phenotypes that are both heritable and related to the illness under study, may facilitate detection of these genes (Glahn et al. 2007). Indeed, brain structure may serve as such an intermediate (or endo)phenotype since some of the brain abnormalities associated with schizophrenia have been found to be related to the genetic risk to develop the disease. A variant in the gene encoding brain-derived neurotrophic factor (BDNF) has been studied extensively for association with schizophrenia. Although findings from several meta-analyses have been equivocal (Gratacos et al. 2007; Jonsson et al. 2006; Kanazawa et al. 2007; Naoe et al. 2007; Qian et al. 2007; Xu et al. 2007), some favour the existence of an association, albeit of low increased risk and likely including multiple causal variants.

The BDNF gene is a member of the growth factor family and plays an important role in neuronal differentiation during development as well as in synaptic plasticity, and neuronal survival in the adult brain (Angelucci et al. 2005; Binder and Scharfman, 2004). A single nucleotide polymorphism (SNP rs6265) in the BDNF gene that produces a G/A amino acid substitution (Valine to Methionine) at codon 66 (Val66Met) has been reported to affect the activity-dependent secretion of BDNF in neuronal cell cultures (Chen et al. 2004; Egan et al. 2003), human hippocampal function (Egan et al. 2003; Hariri et al. 2003) and episodic memory (Dempster et al. 2005; Egan et al. 2003; Tan et al. 2005).

So far, most studies that investigated the association between the BDNF gene and brain morphology in schizophrenia focused on the abovementioned "Val/Met" polymorphism. Although these cross-sectional studies used different approaches (voxel-based morphometry or volumetry), findings indicate that Met-allele carriers tend to have smaller (para)hippocampal (Ho et al. 2006; Szeszko et al. 2005; Takahashi et al. 2008), prefrontal, temporal and occipital (gray matter) volumes (Agartz et al. 2006; Ho et al. 2006; Ho et al. 2007) relative to Val-homozygotes. However, little is known about the association

	Patients with schizophrenia			Healthy comparison subjects		
	T0	T0 (FU only)	T5	T0	T0 (FU only)	T5
	N = 87	N = 68		N = 90	N = 84	
Gender (m/f)	71/16	54/15		56/34	56/28	
Age (yr) at baseline and follow-up [range]	36.05 (12.78) [17.17-67.53]	34.54 (11.44) [17.17-57.05]	39.40 (11.45) [22.16-61.29]	38.19 (13.55) [16.75-64.87]	37.89 (13.53) [16.75-64.87]	42.79 (13.49) [21.97-69.84]
Handedness (r/l/both0	74/11/2	58/8/2		77/11/2	72/10/2	
Illness duration (yr) at baseline and follow-up [range]	14.45 (12.03) [0.40-51.53][a]	12.65 (10.07) [0.40-36.25]	17.50 (10.08) [5.53-41.25][b]			
Follow-up duration (yr) [range]			4.86 (0.48) [3.85-6.21]			4.89 (0.30) [4.17-5.69]
PANSS						
Positive symptoms	15.58 (6.10)	15.21 (5.93)	13.29 (4.87)			
Negative symptoms	16.46 (5.60)	15.93 (5.24)	12.46 (5.49)			
General psychopathology	32.87 (10.99)	32.12 (10.74)	25.80 (8.03)			
BDNF genotype						
Met-Met	4	2		5	5	
Val-Met	28	22		26	24	
Val-Val	55	44		59	55	
Allele frequency (%)						
Met	21	19		20	17	
Val	79	81		80	83	

Table 1. Demographics and clinical variables of all subjects at baseline and follow-up

Abbreviations: Met, Methionine; Val, Valine; FU, follow-up
[a] Data not available for 4 patients
[b] Data not available for 1 patient

between progressive brain volume change in schizophrenia and BDNF genotype. In a study examining only patients with schizophrenia, Met-carriers showed a larger frontal gray matter volume decrease and excessive increases in lateral ventricle and sulcal CSF volume over time compared with Val-homozygotes (Ho et al. 2007). The aim of the present study was to investigate the relationship between hippocampal volume change in patients with schizophrenia and healthy control subjects and BDNF genotype. We hypothesized that the putative neuroprotective effect of BDNF (i.e. Val-homozygotes) will be associated with hippocampus volume change, indicating volume preservation or less volume loss in Val-homozygotes compared to Met-carriers in both groups.

Materials & Methods

Subjects

A 5-year follow-up magnetic resonance imaging (MRI) study was carried out, including patients with schizophrenia and healthy comparison subjects. Eighty-seven patients and 90 healthy subjects were selected from two larger samples (Hoogendoorn et al. 2008; van Haren et al. 2007) based on the presence of blood samples available for research purposes and at least three Caucasian grandparents. There was considerable overlap with our previous study reporting on hippocampal volume change in schizophrenia (Koolschijn et al. 2009). A total of 68 patients and 84 healthy comparison subjects completed the longitudinal study. Since the Met-allele is relatively rare in western populations, subjects were classified as either Val-homozygotes (baseline: 55 patients, 59 controls; follow-up: 44 patients, 55 controls) or Met-carriers (baseline: 32 patients, 31 controls; follow-up: 24 patients, 29 controls) (for demographic information see Table 1). The study was approved by the Human Ethics Committee of the University Medical Center Utrecht. Written informed consent was obtained for all subjects.

Criteria for inclusion in the study and clinical assessment were discussed previously (Hoogendoorn et al. 2008; Hulshoff Pol et al. 2001; van Haren et al. 2007; van Haren et al. 2008) and will be described only briefly. All subjects were between 16 and 70 years of age. From all subjects, 20 ml of whole blood was obtained. Both at baseline and follow-up, the presence or absence of

psychopathology was established by using the Comprehensive Assessment of Symptoms and History (CASH; (Andreasen et al. 1992)). Diagnostic consensus was achieved in the presence of a psychiatrist. All patients met DSM-IV criteria for schizophrenia or schizophreniform disorder at time of first measurement. The presence of symptoms was measured with the Positive and Negative Syndrome Scale (PANSS; (Kay et al. 1987)). 'Age at onset of illness' was defined as the first time patients experienced psychotic symptoms, as obtained from the CASH interview. Duration of illness was defined as time between age at onset of illness and age at first MRI scan. Information on number of hospitalizations and total duration of hospitalization during the scan interval was obtained from the CASH interview.
Patients received typical and atypical antipsychotic medication prior to and during the scan-interval. Clozapine and olanzapine were the types of atypical antipsychotics most often prescribed. All healthy comparison subjects met Research Diagnostic Criteria (Pfohl et al. 1995) for 'never mentally ill' and had no first-degree family members with a psychotic illness. The comparison subjects were matched for age, gender, handedness, height, socioeconomic status of their parents (expressed as the highest level of education completed by one of the parents), and follow-up duration.

Brain Imaging

Magnetic Resonance Imaging brain scans on baseline and follow-up were acquired on a Philips NT scanner operating at 1.5 T (Best, the Netherlands) with the same scanning protocol for all subjects at both visits. The acquisition protocol for the T1- and T2-weighted images and the pre-processing of the scans has been described in detail by Hulshoff Pol et al (Hulshoff Pol et al. 2001). Briefly summarized, scans were put into Talairach frame (no scaling) and corrected for inhomogeneities in the magnetic field. Quantitative assessments of intracranial and total brain volumes were performed on the basis of histogram analyses and series of mathematical morphological operators to connect all voxels of interest (Schnack et al. 2001a; Schnack et al. 2001b). The hippocampus was manually segmented on the non-uniformity corrected T1 image using Display imaging software (http://www.bic.mni.mcgill.ca/software/Display/) according to a fixed set of rules. This hippocampus segmentation procedure has been published previously (Koolschijn et al. 2009). In short, the hippocampus is part of the parahippocampal gyrus but it is segmented separately. Segmentation is done in coronal slices from anterior to posterior. The first coronal slice in which the characteristic oval shape of mamillary bodies was visible for the first time was taken as the anterior border. Posterior, the slice before the slice in which the fornix forms a continuous

tract for the first time is the last one to be segmented. The superior border was formed by the inferior horn of the lateral ventricle, while the inferior border consisted of the white matter. The subicular complex and the uncal sulcus were included in the segment. Every segment was checked in all dimensions after the initial segmentation. To be certain that there were no voxels containing the temporal horns and cerebrospinal fluid included in the hippocampus segmentation, the segments were multiplied with the total brain segment.
The interrater reliability of the volume measurements from a trained rater (P.C.M.P.K.) determined by the intraclass correlation coefficient (ICC) (Bartko and Carpenter, Jr., 1976) in 13 brains for the left and right hippocampus was 0.87 and 0.91 respectively. Intrarater reliabilities for left and right hippocampus were 0.86 and 0.96 respectively. Due to poor quality of a scan, no hippocampal segmentations were obtained from one control at follow-up, resulting in hippocampal volumes of 87 patients and 90 healthy controls at baseline, of which 68 patients and 83 healthy controls had a follow-up measurement.

BDNF Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures. The BDNF Val/Met polymorphism (rs6265) was genotyped using an Illumina Bead Station (Illumina, San Diego, CA) according to the manufacturer's protocol. Genotyping was part of a genome-wide association study (GWAS) in a much larger sample of schizophrenia patients and controls. DNA "fingerprints" using multiple markers had been generated for all individuals prior to the GWAS, which allowed identification of possible sample handling errors.

Statistical Analysis

The BDNF Val66Met genotype frequencies between patients with schizophrenia and healthy controls were tested for Hardy-Weinberg Equilibrium using a chi-square test. Since Met-carrier status is associated with impaired BDNF secretion and the small number of available Met-homozygous subjects in this study, heterozygotes and Met-homozygotes were categorized as Met-carriers and compared against Val-homozygotes.

Hippocampal volume (change)

All analyses were performed for total hippocampal volume at baseline, and hippocampal volume change over time. In case of significant findings, analyses were repeated for left and right hippocampal volume (change) separately. Hippocampal volume change per year was calculated by subtracting baseline

volume from follow-up volume, dividing it by the duration of the scan interval in years ((T5-T0)/interval) and is thus expressed as milliliter change per year. Correlation analyses showed that hippocampal volume change was significantly associated with hippocampal volume at baseline ($r=-0.332$, $p<0.0001$) and change in total brain volume ($r=0.329$, $p<0.0001$).
Linear regression was used to correct hippocampal volume change per year for hippocampal volume at T0, change in cerebral brain volume per year, sex and age at baseline, and unstandardized residuals were saved (i.e. further referred to as corrected hippocampal volume change). In addition, linear regression was used to correct hippocampal volume at baseline for age at baseline, sex and cerebral brain (or intracranial) volume at baseline, and unstandardized residuals were saved (i.e. further referred to as corrected baseline hippocampal volume). The unstandardized residuals were checked for normal distributions. To investigate the effects of BDNF genotype on corrected hippocampal volume change, multiple regression analyses were used to detect group (Met-carriers compared with Val-homozygotes) and diagnosis (patient or healthy control) differences. In addition, the interaction between genotype and diagnosis was added to the analysis. Moreover, to investigate the effects of BDNF genotype on corrected baseline hippocampal volume, multiple regression analyses were used to detect group (Met-carriers compared with Val-homozygotes) and diagnosis (patient or healthy control). Again, the interaction between genotype and diagnosis was added to the analysis. If significant interactions were found between genotype and diagnosis, pairwise comparisons were performed to identify the source of the significant outcome.

Results

For demographic information at baseline and follow-up as well as BDNF allele and genotype frequencies see Table 1. Mean (sd) hippocampal volumes at baseline and follow-up are presented in Table 2. Genotype distributions in patients with schizophrenia and healthy controls did not deviate from Hardy-Weinberg Equilibrium on both measurements (baseline: $\chi=0.61$, $p=0.44$; follow-up: $\chi=0.31$, $p=0.58$).

Hippocampal volume change

At follow-up, hippocampal volumes were available for 151 subjects ($N_{Pt} = 68$, $N_{Nc} = 83$). Healthy controls showed excessive volume loss relative to the patient group (total: $F = 11.091$, $p<0.001$; left: $F = 9.939$, $p=0.002$; right: $F = 9.035$, $p=0.003$). No significant BDNF genotype main or interaction effects were associated with hippocampal volume change.

Table 2. Mean hippocampal volumes (SD) at baseline and follow-up of patients with schizophrenia and control subjects

Volumes (ml) per Genotype	Patients with schizophrenia			Healthy control subjects		
	T0	T0 (FU only)	T5	T0	T0 (FU only)	T5
	N = 87	N = 68		N = 90	N = 83	
	32M/55V	24M/44V		31M/59V	29M/54V	
Left Hippocampus	3.50 (0.64)	3.55 (0.65)	3.47 (0.69)	3.64 (0.56)	3.64 (0.55)	3.31 (0.69)
Met-carrier	3.60 (0.71)	3.60 (0.79)	3.44 (0.76)	3.57 (0.50)	3.56 (0.46)	3.25 (0.68)
Val-homozygote	3.43 (0.59)	3.53 (0.57)	3.49 (0.66)	3.67 (0.58)	3.69 (0.60)	3.35 (0.70)
Right Hippocampus	3.47 (0.72)	3.52 (0.71)	3.44 (0.68)	3.67 (0.58)	3.69 (0.59)	3.34 (0.76)
Met-carrier	3.64 (0.79)	3.62 (0.83)	3.44 (0.75)	3.54 (0.46)	3.54 (0.44)	3.19 (0.63)
Val-homozygote	3.37 (0.66)	3.46 (0.64)	3.43 (0.65)	3.75 (0.63)	3.77 (0.65)	3.43 (0.81)
Total Hippocampus	6.97 (1.31)	7.07 (1.31)	6.90 (1.31)	7.31 (1.08)	7.33 (1.09)	6.66 (1.40)
Met-carrier	7.25 (1.47)	7.22 (1.59)	6.88 (1.48)	7.11 (0.92)	7.10 (0.86)	6.44 (1.28)
Val-homozygote	6.81 (1.19)	6.99 (1.15)	6.92 (1.22)	7.42 (1.15)	7.46 (1.19)	6.78(1.46)

Abbreviations: M, Met-carrier; V, Val-homozygote; FU, follow-up

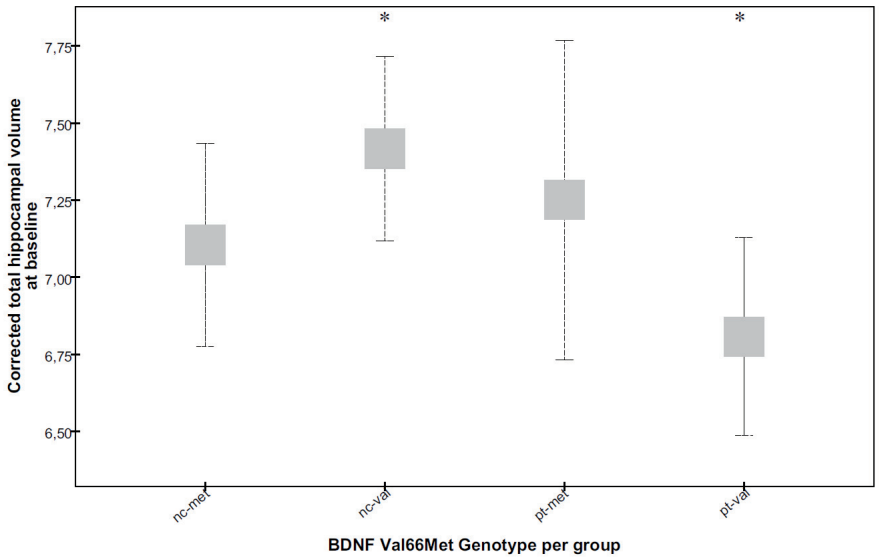

Figure 1. Hippocampal volume at baseline for diagnosis and genotype.
Abbreviations: Met, Met-carrier; Val, Val-homozygotes; nc, healthy control and pt, patient. Error bars indicate the standard error.
* ncval >ptval; p<0.001

Hippocampal volume at baseline

Hippocampal volumes were available for 177 subjects (N_{Pt} = 87; N_{Nc} = 90) at baseline. A significant main effect of diagnosis was found on corrected hippocampal volume, showing smaller volumes in patients compared with healthy controls (total: F = 5.102, p=0.025; left: F = 4.031, p=0.046; right: F = 5.171, p=0.024). A significant genotype-by-diagnosis interaction was found (F = 4.366, p=0.014), reflecting larger total hippocampal volumes in healthy Val-homozygous subjects compared with Val-homozygous patients (Figure 1). However, when we corrected for cerebral brain volume instead of intracranial volume, the interaction showed a trend towards statistical significance (total: F = 2.784, p=0.065). The same pattern was observed as displayed in Figure 1. No significant BDNF genotype main effects were associated with hippocampal volume at baseline.

Discussion

The aim of the present study was to investigate the relationship between hippocampal volume change and BDNF genotype in patients with schizophrenia and healthy control subjects. In contrast to our hypothesis, we did not find an effect of BDNF genotype on hippocampal volume change over time irrespective of diagnosis nor could we replicate earlier findings on smaller hippocampal volume in Met-carriers. However, we did find a genotype-by-diagnosis interaction demonstrating smaller hippocampal volumes in patients homozygous for the Val-allele relative to healthy Val-homozygotes. Also, an effect of diagnosis in general was found at baseline, reflecting smaller hippocampal volumes in patients relative to healthy controls irrespective of genotype.

In the full baseline sample, our study confirms previous findings from cross-sectional studies reporting on decreased hippocampal volumes in schizophrenia patients relative to healthy controls (Nelson et al. 1998; Wright et al. 2000). In the present study, hippocampal volume decreased over time in both patients and controls. In the healthy control group, the hippocampal volume loss over time was found to be significantly larger than that of the patient group. Due to considerable overlap in our population compared with our previous study, these findings are not surprising (Koolschijn et al. 2009). In that study we reported on age-related hippocampal volume changes, demonstrating progressive volume loss in patients with schizophrenia before the age of 26 relative to healthy controls. However, after age 40 healthy controls showed progressive hippocampal volume loss compared to the patients. In normal aging the hippocampus shows an initial increase in volume with a peak volume around 30-40 years of age, before accelerated volume loss sets in. Evidence from most studies examining normal aging indicates that the hippocampus shows a nonlinear pattern with age, with volume loss occurring between 40-50 years of age, and rapid acceleration after age 50 (Kennedy et al. 2008; Raz et al. 2004; Walhovd et al. 2005; Walhovd et al. 2009). The current finding underscores the excessive hippocampal volume loss after age 40 with normal aging and is in line with these earlier findings.

Despite evidence from most MRI studies in healthy controls suggesting larger hippocampal volumes in Val-homozygotes relative to Met-carriers (Bueller et al. 2006; Miyajima et al. 2008; Pezawas et al. 2004; but see (Nemoto et al. 2006)), we failed to replicate this finding even though our sample size is larger than most previous studies. Inspection of Figure 1 indicates that healthy Val-homozygotes tend to have a larger hippocampus volume relative to Met-carriers, but this difference was not significant. Our findings from the baseline

analyses suggest that a smaller hippocampal volume is specific for patients with schizophrenia who are homozygous for the Val-allele and is not present in Met-carrier patients. This could indicate that Val-homozygous patients are more susceptible for decline in hippocampal volumes compared with patients who are hetero- or homozygous for the Met-allele. Given the susceptibility of the hippocampus to extraneous influences, one could speculate that having this particular genotype makes a patient more vulnerable to the effects of environment or the illness itself. As no genotype effect was found on hippocampal volume change over time, this susceptibility possibly occurs early in the illness or before illness onset. Indeed smaller hippocampal volumes are already present in first episode patients (Steen et al. 2006; Vita et al. 2006). Less is known about the period of transition to illness, since both smaller and larger hippocampal volumes have been reported (Pantelis et al. 2007). It would be of interest to investigate the effect of BNDF in these samples. Interestingly, both acute and chronic stress has been shown to decrease the BDNF expression in various animal models (Duman & Monteggia, 2006). In addition, it has been demonstrated that increased levels of circulating cortisol have been associated with atrophy and loss of neurons in the hippocampus (Czeh & Lucassen, 2007; de Kloet et al. 2005; Lee et al. 2002; Sapolsky, 2000). One may speculate that hippocampal volume loss may be the result of increased (psychological) stress that accompanies the onset of and relapse of psychosis due to decreased BDNF expression in the hippocampus.

Our findings seem to be in contrast to earlier reports demonstrating larger hippocampal (Szeszko et al. 2005) and parahippocampal (Ho et al. 2006; Takahashi et al. 2008) volumes in Val-homozygous patients relative to Met-carriers. However, other studies showed no difference of genotype on hippocampal volume in patients with schizophrenia (Agartz et al. 2006; Takahashi et al. 2008). Differences may be due to relatively small sample sizes. It is also important to note that the abovementioned studies used different methodological (voxel-based morphometry vs. volumetry) approaches.
In addition, most studies compared the genotypes within patients or controls separately, without actually testing an interaction between genotype and diagnosis. Stating that genotype has a different effect in two groups does not mean that these differences are actually significant, i.e. this has not been formally tested. There has been little discussion about the issue of which statistical approach is appropriate to use in studies like these. In our study BDNF was chosen as a candidate gene for its possible effect on the hippocampus. However, often candidate genes are by their nature assumed to be associated with the susceptibility to develop the illness, and hence the grouping variables

diagnosis and genotype may not be completely independent. Therefore, the most appropriate statistical measures seem to be a multiple regression analysis or a general linear modelling (GLM) approach. What needs to be tested is whether the unstandardized residuals of the analyses are normally distributed (in case of multiple regression), or the significance of the Levene's test (GLM). Off note, in this sample there was no evidence for BDNF and the Val66Met polymorphism to be a susceptibility gene for schizophrenia, since genotype distributions did not differ between patients and healthy controls. This is in agreement with our previous findings in a much larger case-control sample, which included many of the individuals in the current study (de Krom et al. 2005). It remains to be elucidated whether current statistical methods are sufficient enough to deal with this kind of research or that other more appropriate (or new) methodologies have to be considered.

Several other factors may play an important part in explaining the inconsistency between studies examining the influences of BDNF genotype on brain morphology (especially the hippocampus). First, ethnic differences may account for conflicting results, because allelic frequencies differ between Caucasian and Asian people (Val-allele frequency: 80% vs. 56% resp.)(Gratacos et al. 2007). Second, subjects in the studies examining the BDNF Val66Met polymorphism vary largely in age range. It could be argued that BDNF expression affecting the hippocampus occurs only on certain critical periods during the life span. Indeed, BDNF influences almost all aspects of development in the central nervous system (CNS) during early life and is involved later in life in the survival, differentiation, and plasticity of the CNS (Lipsky & Marini, 2007). Since we did not find any association between genotype and hippocampal volume change, this could indicate a lack of power to detect such genotype driven volume changes that are age-related.

Third, from animal research we know that olanzapine and quetiapine have been associated with increased hippocampal cell proliferation and prevention of decreased BDNF levels compared to typical antipsychotics such as haloperidol (Parikh et al. 2004; Park et al. 2006; Xu et al. 2002). Furthermore, we have recently demonstrated that antipsychotic medication intake appears to be an confounder when investigating hippocampal volume over time (Koolschijn et al. 2009): our results indicated that patients who were exposed to a higher dose of atypical antipsychotics during the scan-interval showed less decrease or even small increases in hippocampal volume. Finally, a number of environmental factors such as physical exercise (Adlard et al. 2005; Cotman et al. 2007; Rojas Vega et al. 2006) and caloric restriction (Mattson et al. 2003) influence BDNF translation (RNA to protein) and concentration in rodents and serum in humans. In addition, physical exercise in

patients with schizophrenia was found to increase the hippocampus volume and to improve psychopathology (Pajonk et al. 2009). Interactions between the BDNF polymorphism and any of these environmental factors could be influencing hippocampal morphology.

The primary limitation of this study is that we could not control for antipsychotic medication intake. Since all the schizophrenia patients in this study were on antipsychotic medication, it is possible that (atypical) antipsychotic medication intake solely or by interacting with BDNF genotype have affected our measurements. Unfortunately, it was not possible to examine the influences of antipsychotic medication intake, even though it is one of the largest samples that measured hippocampal volume manually, because sample size limits our statistical power.

In summary, our results suggest that the BDNF Val66Met polymorphism is not associated with hippocampal volume change over time. Nevertheless, our findings may support the possibility that BDNF affects brain morphology differently in schizophrenia patients and healthy subjects. The present findings require replication in a larger sample to control for antipsychotic medication intake. Furthermore, possible interaction effects between schizophrenia-susceptibility genes such as the functional polymorphisms of the serotonin (5-HT) transporter and BDNF should be included in future studies with sufficient power to detect gene–gene interaction effects on the pathogenesis of schizophrenia (Mattson et al., 2004; Pezawas et al. 2008).

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4 Hippocampal volume change in schizophrenia

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Abstract

Patients with schizophrenia show reductions in hippocampal volume. However, the time course of these changes is still unresolved. Furthermore, it is unknown to what extent these findings are confounded by effects of age and/or antipsychotic medication. Two structural Magnetic Resonance Imaging brain scans were acquired of 96 patients with schizophrenia and 113 healthy subjects with an interval of approximately 5 years. Hippocampal volume change was measured and related to age and cumulative medication intake during the scan interval. Patients and healthy controls demonstrated significantly different age-related trajectories of hippocampal volume change. Before the age of 26 patients show increased volume loss relative to controls. In contrast, after the age of 40 years, controls showed larger volume loss than patients. A higher exposure to atypical antipsychotic medication was related to a smaller decrease in hippocampal volume over time. Our findings suggest progressive hippocampal volume loss in the early course of the illness in patients with schizophrenia, but not in the more chronic stages of the illness. The relationship between larger exposure to atypical antipsychotic medication and smaller hippocampal volume loss during the interval may suggest neuroprotective effects of these agents on hippocampal volume.

Patients with schizophrenia show reductions in hippocampal volume relative to healthy subjects (for meta-analyses see (Boos et al. 2007; Nelson et al. 1998; Wright et al. 2000)). This is of interest since decreased hippocampal volumes have been associated with decreased memory and poorer executive function (Antonova et al. 2004) and aberrant cognitive function is one of the key features of schizophrenia (Barch, 2005). Despite the volume differences reported in cross-sectional studies, longitudinal brain imaging studies have failed to find hippocampal volume change over time in patients with schizophrenia as compared to healthy controls (DeLisi et al. 1997; Whitworth et al. 2005; Wood et al. 2001). However, these studies examined first episode patients and chronic patients with schizophrenia within a limited age range [20-35 yrs], making it difficult to disentangle the influence of age-related and illness-related changes on hippocampal volume. In addition, it is unclear to what extent hippocampal volume loss is affected by antipsychotic medication. Indeed, treatment with olanzapine and risperidone has been associated with larger hippocampal volumes in patients with schizophrenia as compared to those on haloperidol in a cross-sectional study (Chakos et al. 2005), but this finding was not replicated in another study with chronically ill patients (Arango et al. 2003). In addition, two follow-up studies with short scan intervals (both less than a year) found no relationship between type of antipsychotic medication and hippocampal volume change (McClure et al. 2006; Panenka et al. 2007).

Earlier we reported on excessive global brain volume change in patients with schizophrenia relative to healthy individuals (van Haren et al. 2008). In the current study we used the same data set to compare age-related hippocampal volume change between 96 patients with schizophrenia and 113 healthy individuals. Furthermore, the relationship between cumulative dose of antipsychotic medication during the scan interval and hippocampal volume change in patients was investigated.

Methods

Subjects

A 5-year follow-up magnetic resonance imaging (MRI) study was carried out, including patients with schizophrenia and healthy comparison subjects. At baseline (T0), 159 patients (112 male/47 female) and 158 (106 male/52 female) healthy individuals were included.(Hulshoff Pol et al. 2001) A total of 96 patients (70 male/26 female) and 113 healthy comparison subjects (76 male /37 female) completed the longitudinal study and were rescanned after an interval of 5 years (T5) (van Haren et al. 2007; van Haren et al. 2008). The study was approved by the Human Ethics Committee of the University

Medical Center Utrecht. Written informed consent was obtained for all subjects.

Criteria for inclusion in the study and clinical assessment were discussed previously (Hulshoff Pol et al. 2001; van Haren et al. 2007; van Haren et al. 2008) and will be described only briefly. At baseline measurement subjects with a major medical or neurological illness including migraine, epilepsy, hypertension, cardiac disease, diabetes mellitus, endocrine disorders, cerebrovascular disease, alcohol or other drug dependence in the 6 months before entry in the study, head trauma in the past, or an IQ below 80 were excluded from this study. Both at baseline and follow-up, the presence or absence of psychopathology was established by using the Comprehensive Assessment of Symptoms and History (CASH; (Andreasen et al. 1992)) and was assessed by two independent raters. Patients gave permission to contact their treating physician or nurse for further information and medical records were used when necessary. In case the information provided by the patient, medical records, treating physician, or nurse was not reliable, the patient was excluded from the analysis. Diagnostic consensus was achieved in the presence of a psychiatrist. All patients met DSM-IV criteria for schizophrenia or schizophreniform disorder at time of first measurement; those with schizophreniform disorder were reassessed and met the criteria for a diagnosis of schizophrenia after one year of illness. At follow-up, all patients met criteria for schizophrenia except four patients who received a diagnosis of schizoaffective disorder. Severity of illness was measured with the Positive and Negative Syndrome Scale (PANSS; (Kay et al. 1987)). Outcome at follow-up was measured using the Camberwell Assessment of Need (CAN: sum of all relevant needs as rated by the treating physician divided by the number of relevant needs; (Phelan et al. 1995)) and Global Assessment of Functioning (GAF; (Hall, 1995)) scales. 'Age at onset of illness' was defined as the first time the patients experienced psychotic symptoms, as obtained from the CASH interview (Andreasen et al. 1992). Duration of illness was defined as time between age at onset of illness and age at first MRI scan. Information on number of hospitalizations and total duration of hospitalization during the scan-interval was obtained from the CASH interview and medical records.

To calculate the cumulative dosage of typical antipsychotics during the scan interval, a table from the Dutch National Health Service (Commissie Farmaceutische Hulp, 2002) was used to derive the haloperidol equivalents (similar to guidelines from the American Psychiatric Association (American Psychiatric Association, 2004)). For atypical antipsychotics, the respective pharmaceutical companies suggested conversion rates into haloperidol equivalents (clozapine, 40:1; olanzapine, 2.5:1; risperidone, 1:1; sulpiride, 170:1; quetia-

pine, 50:1; and sertindole, 2:1). No reliable information on medication intake during the scan interval was available for six patients. Ten patients had been taking typical antipsychotic medication exclusively and 27 patients atypical antipsychotic medication exclusively over the entire 5-year period. Forty-three patients switched between typical and atypical medication during the scan interval. Thirty out of these 43 patients switched from typical to atypical antipsychotic medication, one patient from atypical to typical antipsychotic medication and 12 patients changed several times between the two types of drug during the 5-year interval. Clozapine and olanzapine were the types of atypical antipsychotics most often prescribed

All healthy comparison subjects met Research Diagnostic Criteria (Pfohl et al. 1995) for 'never mentally ill' and had no first-degree family members with a psychotic illness. The comparison subjects were matched for age, sex, handedness, height, socioeconomic status of their parents (expressed as the highest level of education completed by one of the parents), and scan interval.

Brain Imaging

Magnetic Resonance Imaging brain scans on baseline and follow-up were acquired on a Philips NT scanner operating at 1.5 T (Best, The Netherlands) using the identical scanning protocol for all subjects on both measurements. Details of the MRI acquisition protocol and processing of the images have been presented before (Hulshoff Pol et al. 2002; Schnack et al. 2001a; Schnack et al. 2001b). Briefly summarized, quantitative assessments of intracranial, cerebrum (=gray and white matter of the cerebrum excluding the cerebellum and brainstem), lateral and third ventricles, and peripheral cerebrospinal fluid volumes were performed on the basis of histogram analyses and series of mathematical morphological operators to connect all voxels of interest. The hippocampus was manually segmented using Display imaging software (http://www.bic.mni.mcgill.ca/software/Display/) according to a fixed set of rules. This hippocampus segmentation procedure has been published previously (Baaré et al. 2001; Janssen et al. 2004; Staal et al. 2000). In short, the hippocampus is part of the parahippocampal gyrus but it is segmented separately. Segmentation is done in coronal slices from anterior to posterior (see Table 1). Every segment was checked in all dimensions after the initial segmentation. To be certain that there were no voxels in the hippocampal segment that were actually part of the cerebrospinal fluid in the temporal horns; the segment was multiplied with the cerebrum segment.

Table 1. Segmentation procedure of the hippocampus

Borders	
Anterior	The first coronal slice in which the characteristic oval shape of the mamillary bodies is visible
Posterior	The slice before the slice in which the fornix forms a continuous tract for the first time is the last one to be segmented
Superior	The inferior horn of the lateral ventricle
Inferior/Medial	The surrounding white matter. The subicular complex and the uncal sulcus are included in the segment

The interrater reliability of the volume measurements between three trained raters (P.C.M.P.K.; F.K. and J.J.) determined by the intraclass correlation coefficient (ICC; (Bartko and Carpenter, Jr., 1976)) in 13 brains for the left and right hippocampus were at least .85 or higher (range: 0.85 - 0.95). Intra-rater reliabilities for left and right hippocampus were .85 or higher (range: 0.85 - 0.96).

Due to poor quality of the scans, no hippocampal segmentations were obtained from 6 patients (5 at baseline) and 2 healthy controls (both at baseline), resulting in hippocampal volumes of 153 patients and 156 healthy controls at baseline, of which 95 patients and 113 controls had a follow-up measurement.

Statistical Analysis

Data were checked for outliers, extreme values and the normality of the distribution. Except for the different medication variables all variables were normally distributed. Nonparametric testing was used in case the medication variables were included into the analysis. All analyses were performed for left, right and total hippocampal volume (change).

Hippocampal volume change per year was calculated by subtracting baseline volume from follow-up volume, dividing it by the duration of the scan-interval in years ((T5-T0)/interval) and is thus expressed as milliliter change per year.

Correlation analyses showed that hippocampal volume change was significantly associated with hippocampal volume at baseline ($r=-0.335$, $p<0.0001$) and change in cerebral brain volume ($r=0.305$, $p<0.0001$). Linear regression was used to correct hippocampal volume change per year for hippocampal volume at T0, change in cerebral brain volume per year, sex and age at baseline, and unstandardized residuals were saved (i.e. further referred to as corrected hippocampal volume change). In addition, linear regression was used to correct hippocampal volume at baseline for age at baseline, sex and cere-

bral brain volume at baseline, and unstandardized residuals were saved (i.e. further referred to as corrected baseline hippocampal volume).

Group differences

First, we used a General Linear Model-univariate analysis to detect cross-sectional group differences, using age at baseline, sex and cerebral brain volume as covariates. This analysis was done in the total baseline sample (N_{Pt}=153 patients and N_{Nc}=158 controls), and on the sub-sample of only those subjects that participated at follow-up (N_{Pt}=95, N_{Nc}=113). Moreover, corrected hippocampal volume change was compared between the groups.

Since in this study we were particularly interested in the relationship between age and hippocampal volume change in both groups, a regression analysis in the form of a locally-weighted running-line smoother (Cleveland and Devlin, 1988; Hastie and Tibshirani, 1990) was used to obtain the dependence of volume changes on age (see also (van Haren et al. 2008)). Software for these analyses was developed in house. Fits with different degrees of freedom (df) were calculated for each group to find the one that described the data best. Standard-error (SE) bands were calculated to show at which age volume change differed significantly between patients and healthy subjects.

The analyses were done on corrected and uncorrected hippocampal volume change, with and without correcting for sex. The results from these analyses were similar; therefore, the findings of the uncorrected volume changes per year are reported here.

Relationship with clinical variables

In the patient group only, Pearson and Spearman Rank correlations were calculated between corrected hippocampal volume change and 1) medication intake (cumulative intake of typical antipsychotic medication in haloperidol equivalents, atypical antipsychotic medication in haloperidol equivalents per year during the scan interval (hal. eq./scan interval), and clozapine and olanzapine in milligrams per year during the scan interval (mg./scan interval)) and 2) outcome (i.e., GAF-score at follow-up, CAN-score (a square-root transformation was performed to create a normal distribution of the data) at follow-up, number of hospitalizations and total duration of hospitalization during the scan interval, and scores on the positive, negative and general symptom scales of the PANSS at follow-up).

A two-tailed alpha level of 0.05 was used to determine significance of the effect.

	Patients with schizophrenia			Healthy comparison subjects		
	T0 N = 153	T0 (FU only) N = 95	T5	T0 N =156	T0 (FU only) N = 113	T5
Sex (m/f)	108/45	69/26		105/51	76/37	
Age (yr) at baseline and follow-up [range]	34.82 (12.31) [16.88-67.53]	32.16 (11.14) [16.88-56.25]	36.98 (11.25) [21.32-61.25]	37.33 (13.87) [16.75-67.79]	35.28 (12.25) [16.75-56.27]	40.22 (12.21) [21.97-61.54]
Height	176.27 (9.3)	176.69 (9.5)		178.05 (8.7)	178.40 (8.4)	
Handedness (r/l/both)	131/19/3	82/10/3		131/23/2	96/15/2	
Level of education (yr) [a b]	10.81 (2.95)	12.03 (2.77)		12.07 (2.97)	12.81 (2.57)	
Parental level of education (yr)	10.67 (3.29)	11.08 (3.08)		10.66 (2.87)	10.92 (2.69)	
Follow-up duration (yr) [range]			4.83 (0.55) [3.48-6.34]			4.94 (0.32) [4.15-5.71]
Age of first psychotic symptoms (yr) [range]	20.97 (5.36) [9-36]	21.29 (5.43) [9-36]				
Duration of illness (yr) at baseline [range] (N=152)[c]	13.94 (12.28) [0.40-51.53]	10.86 (10.25) [0.40-36.25]				
< 1 year	10	10				
1-2 years	16	9				
2-5 years	31	23				
5-10 years	18	15				
10-20 yuears	29	18				
> 20 years	48	20				
PANSS						
Positive symptoms	18.47 (5.65)	17.78 (5.52)	13.22 (6.03)			
Negative symptoms	17.04 (5.61)	16.87 (5.83)	13.74 (5.23)			
General psychopathology	36.24 (9.33)	35.74 (8.36)	26.75 (8.32)			
Global Assessment of functioning at follow-up [range] (N = 93)			52.62 (17.20) [11-90]			
Cumulative medication intake per year scan-interval [d]						
N = 10 only typical antipsychotic medication (HEQ)			1828 (1238)			
N = 27 only atypical antipsychotic medication (HEQ)			1403 (1031)			
N = 52 patients who switched and used among others [e]:						
- typical (N = 43, HEQ)			366 (451)			
- atypical (N = 36, HEQ)			789 (693			

Table 2. Demographics and clinical variables of all subjects at baseline and follow-up

Abbreviations: FU, follow-up; HEQ, Haldol equivalent

[a] Level of education was significantly lower in patients than in comparison subjects ($F=15.511$; $p<0.001$).

[b] Level of education was significantly lower in patients who only participated at baseline compared with patients included at follow-up ($F=4.292$; $p=0.04$)

[c] Duration of illness was significantly longer in patients who only participated at baseline compared with patients included at follow-up ($F=17.736$; $p<0.001$) Not available for 1 patient

[d] Cumulative typical and atypical antipsychotic medication intakes are in haloperidol equivalents (HEQ) per year during the scan interval. Cumulative olanzapine intake is in milligram (mg) per year during the scan interval

[e] During the scan interval, 52 patients switched between at least two of typical antipsychotics, atypical antipsychotics. For example, 43 of these patients used typical antipsychotic medication at some point during the scan interval, but have also been taken atypical antipsychotics.

Selection bias at follow-up

At baseline, the patients included at follow-up were younger, had a shorter duration of illness, fewer negative symptoms, and larger volumes of cerebral (gray) matter than the patients who did not complete the follow-up (for further details see (van Haren et al. 2007)). Moreover, the number of years of education was significantly lower in patients who only participated at baseline compared with patients included at follow-up. A linear regression analysis was performed to compare corrected baseline hippocampal volume between patients who participated at follow-up and patients who did not. This analysis was repeated to investigate the confounding effects of negative symptoms, duration of illness, or years of education.

Results

Group differences

For demographic information at baseline and follow-up see Table 2. Mean (sd) hippocampal volumes at baseline and follow-up are presented in Table 3.

Table 3. Mean hippocampal volumes (SD) at baseline and follow-up of patients with schizophrenia and comparison subjects

Hippocampal volumes (ml)	Patients with schizophrenia			Healthy comparison subjects		
	T0	T0 (FU only)	T5	T0	T0 (FU only)	T5
	N = 153	N = 95		N = 156	N = 113	
Left	3.57 (0.57)[a]	3.66 (0.52)	3.54 (0.55)	3.77 (0.57)[a]	3.75 (.57)	3.57 (0.58)
Right	3.57 (0.58)[b]	3.61 (0.58)	3.50 (0.55)	3.76 (0.59)[b]	3.77 (0.60)	3.63 (0.62)
Total	7.15 (1.09)[c]	7.27 (1.04)	7.05 (1.03)	7.53 (1.09)[c]	7.51 (1.11)	7.20 (1.14)

Abbreviations: FU, follow-up
Patients in the total baseline sample showed bilateral smaller corrected baseline hippocampal volumes compared to healthy comparison subjects at T0
[a] Left hippocampus: (F=8.205; p=.004)
[b] Right hippocampus: (F=5.49; p=.02)
[c] Total hippocampus: (F=7.81; p=.006)

In the total baseline sample (N_{Pt}=153; N_{Nc}=156) patients had significantly smaller bilateral hippocampus volumes compared to healthy controls after correction for age, sex and cerebral brain volume (left: F = 8.205, p=0.004; right: F = 5.49, p=0.02; total: F = 7.81, p=0.006). However, when including only those subjects who participated at follow-up, the difference in baseline hippocampus volume between patients and controls was no longer significant (left: F = 0.665, p=0.416; right: F = 1.863, p=0.174; total: F = 1.386, p=0.24). Moreover, at follow-up no significant difference in hippocampal volume between the groups was present (left: F = 0.071, p=0.79; right: F = 1.21, p=0.291; total: F = 0.182, p=0.67)
Finally, no differences were found in the rate of volume change in the patient group compared to the control group (left: F = 1.131, p=0.289; right: F = 0.143. p=0.705; total: F = 0.229, p=0.633).

Our main interest concerned the association between age and hippocampal volume change and possible differences in this relationship between patients and healthy individuals. Healthy controls showed a linear relationship between hippocampal volume change and age, representing a larger decrease of hippocampal volume with increasing age (df=2, Figure 1 in blue). Around the age of 20 almost no hippocampal volume change was present, while

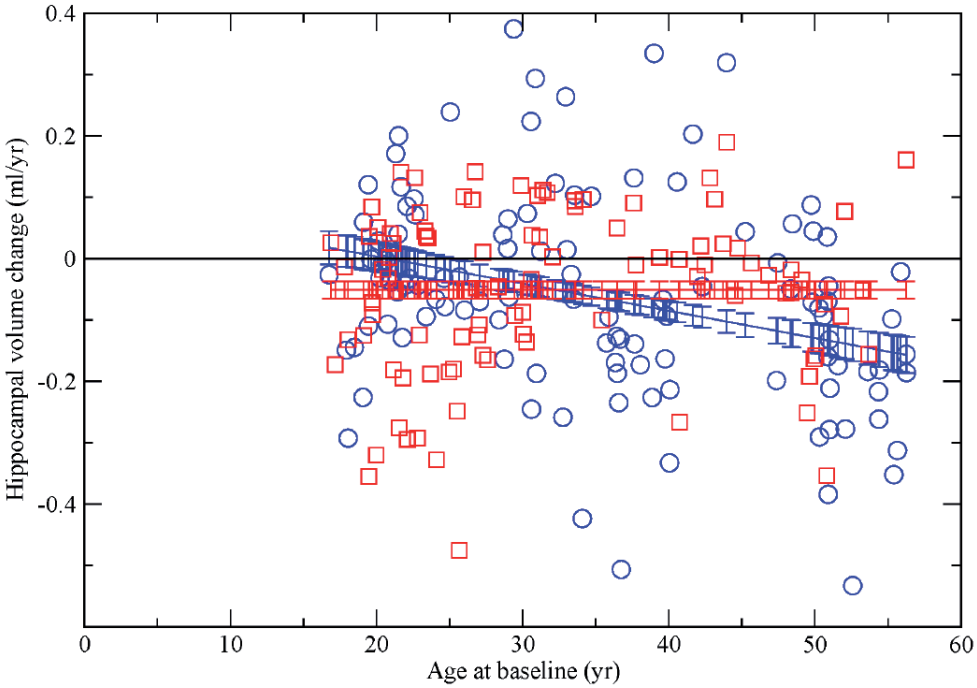

Figure 1. Age related trajectory of hippocampal volume change in patients with SZ and healthy subjects

Change in hippocampal volume (ml/year) as a function of age for patients with schizophrenia (red squares, linear df = 1) and healthy comparison subjects (blue circles, linear df = 2). The x-axis represents the age of the subject at baseline measurement. The y-axis represents the average volume change per year during the interval which started at this particular age. Correction for change in cerebral brain volume, hippocampal volume at baseline and sex did not change the results.

around the age of 45 the decrease was 0.1 ml/yr, showing a further decrease to approximately 0.15 ml/yr around age 55. In contrast, hippocampal volume loss in patients remained stable across the entire age range (df=1; Figure 1 in red), decreasing approximately 0.05 ml/yr. The slopes of patients and healthy controls were significantly different before the age of 26 (non-overlapping standard error bands), showing increased volume loss in patients relative to controls; conversely after the age of 40 years it showed significant progressive volume loss in controls relative to patients.

Results for age-related volume change in the left and right hippocampus were similar to those found for total hippocampal volume change.

Relationship with clinical variables

Correlations between unstandardized residuals of corrected hippocampal volume change (corrected for baseline hippocampal volume, change in cerebral volume, sex and age at baseline) and cumulative dose of antipsychotic medication per year were calculated. A significant positive association was found between hippocampal volume change and cumulative intake of atypical antipsychotics per year during the scan interval (N=49: rho=.31, p=0.028: Figure 2A); higher exposure to atypical antipsychotics was associated with less decrease in hippocampal volume. Olanzapine in particular showed a positive association between atypical antipsychotic exposure and hippocampal volume change that reached trend level significance (N=37: rho=0.32 p=0.056;). Moreover, a negative correlation between cumulative intake of typical antipsychotics and hippocampal volume change (N=51: rho=-.27, p=0.058; Figure 2B) was significant at trend level, indicating that a larger dose of typical antipsychotics during the scan interval was correlated with a larger decrease in hippocampal volume. From the correlation analyses with cumulative intake of typical antipsychotics one patient, who was prescribed a large dose of typical antipsychotics compared to all other patients was excluded. This did not change our findings (N=52; rho=-.26 p=0.062). After bonferroni correction for multiple comparisons our findings were no longer significant.

No significant differences were found between good and poor outcome patients (as defined by the GAF score at follow-up). In addition, no significant correlations were found between scores on the negative, positive or general symptom scales of the PANSS, CAN scores at follow-up, number and total duration of hospitalisations during the interval and corrected hippocampal volume change at the 0.05 significance level.

Selection bias at follow-up

No significant differences were found between corrected baseline hippocampal volumes between patients who participated at follow-up and patients who did not (F = 2.062; p=0.153). Moreover, adding level of negative symptoms (F = 1.359; p=0.246), duration of illness (F = 1.002; p=0.318) or years of education (F = 1.684; p=0.196) as covariate did not change this finding.

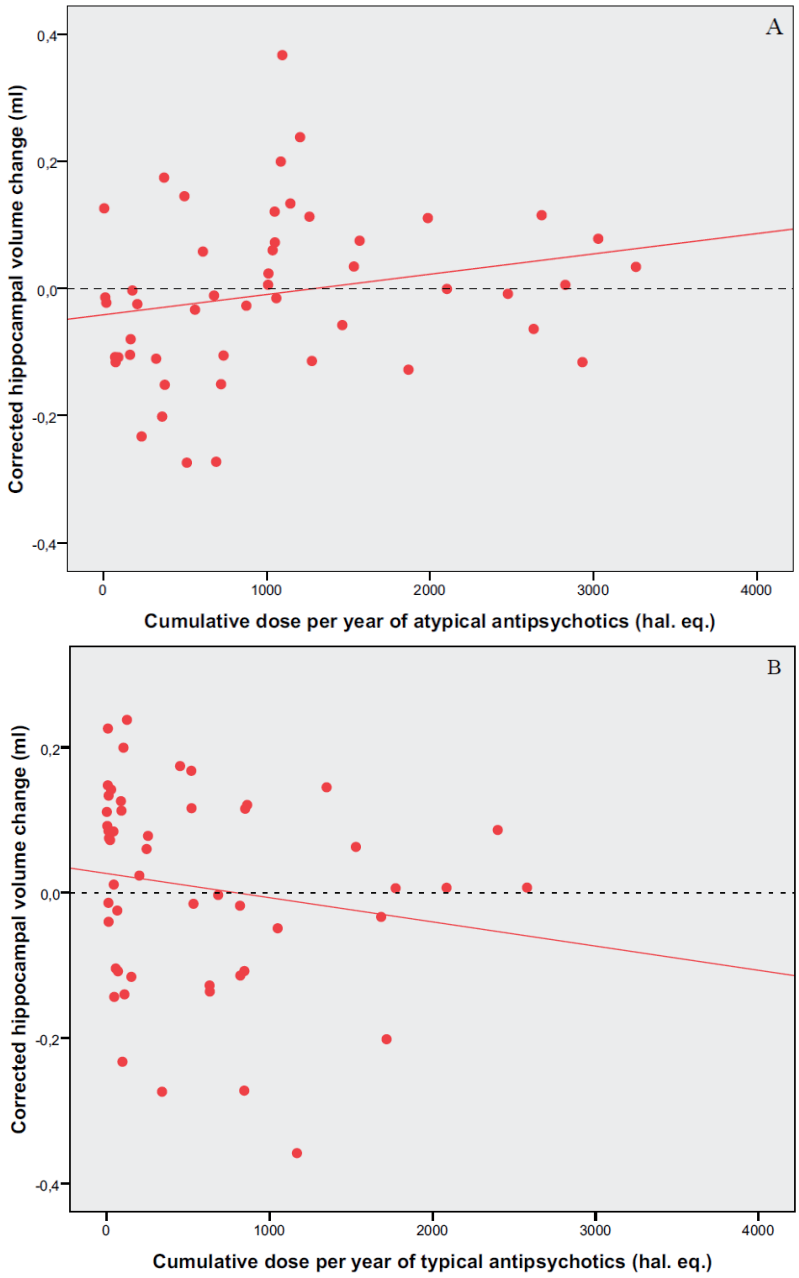

Figure 2. Association between hippocampal volume change and cumulative dose of typical and atypical antipsychotics.

Correlations between corrected hippocampal volume change and cumulative dose of A: Atypical antipsychotics per year (Haldol equivalents) and B: typical antipsychotics per year (Haldol equivalents). One patient who was prescribed a large dose of typical antipsychotics compared to all other patients was excluded from the analysis.

Discussion

This five-year follow-up study compared age-related hippocampal volume change in 95 patients with schizophrenia relative to 113 healthy control subjects. The main finding is that the trajectory of hippocampal volume change over time differs between patients with schizophrenia and healthy individuals. Before the age of 26, patients demonstrated a pattern of larger hippocampal volume loss relative to healthy controls, but thereafter patients did not show excessive volume loss compared with healthy controls. In fact, after age 40 healthy individuals showed a larger volume loss relative to the patients, suggesting that progressive brain abnormalities are present (only) in the early course of the disease.

Our results are consistent with those of cross-sectional studies reporting decreased hippocampal volumes in first episode patients with schizophrenia (Steen et al. 2006) with effect sizes twice as large as those found in chronically ill patients (Nelson et al. 1998). One may speculate that this early volume loss is the result of increased (psychological) stress that accompanies the onset of psychosis, since it has been demonstrated that increased levels of circulating cortisol have been associated with atrophy and loss of neurons in the hippocampus (Czeh and Lucassen, 2007; de Kloet et al. 2005; Lee et al. 2002; Sapolsky, 2000). Interestingly, individuals at high-risk for psychosis who subsequently developed frank psychosis display higher levels of anxiety and depressive symptoms than those who do not go on to develop a psychosis (Phillips et al. 2006). However, brain changes during the period of transition to illness are inconsistent (Pantelis et al. 2007). Since depressive symptoms and depression are highly prevalent in schizophrenia (Häfner et al. 2005) and have been related to decreased hippocampal volume (Campbell et al. 2004; Videbech and Ravnkilde, 2004), this factor could be a potential confounder. Although in our sample a small number of patients showed minor depressive symptoms (as measured with the depressive scale of the PANSS) and were treated with antidepressants, no significant different hippocampal volume (change) was found compared to those solely on antipsychotic medication.

In contrast to the progressive hippocampal volume loss before age 26 in patients, healthy individuals demonstrated a progressive volume loss after the age of 40 relative to the patient group. The linearly increasing hippocampal loss with increasing age is in line with earlier findings in normal aging demonstrating accelerated hippocampal volume loss in later life (Kennedy et al. 2008; Walhovd et al. 2005).

Antipsychotic medication intake appears to be an important confounder when investigating hippocampal volume over time. A significant positive association was found between cumulative intake of atypical antipsychotics, in particular olanzapine, and hippocampal volume change. Patients who were exposed for a longer period or received a higher dose of atypical antipsychotics over time showed less decrease or even small increases in hippocampal volume. In contrast, a negative correlation (although only significant at trend level) was found between cumulative intake of typical antipsychotics and hippocampal volume change, suggesting that patients who received more typical antipsychotic medication during the scan interval showed larger decreases in hippocampal volume. Although our findings indicate a positive association between atypical antipsychotic medication intake and hippocampal volume change, suggesting possible neuroprotective properties of atypical antipsychotics similar with previous reports (Dazzan et al. 2005; Lieberman et al. 2005), these findings should be interpreted with caution since many of the patients currently receiving atypical medication may have been prescribed typical medication at an earlier stage of their illness.
Evidence from animal studies indicates that atypical antipsychotics such as quetiapine and olanzapine increase neurogenesis in the hippocampus (Kodama et al. 2004; Wakade et al. 2002; Xu et al. 2006; but see (Schmitt et al. 2004)). Moreover, olanzapine and quetiapine have been associated with increased hippocampal cell proliferation and prevention of brain-derived neurotrophic factor (BDNF) decrease compared to typical antipsychotics such as haloperidol (Parikh et al. 2004; Park et al. 2006; Xu et al. 2002). Interestingly, BDNF regulates neuronal cell survival, differentiation, synaptic strength and morphology (Ghosh et al. 1994), and emerging evidence suggests that several polymorphisms of the BDNF gene play a role in several neuropsychiatric disorders including schizophrenia (Angelucci et al. 2005).

Although hippocampal volume was significantly smaller in the (larger) baseline schizophrenia sample than in the controls, the difference no longer reached significance after including only those subjects that participated at follow-up. Inspection of Table 3 indeed indicates that hippocampus volume in the total sample of controls and the sub-sample of control subjects that participated at follow-up is almost similar, while for the patients it is not. Patients that participated at follow-up showed a larger baseline hippocampal volume than patients that participated only at baseline; although this difference was not significant, this might suggest a selection bias in our follow-up sample. Indeed, as was presented earlier (van Haren et al. 2007) those patients that were lost for follow-up were older, hence had a longer illness duration,

showed more negative symptoms and had smaller cerebral gray matter volume at baseline. Moreover, those patients included at follow-up had a higher level of education compared to those lost for follow-up. However, these dissimilarities could not explain the lack of difference in baseline hippocampal volume between included and excluded patients.

Several other limitations have to be taken into consideration when interpreting these findings. Most patients changed medication during the scan interval, making it difficult to reliably investigate the specific effects of different types of antipsychotics. Only 10 patients were exclusively taking olanzapine during the scan interval, therefore it cannot be ruled out that the protective effect can be explained by the release of exposure to typical antipsychotics. Moreover, it should be noted that patients differed in the amount of medication that they had used prior to inclusion in the study while reliable information on their lifetime cumulative medication use was not available.
Similar to Whitworth et al. (Whitworth et al. 2005), we found no association between hippocampal volume change and clinical variables such as symptom and outcome measurements at follow-up. However, it must be noted that CAN and GAF scores were not available at baseline. Therefore, whether improvement in daily life functioning between baseline and follow up was associated to hippocampal volume change could not be assessed.

In summary, the age-related trajectories of hippocampal volume change differ significantly between schizophrenia patients and healthy control subjects, with patients showing an excessive volume decrease in the early course of the illness. In contrast, after age 40 the control group showed a progressive decrease of hippocampal volume with increasing age relative to the patients. Speculatively, these differences could be taken to suggest that the high levels of stress that accompany the onset of psychosis result in decreases in hippocampal volumes. Moreover, our findings suggest a differential influence of typical and atypical antipsychotic medication since a larger dose of atypical antipsychotic medication during the interval was related to a smaller decrease of hippocampal volume suggestive for neuroprotective effects of atypical antipsychotic medication.

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5 Cortical thickness and voxel-based morphometry in depressed elderly

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Abstract

This is the first study to examine concurrently cortical thickness and voxel-based morphometric (VBM) abnormalities in patients with major depressive disorder (MDD). In the current study we set out to investigate depressed elderly patients to determine whether a previous depression is related to neurobiological abnormalities in older age. Cortical thickness measures and VBM were applied to the same magnetic resonance imaging data set of 28 female elderly subjects with MDD and 38 age-matched control subjects. Two principal findings emerge from this study. First, no effect of illness on cortical thickness or gray matter density measurements was found. Moreover, life time depression, severity of illness and the number of depressive episodes were not associated with neurobiological abnormalities in older age in our patient group. Second, a diffuse pattern of highly significant age effects were found in cortical thickness as well as in the VBM measurements in the same areas, irrespective of diagnosis.

Nearly one in five people will experience a major depressive episode at some point in their lives (Kessler et al. 2003). Major depressive disorder (MDD) has traditionally been viewed as an illness in which depressive episodes are followed by periods of euthymic mood. Nonetheless, patients in remission may show persistent neurobiological abnormalities. In the current study we set out to investigate elderly patients with MDD to determine whether a previous depression is related to neurobiological abnormalities in older age.
We recently showed in a meta-analysis that MDD is characterized by volume reductions particularly in those brain areas that are involved in emotion processing and stress-regulation (Koolschijn et al. 2009). The vast majority of structural neuroimaging studies in MDD have used a region of interest (ROI) approach. The ROI approach may have biased study outcomes as most MDD studies have a priori focused on brain areas that are involved in emotion- and stress regulation. We intend to use an unbiased approach, i.e. comparing the thickness of the cortex and the density of the whole brain gray matter without any a priori assumptions of localization of structural brain deficits, between elderly patients with MDD and matched elderly controls. Given the known effect of aging on brain development in the later life-span (Galluzzi et al. 2008; Resnick et al. 2003; Rettmann et al. 2006), we also wanted to determine whether age differentially affects cortical thickness and/or gray matter density in both groups.

Methods

Subjects

In this study 28 female outpatients with a lifetime diagnosis of a DSM-IV major depressive disorder were included. Patients were 45 years and older and the first depressive episode had occurred before the age of 45 years. Sample characteristics and inclusion criteria have been published previously (Janssen et al. 2004; Lampe et al. 2003) and will be described briefly (see also Table 1). Diagnoses were established with the Mini-International Neuropsychiatric Interview (Sheehan et al. 1998). Subjects were screened with a self-report health questionnaire that reviewed demographic data and medical history. Exclusion criteria were comorbid psychiatric disorders, a history of central nervous system disease, cerebrovascular disease, dementia, and substance dependence. Depression duration (months) was assessed in an interview by using life-chart methodology. Depression severity at the time of the scan was measured with the Montgomery-Åsberg Depression Rating Scale (MADRS; (Montgomery and Asberg, 1979)). Global cognitive functioning was assessed with the Mini-Mental State Examination (MMSE; (Folstein et al. 1975)). At the time of the scan, 22 patients were receiving medication:

antidepressants (N=11), lithium (N=4), benzodiazepines (N=1), or a combination of antidepressants with neuroleptics (N=3), lithium (N=2), or both (N=1). In addition, eight patients were currently depressed at time of the scan. Mean duration of illness was 93.5 months (SD=17.5 months); we did not have information on duration of illness for five patients. Thirty-eight healthy female control participants, between 45 and 85 years were recruited within the community from general practitioners' practices situated in the city of Utrecht and from advertisements in regional newsletters. Comparison subjects were matched to the patients on age, handedness, and level of education. Furthermore, comparison subjects were given the same self-report health questionnaire as the patients enabling matching on health status. Exclusion criteria were similar to the patient group, with the addition of excluding those with any current or past Axis I psychiatric diagnosis as established by the MINI-Plus interview. Two independent clinical neuroradiologists examined brain MRIs; no gross abnormalities were reported in any participant.

Table 1. Demographics and clinical data of female subjects with MDD and healthy comparison subjects

	Patients with MDD	**Healthy comparison subjects**
	N = 28	**N = 38**
Age (yrs) (mean, sd)	64.04 (10.90)	61.89 (11.03)
Handedness (r/l/both)	26/2/0	34/1/3
Level of education (yr) (mean, sd)	10.89 (4.05)	11.08 (2.93)
MADRS score (mean, sd)	18.32 (13.02)	3.71 (3.86)
MMSE score (mean, sd)	27.39 (2.46)	28.54 (1.56)
Age of onset (yrs) (mean, sd)	33.04 (9.48)	
No of episodes (mean, sd, range; median)	11.14 (14.12) [1-49] 4.00	
Cumulative duration of illness (months)(mean, sd) [a]	93.50 (17.50)	

Abbreviations: MADRS, Montgomery Åsberg Depression Rating Scale; MMSE, Mini-Mental State Examination

[a] Missing data for 5 patients

Brain imaging

Magnetic Resonance Imaging brain scans were acquired on a Philips NT scanner operating at 1.5 T (Best, the Netherlands) with the same scanning protocol for all subjects. The acquisition protocol for the T1- and T2-weighted images and the pre-processing of the scans has been described in detail by Hulshoff Pol et al (Hulshoff Pol et al. 2001). Briefly summarized, scans were put into Talairach frame (no scaling) and corrected for inhomogeneities in the magnetic field. Quantitative assessments of intracranial and total brain volumes were performed on the basis of histogram analyses and series of mathematical morphology operators to connect all voxels of interest. Intensity histogram analysis on the T1 image yielded thresholds for separating brain tissue from cerebrospinal fluid and, within the brain, grey matter from white matter. Grey and white matter segments were created by applying these thresholds to the images (Schnack et al. 2001a; Schnack et al. 2001b).

Cortical thickness

To analyse the cortical thickness, the CLASP-algorithm developed at the McConnell Brain Imaging Centre of the Montreal Neurological Institute was employed (Kabani et al. 2001; Kim et al. 2005; Lerch et al. 2008; Lyttelton et al. 2007; MacDonald et al. 2000). A 3D-surface comprising 81,920 polygons and 40,962 vertices was fitted to the white matter/gray matter intersection, which created the inner surface of the cortex. To create the outer cortical surface, the inner surface was expanded out to fit the gray matter/cerebrospinal fluid intersection. Cortical thickness was estimated by taking the distance between the two surfaces such that each polygon vertex on the outer surface had a counterpart vertex on the inner surface. For each subject the cortical thickness was calculated for every vertex and smoothed across the surface using a 20-mm surface-based blurring kernel (Chung and Taylor, 2004). This method of blurring improves the chances of detecting population differences, but also follows the curvature of the surface to preserve any anatomical boundaries within the cortex. The surfaces of the subjects were registered to an average surface created from 152 subjects (ICBM) (Lyttelton et al. 2007). This registration allowed us to compare cortical thickness locally between subjects.

Voxel-based morphometry

The gray and white matter segments were blurred by a 3D Gaussian kernel (full-width at half maximum (FWHM) = 8 mm) to conform the data to the Gaussian field model underlying the statistical procedures used for making inferences about significance. The voxel values of these blurred gray and

white matter segments reflect the local presence, or concentration, of gray or white matter, respectively, and these images are referred to as 'density maps'. To compare differences in brain tissue at the same anatomical locations in all subjects, the individual density maps were transformed into a standard coordinate system. First, the images were linearly transformed to the model brain, the previously determined 'most average' brain (Hulshoff Pol et al. 2001). In this linear step, a joint entropy mutual information metric was optimized. In the second step, nonlinear (elastic) transformations were calculated to warp the linearly transformed images to the model brain using the ANIMAL program (Collins et al. 1995). The nonlinear registration sampling and stiffness parameters were chosen to minimize global shape differences between brains, although retaining most of local differences. Sampling was limited to a scale of 4 mm (FWHM), with deformation vectors estimated every 2 mm throughout the volume. Stiffness was kept high to have a smooth transformation, thus minimizing changes to local volume. Registration with these parameters corrected for overall brain shape without significantly modifying local dimensions. Finally, the density difference maps were resampled to voxels of size 2x2x2.4 mm^3.

Statistical Analysis

Cortical thickness

To evaluate the differences in cortical thickness at each point between the two groups a vertex-by-vertex analysis was carried out. Group differences in cortical thickness (per vertex) were calculated by using regression analyses with age and handedness as covariates. Diagnosis (patient vs. control) was included in the analysis as an independent variable. This produced F-statistics at each vertex, one for the effect of diagnosis (patients vs. healthy controls), one for the effect of age, and one for the effect of handedness (left/right). Statistical maps were created for differences in cortical thickness in left and right hemisphere between patients and healthy comparison subjects. Earlier Lampe and co-workers reported a significant relationship between total illness duration and cerebral gray matter volume (Lampe et al. 2003). Interestingly, total illness duration was not correlated with age ($r=0.257$; $p=0.237$; $N=23$). Therefore, we repeated the analysis with duration of illness in months as an independent variable in the patient group only. Similarly, the effect of the number of episodes was investigated. Due to the broad range of the number of episodes (see Table 1), we divided patients in two groups based on the median split of the number of episodes (less than five vs. five or more episodes). Furthermore, to investigate possible trait or state effects, we compared depressed patients with remitted patients at time of the scan. To

explore possible age-related differences between both groups a diagnosis-by-age-interaction was added to our analyses.
For those cortical areas that showed significant main or interaction effects the peak-vertex was identified using the software package BRAINVIEW developed at the Montreal Neurological Institute. Given the number of vertices in the brain, a correction for multiple comparisons was carried out according to the false discovery rate (FDR) (α= 0.05, two-tailed), allowing for an overall 5% chance of false positives (Genovese et al. 2002). The critical F-values were 4.95 and 5.06 for the left and right hemisphere respectively.

Voxel-based morphometry

Linear regression analysis was done through all brains for each voxel separately in the gray and white matter density maps. Group (patients vs. healthy controls) and handedness (right/left) entered the analysis as predictor variables; age served as covariate. Similar to the cortical thickness analyses, we repeated the analysis with duration of illness in months as an independent variable in the patient group only. Similarly, the effect of the number of episodes was investigated. Due to the broad range of the number of episodes, we divided patients in two groups based on the median split of the number of episodes (less than five vs. five or more episodes). Furthermore, to investigate possible trait or state effects, we compared depressed patients with remitted patients at time of the scan. To explore possible age-related differences between both groups a diagnosis-by-age-interaction was added to our analyses. Given the number of subjects, data resolution, voxel size, and volume of the search regions we used the false discovery rate (FDR) (α= 0.05, two-tailed), allowing for an overall 5% chance of false positives (Genovese et al. 2002). The critical threshold *t*-value for a two-tailed significance level of 0.05 after correcting for multiple comparisons was $|t|=2.26$ (df=62).

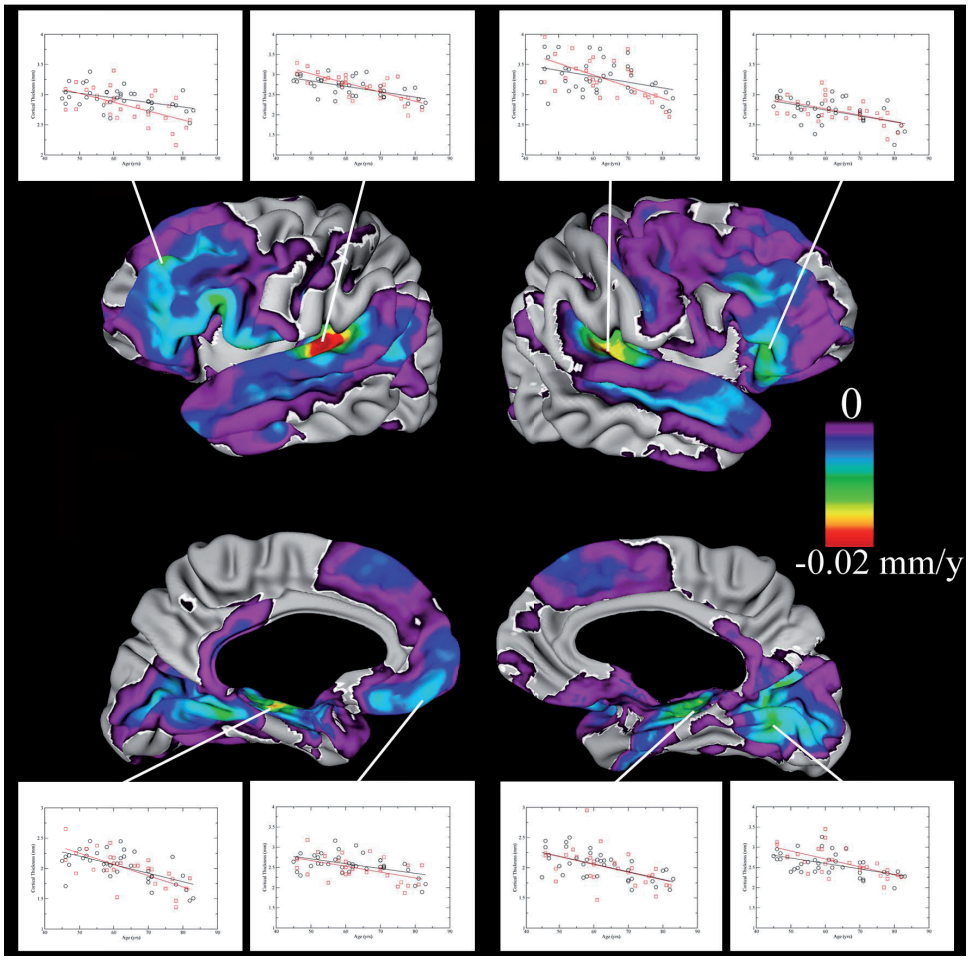

Figure 1. Age-related cortical thinning of the whole cortex irrespective of diagnosis

The critical F-values were 4.95 and 5.06 for the left and right hemisphere respectively, corrected for multiple comparisons according to the false discovery rate, α=0.05, two-tailed. Significant voxels are overlaid on the 152 ICBM template.

Results

Cortical thickness

No significant differences were found between patients and controls on cortical thickness across the cortical surface. Moreover, duration of illness, the number of episodes or current depression state was not associated with cortical thinning in the patient group. In addition, the effect of age on cortical thickness was similar in both groups.

As no significant interaction was found between diagnosis and age on cortical thickness, the effect of age on cortical thickness was investigated in the whole

sample. Highly significant age effects were found in cortical thickness. The statistical maps of age effects are shown in Figure 1. These maps show a pattern of cortical thinning irrespective of diagnosis in widespread areas of the prefrontal and (medial) temporal cortex in both hemispheres. Large age effects were found bilaterally in the posterior parts of the superior and transverse temporal gyrus (Heschl's gyrus), the parahippocampal gyrus and the inferior and middle frontal gyrus. Also, thinner parts of the cortex were found in the inferior, medial and superior frontal gyrus and the rectal gyrus, the lingual gyrus and the posterior cingulate. In Figure 1, for some peak vertices age-related effects in both groups are plotted.

Voxel-based morphometry

No significant main effect of diagnosis was found in gray matter density maps across the brain. In addition, no significant relationship was found between gray matter density and duration of illness, the number of episodes or current depression state in the patient group only. No significant age-by-diagnosis interaction was found.

As no significant interaction was found between diagnosis and age on gray matter density the effect of age on cortical thickness was investigated in the whole sample. Widespread age-related density decreases were found in gray matter density maps after correcting for multiple comparisons with FDR (Figure 2). Highly significant decreased gray matter density is found with increasing age in superior temporal regions, middle and inferior frontal cortices, parahippocampal gyrus, insula and caudate nucleus. Due to the widespread and diffuse pattern of age-related gray matter density decreases, we report only the peak voxels as depicted in Table 2.

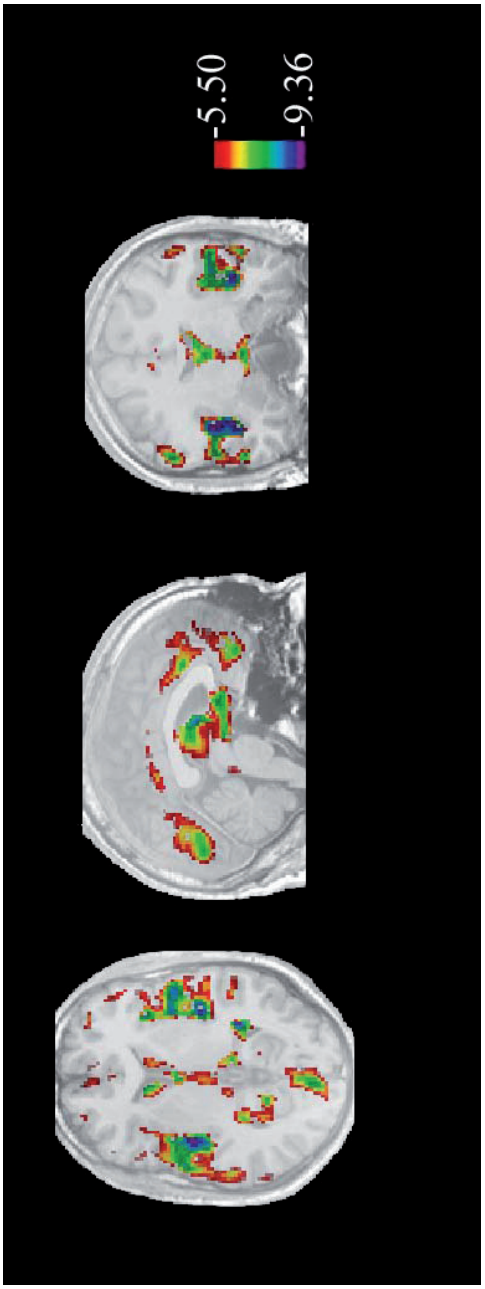

Figure 2. Age-related focal gray matter density decreases irrespective of diagnosis.
Images are oriented according to neurological convention (left=left). Critical level of significance is $|t|=2.26$ (for illustration purposes, $|t|$ is set at 5.5) corrected for multiple comparisons according to the false discovery rate, $\alpha=0.05$, two-tailed. Significant voxels are overlaid on our model brain.

Table 2. Focal gray matter density decreases with age irrespective of diagnosis (peak |*t*|-values)

		Talairach coordinates				
Brain area	**Side**	**x**	**y**	**z**	**t**	**BA**
Angular Gyrus	L	-53	-66	37	-6.51	39
Anterior Cingulate	L	-1	7	-3	-7.70	25
	L	-1	27	21	-7.29	24
Caudate Nucleus	L	-9	14	11	-7.96	
Cuneus	L	-3	-77	9	-6.93	18
	L	-9	-69	11	-6.68	30
Inferior Frontal Gyrus	L	-37	29	-11	-8.53	47
	L	-27	27	15	-6.57	11
Insula	L	-41	-17	13	-9.36	13
Medial Frontal Gyrus	L	-3	39	-13	-6.92	11
Middle Frontal Gyrus	L	-43	36	15	-7.53	46
Middle Temporal Gyrus	L	-52	-2	-7	-8.79	21
Parahippocampal gyrus	L	-25	-45	-7	-8.32	37
	L	-19	-21	-11	-7.98	35
	L	-25	-53	5	-6.82	30
Posterior Cingulate	L	-9	-65	13	-6.66	30
Precentral Gyrus	L	-59	-12	33	-7.28	4
Superior Frontal Gyrus	L	-24	46	25	-6.52	10
Superior Temporal Gyrus	L	-57	-29	17	-8.34	42
	L	-47	9	-5	-8.05	22
	L	-33	12	-23	-6.76	38
Caudate Nucleus	R	9	3	17	-6.45	
Inferior Frontal Gyrus	R	41	26	-7	-7.60	47
Insula	R	39	-19	11	-9.04	13
Lingual Gyrus	R	1	-82	1	-6.61	18
Middle Frontal Gyrus	R	33	50	9	-6.21	10
Middle Temporal Gyrus	R	55	-11	-6	-6.98	22
Parahippocampal gyrus	R	27	3	-11	-7.23	34
Postcentral Gyrus	R	53	-24	17	-8.49	40
Posterior Cingulate	R	7	-56	15	-7.65	23
Posterior Lobe (Declive)	R	-37	-65	-17	-7.11	
Precentral Gyrus	R	49	-4	9	-8.36	6
	R	53	-11	13	-7.86	43
Superior Temporal Gyrus	R	39	5	-13	-6.80	38
	R	49	-33	5	-6.71	22
Thalamus (pulvinar)	R	7	-26	13	-6.66	

Abbreviations: L. left; R, right; BA, Brodmann area.
Critical threshold: FDR: |*t*|=2.26

Discussion

To our knowledge, this is the first study to examine concurrently cortical thickness and voxel-based morphometric abnormalities in patients with MDD in comparison with matched healthy subjects. Two principal findings emerge from this study. First, in contrast to our hypothesis, we did not find an effect of illness on cortical thickness or gray matter density measurements. Second, a diffuse pattern of highly significant age effects were found in cortical thickness as well as in the VBM measurements in the same areas, irrespective of diagnosis.

The lack of illness-related cortical thinning or GM density decreases seems surprising, since we recently showed in our meta-analysis that especially frontal and temporal regions were decreased in volume in patients compared with healthy controls (Koolschijn et al. 2009).

It is difficult to compare our findings with previous VBM studies, because of differences in patient samples. Three VBM studies reported reduced gray matter density in frontal and temporal regions (Egger et al. 2008; Yuan et al. 2008) and in the right hippocampus (Bell-McGinty et al. 2002; Egger et al. 2008) in elderly depressed patients. However, in these studies patients were diagnosed with late onset depression in contrast to our early onset subject sample.

Another possible explanation for the absence of illness-related differences is the fact that patients had relative low scores on the MADRS symptom questionnaire (with only eight patients with a current depression). The MADRS score range is from 0-60; a score of 20 or higher indicates moderate depression with a probable need for treatment (Snaith et al. 1986). So, patients were not severely depressed at time of the scan. State-trait issues have received less attention in the structural imaging literature, but it is suggested that MDD patients who are capable of spontaneous remission, and remain well for years at a time, are clinically different from their counterparts who are chronically ill (Koolschijn et al. 2009; Savitz and Drevets, 2009).

Although we did not find differences in cortical thickness between patients and healthy controls, histopathologic evidence has revealed cellular changes in the forebrain in depression (Rajkowska, 2003). In other postmortem research decreases were found in cortical thickness, neuronal sizes, and neuronal and glial densities in the orbitofrontal cortex , dorsolateral prefrontal and anterior cingulate cortex (Cotter et al. 2001; Cotter et al. 2002; Rajkowska et al. 1999). Besides decreases in glial density, reduced glial numbers have also been reported in regions of the anterior cingulate cortex in familial MDD (Ongur et al. 1998). Thus, both neurons and glia appear to participate in the

neuropathology of depression. Although cellular characteristics cannot be quantified directly in neuroimaging data, cortical thickness may more closely reflect cytoarchitectural abnormalities than cortical volume does. However, it is possible that the pathological differences are too small to detect with MRI (in our small sample).

One of the main goals of this study was to determine whether there were age-related differences in thinning of density between patients with MDD and healthy controls. However, diagnosis was not found to be associated with different aging patterns. This finding is similar to a previous study, investigating only hippocampal volumes, showing that age itself does not predict hippocampal volume in depressed subjects, but rather it may be predicted by length of illness and other variables associated with past burden of illness (Koolschijn et al. 2009; Sheline et al. 1999). Since there were no differences between patients and healthy controls, we examined the effects of age on the whole sample. Age-associated cortical thinning was found throughout the whole cortex irrespective of diagnosis. In particular superior and transverse temporal (Heschl's) gyri and the parahippocampal gyrus showed excessive thinning with increasing age. These regions encompass the primary auditory cortex and are believed to be a major anatomical substrate for speech, language and communication. Furthermore, superior, middle and inferior frontal gyri showed strong age-related thinning. The thinning of the frontal cortex supports the selective vulnerability of the prefrontal cortex in aging, suggesting that brain areas that are latest to develop are most affected in later life (Raz, 2000). Recently, cortical thickness was associated with aging in a large sample of healthy individuals (Fjell et al. 2009). Our results confirm their findings indicating robust cortical thinning in frontal and temporal regions in this age range. Interestingly, Fjell and colleagues also reported the most pronounced excessive thinning in superior and transverse temporal gyri with increasing age.

On the gray matter density maps, diffuse reductions of gray matter density were seen with age in the same regions as the cortical thickness measurements in the frontal and temporal cortex. In addition, reduced basal ganglia gray matter density was found with increasing age. These findings are comparable with other VBM and volumetric data in older healthy individuals showing normal age-related decreases in gray matter density in these areas (Raz and Rodrigue, 2006; Resnick et al. 2003; Salat et al. 2004; Smith et al. 2007).

Our findings may be limited by a relatively small sample size and the fact that only female subjects were included in our study. Since all the patients in this study were on antidepressant medication, the effect of cumulative intake of medication cannot be ruled out.

In summary, in this study we did not find evidence for differences in cortical thinning or gray matter density in elderly female patients with MDD compared with healthy controls. Moreover, life time depression and severity of illness were not associated with neurobiological abnormalities in older age in our patient group.

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6 Cigarette smoking and brain volume loss in schizophrenia

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Abstract

Despite the well-known health hazards of cigarette smoking, its effect on the morphology of the brain has hardly been studied. Moreover, it is unknown whether the reported brain loss in schizophrenia can be attributed to the effects of tobacco smoking.

96 Patients with schizophrenia (54 smokers, 42 non-smokers) and 113 healthy control subjects (35 smokers, 78 non-smokers) were included in a 5-year longitudinal magnetic resonance imaging study. Smoking behavior at time of follow-up measurement was obtained. Multiple regression analyses were performed to investigate whether brain volume change in patients and controls was differentially influenced by smoking behavior.

Significantly more patients smoked than did healthy subjects. Healthy smokers and nonsmokers did not differ in the extent of brain volume change over time. Moreover, no diagnosis–by-smoking status interaction was found on change in brain volume.

In conclusion, we showed that, despite the higher prevalence of smoking behavior and the higher number of cigarettes consumed per day in the patient sample, cigarette smoking did not explain the excessive decreases in cerebral (gray matter) volume in the patients. Moreover, smoking was not associated with brain volume change in the healthy subjects.

Despite the well-known health hazards of cigarette smoking its effect on the morphology of the brain has hardly been studied. Cross-sectional brain imaging studies suggest that healthy subjects who smoke cigarettes show smaller gray matter volumes and/or densities in the prefrontal, anterior cingulate, occipital, and temporal cortices (including parahippocampal gyrus), thalamus, substantia nigra and cerebellum as compared to non-smokers (Brody et al. 2004; Gallinat et al. 2005). Moreover, a negative association has been reported between the total number of cigarettes smoked and volume of frontal and temporal lobes and cerebellum (Gallinat et al. 2006). In addition, gray matter density in the bilateral posterior cingulum, precuneus, and frontal cortex, as well as in the right thalamus was found to be decreased in elderly smokers (aged 70-83 years) as compared to similarly aged subjects who had never smoked cigarettes (Almeida et al. 2008).

Patients with a mental illness are about twice as likely to smoke cigarettes as the general population (Lasser et al. 2000) with schizophrenia patients (and patients with substance abuse disorder) displaying the highest prevalence (66% (Poirier et al. 2002)). Since, as indicated, cigarette smoking may be related to smaller brain volumes in healthy subjects and excessive brain loss over time has been convincingly demonstrated in many longitudinal studies in schizophrenia (Hulshoff Pol and Kahn, 2008; Pantelis et al. 2005) the brain volume loss in schizophrenia could be influenced by the (excessive) use of tobacco in these patients. Surprisingly, it has not been studied whether cigarette smoking affects the brain over time, neither in health or disease. This study therefore investigated the effect of cigarette smoking on brain volume change over a 5-year interval in healthy subjects and patients with schizophrenia.

Method

Subjects

Earlier we reported on excessive brain volume loss in patients with schizophrenia relative to control subjects. A total of 96 patients with schizophrenia (54 smokers, 42 non-smokers) and 113 healthy controls (35 smokers, 78 non-smokers) were included in a 5-year longitudinal study ((see Table 1; van Haren et al. 2008; van Haren et al. 2007). Both at baseline and follow-up, psychopathology was assessed using the Comprehensive Assessment of Symptoms and History (CASH (Andreasen et al. 1992)). Diagnostic consensus was achieved in the presence of a psychiatrist. Outcome was assessed using the Global Assessment of Functioning (GAF (Hall, 1995)), the Camberwell Assessment of Needs (CAN (Phelan et al. 1995)), and the Positive and Negative Syndrome Scale (PANSS (Kay et al. 1987)).

Smoking behavior was determined by the Comprehensive Assessment of Symptoms and History (CASH (Andreasen et al. 1992)) and Composite International Diagnostic Interview Diagnostic (CIDI (Smitten et al. 1998)) at the day of the follow-up MRI scan. Subjects were asked whether they smoked or not, and if so, how many cigarettes they smoke per day on average.

Table 1: Demographic and illness information (n or mean [SD]) at inclusion (T0) and follow-up (T5) of patients with schizophrenia and comparison subjects

	Patients N=96		**Healthy subjects N=113**	
	Smokers N=54	**Non-smokers N=42**	**Smokers N=35**	**Non-smokers N=78**
Number of cigarettes per day [median, range]	23.81 (12.87) [20, 3-60]		10.12 (6.96) [10, 1-25]	
Gender (m/f)	41/13	29/13	26/9	50/28
Age at inclusion (yr)	31.17 (10.78)	33.57 (11.47)	34.90 (12.03)	35.45 (12.42)
Handedness (right/left)	47/7	36/6	32/3	64/14
Education (yr)	10.70 (2.92)	11.62 (3.07)	11.91 (2.76)	12.10 (2.88)
Parental level of education (yr)	12.59 (3.40)	11.24 (3.36)	12.23 (3.40)	12.15 (3.05)
Follow-up duration (yr)	4.80 (0.58)	4.87 (0.51)	4.97 (0.29)	4.92 (0.33)
Age of first psychotic symptoms (yr)	20.69 (5.29)	22.02 (5.53)		
Illness duration at inclusion (yr)	10.48 (10.13)	11.54 (10.45)		
Global Assessment of Functioning at follow-up	51.77 (16.75)	53.29 (17.94)		
PANSS positive at follow-up	14.54 (5.48)	12.69 (4.47)		
PANSS negative at follow-up	13.53 (6.25)	12.79 (5.76)		
PANSS general at follow-up	27.13 (8.57)	26.23 (8.05)		

Brain imaging

T1- and T2-weighted magnetic resonance images were acquired on a 1.5 Tesla Philips NT scanner (Philips, Best, the Netherlands). Quantitative assessments of volumes of the intracranium, cerebrum, cerebellum, lateral and third ventricles, cerebral gray and white matter and cortical gray matter of the four lobes (frontal, parietal, temporal and occipital lobe) were performed. Acquisition parameters and processing procedures have been described before (van Haren et al. 2008).

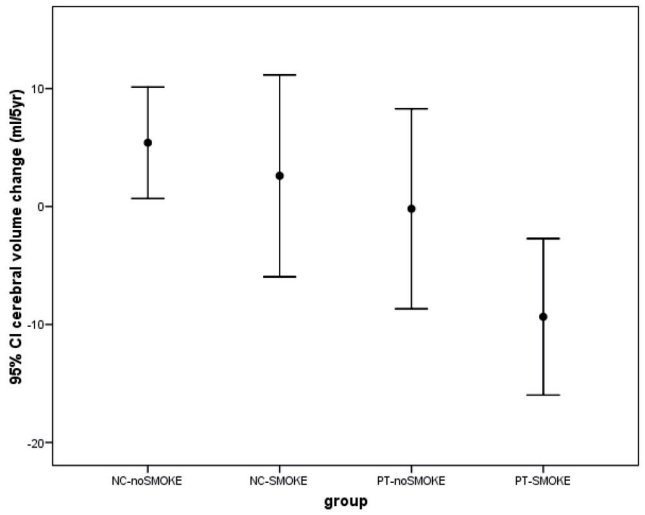
Statistics

Chi-square statistics and ANOVAs were used to compare the groups on the relevant variables, i.e., age, gender distribution, smoking distribution, number of cigarettes smoked per day, and parental education (as a proxy for social economic status). Moreover, smoking and non-smoking patients were compared on cumulative antipsychotic medication intake (typical and atypical antipsychotics in haldolequivalents, olanzapine and clozapine in mgs) and outcome (i.e., PANSS positive and negative symptoms at follow-up, GAF-score at follow-up, CAN-score at follow-up, number of hospitalizations and total duration of hospitalization during the scan-interval).

Main effect of diagnosis on brain volume change was investigated using multiple regression analyses, adding age at baseline, gender and intracranial volume at baseline as covariates. The main analyses were regression analyses investigating the difference between groups in the effect of smoking on brain volume change. Age at baseline, gender and baseline intracranial volume were added as covariates. Main effects for diagnosis and smoking, and the diagnosis-by-smoking interaction were added as independent variables.
Post hoc, smoking was added as a covariate in the first regression analyses, investigating the main effect for illness. Here, *b*-values represent the excessive change in milliliter in patients relative to controls. Comparing the *b*-values before and after correction for smoking behavior provides information on how smoking influences the significance of the diagnosis effect.

Results

A significant overrepresentation of smokers was found in the patient group as compared to the healthy comparison group ($\chi 2 = 13.56$, $p<0.001$). Moreover, the number of cigarettes smoked per day (at follow-up) in smoking patients (mean [sd] = 23.81 [13.01]) was significantly higher than that in smoking controls (mean [sd] = 10.11 [6.96]); $F = 31.65$, $p<0.001$). Information on the number of cigarettes smoked per day was missing in 2 patients and 1 control.



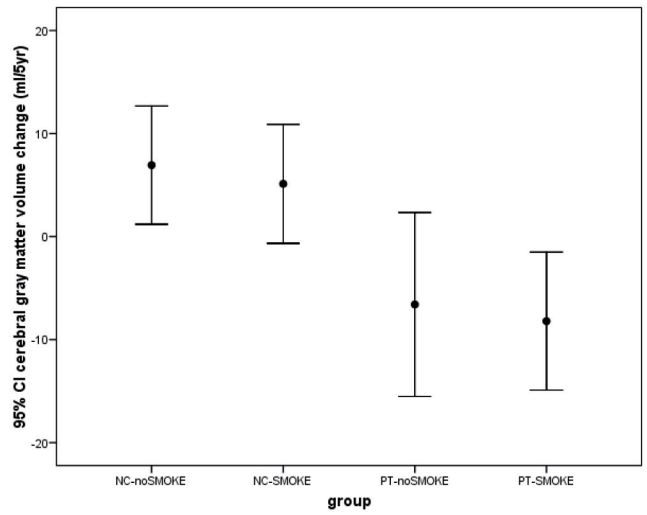


Figure 1. Cerebral (GM) volume change (CI 95%) for smoking and non-smoking patients with schizophrenia and healthy comparison subjects, after correction for intracranial volume at baseline, age at baseline and sex. Patients with schizophrenia show excessive cerebral (GM) volume loss during the interval as compared to healthy individuals. No significant effect of smoking and no significant interaction between diagnosis and smoking was found.

Abbreviations: IC, intracranial volume; NC-noSMOKE, non-smoking controls; NC_SMOKE, smoking controls; PT-noSMOKE, non-smoking patients; PT-SMOKE, smoking patients; 95% CI, 95% confidence intervals

The four groups did not differ with respect to age, level of parental education, gender and handedness distribution. Smoking and non-smoking patients did not differ on any of the medication or outcome measures.

Our previous findings of excessive decreases in cerebral (gray matter) volume, and excessive increases in third and lateral ventricle volumes in patients with schizophrenia relative to control subjects (see Table 3 in (van Haren et al. 2008)) are extended with excessive decreases in the cortical gray matter in all four lobes (see Table 2-first column). No significant effect of smoking on brain volume change was found in healthy individuals (see Table 2-third column). The interaction between diagnosis and smoking did not reveal any significant findings (all p-values > 0.22; see Table 2-fourth column and Figure 1). In a post-hoc analysis we investigated the main effect of diagnosis after controlling for smoking behavior (see Table 2-second column). On average, b-values representing the excess of brain volume change in patients with schizophrenia as compared to healthy subjects were remarkably similar whether or not controlling for smoking.

Discussion

The effect of cigarette smoking on brain volume change over a five year interval was studied in 96 patients with schizophrenia and 113 healthy subjects. Our main finding is that cigarette smoking does not explain the excessive brain tissue loss over time that we found in the patients relative to the healthy controls, despite a significant overrepresentation of smokers among the patients (56.25%) as compared to the healthy individuals (30.97%).

As far as we know there is only one other study investigating the effect of cigarette smoking on brain morphology in patients with schizophrenia (Tregellas et al. 2007). This cross-sectional study, using a voxel-based morphometry approach, reported increased lateral prefrontal and superior temporal gyrus gray matter volumes in 14 patients who smoked cigarettes relative to 18 patients who did not. The relatively low number of subjects and the fact that the effect of smoking could not be investigated in the healthy control group as none of them smoked cigarettes at the time of measurement might explain these findings.

	Relative volume change in patients compared to controls (ml/5yr)			Diagnosis, corrected for smoking		Relative volume change in smoking controls compared to non-smoking controls (ml/5yr)			Excessive volume change in smoking patients (ml/5yr) over and above the effect of smoking in controls		
	b^a (SE)	$t_{(df=204)}$	p	b^b (SE)	p	b^c (SE)	$t_{(df=202)}$	p	b^d (SE)	$t_{(df=202)}$	p
Cerebrum	-10.11 (3.38)	-2.99	0.003	-8.63 (3.47)	0.01	-3.01 (4.90)	-0.62	0.54	-6.60 (7.00)	-0.95	0.34
Cerebellum	-0.25 (0.50)	-2.79	0.62	-0.10 (0.52)	0.85	-0.85 (0.73)	-1.16	0.25	0.36 (1.04)	0.35	0.73
Cerebral GM	-14.19 (3.48)	-4.08	<0.001	-13.71 (3.58)	<0.001	-2.00 (5.08)	-0.40	0.69	-0.02 (7.21)	-0.003	1.00
Cerebral WM	4.08 (2.99)	1.37	0.17	5.08 (3.07)	0.10	-1.01 (4.34)	-0.23	0.82	-6.57 (6.16)	-1.07	0.29
Lateral ventricles	0.82 (0.32)	2.59	0.01	0.83 (0.33)	0.01	-0.18 (0.46)	-0.38	0.70	0.27 (0.66)	0.41	0.68
Third ventricle	0.06 (0.03)	2.38	0.02	0.05 (0.03)	0.04	0.04 (0.04)	0.98	0.33	-0.02 (0.05)	-0.38	0.71
Frontal GM	-4.34 (1.19	-3.66	<0.001	-4.02 (1.22)	0.001	-1.44 (1.26)	-1.14	0.26	0.53 (1.79)	0.30	0.77
Temporal GM	-2.66 (0.68)	-3.89	<0.001	-2.65 (0.70)	<0.001	-0.76 (0.99)	-0.76	0.45	1.48 (1.41)	1.05	0.30
Parietal GM	-1.63 (0.70)	-2.34	0.02	-1.43 (0.72)	0.048	0.02 (1.01)	0.02	0.98	-1.78 (1.44)	-1.24	0.22
Occipital GM	-1.20 (0.47)	-4.16	<0.001	-1.93 (0.48)	<0.001	-0.24 (0.69)	-0.35	0.73	0.31 (0.97)	0.32	0.75

Table 2: Results of the regression analyses showing the effect of diagnosis on brain volume change with and without correction for the effects of smoking (first and second column respectively), the effect of smoking in controls (third column), and the interaction between diagnosis and smoking (fourth column)

[a] Main effect for diagnosis (without adding smoking into the regression): the b-value represents the relative brain volume change in patients with schizophrenia as compared to healthy comparison subjects (ml/5yr)
[b] The *b*-value represents the relative brain volume change in patients as compared to controls (ml/5yr) after correction for smoking (yes/no)
[c] The *b*-value represents the relative brain volume change in healthy smokers as compared to healthy non-smokers (ml/5yr)
[d] Interaction effect between diagnosis and smoking: the *b*-value represents the brain volume change in smoking patients as compared to non-smoking patients (ml). The difference between this *b*-value and the *b*-value of the smoking effect in controls represents the excessive effect of smoking in patients. For example, patients loose -6.60 - -3.01 = -3.59 ml more total brain due to smoking compared to controls. This excessive loss is not significant (p=0.34).

Interestingly, we found no evidence for cigarette smoking to affect brain volume change in healthy subjects. Previous cross-sectional studies showed smaller gray matter volumes and/or densities in the prefrontal, anterior cingulate, occipital, and temporal cortices (including parahippocampal gyrus), thalamus, substantia nigra and cerebellum in smokers compared to non-smokers (Brody et al. 2004; Gallinat et al. 2006). The direction of this effect remains unclear: is the smaller frontal gray matter volume a consequence of smoking cigarettes or do subjects with smaller frontal gray matter volumes have more problems quitting (or are more prone to start smoking) cigarettes. Since we do not find any evidence for excessive brain loss over time as a result of smoking cigarettes the latter explanation seems more likely.
The relationship between cigarette smoking and low socioeconomic status (SES) is well established (for review see (Harwood et al. 2007). Smokers are more likely to be poor and less educated. In addition, there is some evidence of lower SES to be related to smaller brain volume, particularly in lateral inferior frontal gyrus (Raizada et al. 2008) and perigenual anterior cingulate cortex (Gianaros et al. 2007; but see (Eckert et al. 2001; Fotenos et al. 2008). One of the most frequently used measures of SES is level of education (Hackman and Farah, 2009). As patients with schizophrenia often become ill while they are still at school, their level of education is not considered to be a valid measure, therefore, parental level of education is used. As parental level of education did not differ significantly between smokers and non-smokers this could not have influenced our findings.

An important limitation in this study is that information on smoking behavior was only available at follow-up, not at baseline. Although nicotine intake is considered a persistent habit (Foll and Goldberg, 2009), it could well be that subjects either started or stopped smoking during the interval. If anything, it is most likely that subjects stopped smoking during the interval due to increasing pressure from society to quit. In our study, these subjects were included in the non-smokers group (as they reported not to smoke at follow-up measurement). Were the effects of nicotine to be long-lasting or irreversible this could have underestimated the effects of smoking. Secondly, it might be that a 5-year follow-up period is not long enough to pick up subtle effects of smoking cigarettes on brain volume change. If this were the case the effects of smoking would be much smaller in effect size relative to the effect size of brain volume change due to being ill.

In conclusion, we showed that, despite the higher prevalence of smoking behavior and the higher number of cigarettes consumed per day, cigarette smoking did not explain the excessive decreases in cerebral (gray matter) volume in the patients with schizophrenia. Moreover, we found that smoking did not affect brain volume change over time in healthy subjects.

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7 Brain volume abnormalities in major depressive disorder: a Meta-analysis of magnetic resonance imaging studies

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Abstract

So far, there have been no attempts to integrate the growing number of all brain volumetric magnetic resonance imaging studies in depression. In this comprehensive meta-analysis the magnitude and extent of brain volume differences between 2418 patients with major depressive disorder and 1974 healthy individuals from 64 studies was determined. A systematic research was conducted for volumetric magnetic resonance imaging studies of patients with major depressive disorder in relation to healthy control subjects. Studies had to report sufficient data for computation of effect sizes. For each study, the Cohen's d was calculated. All analyses were performed using the random effects model. Additionally, meta-regression analyses were done to explore the effects of potential sources of heterogeneity. Patients showed large volume reductions in frontal regions, especially in the anterior cingulate and orbitofrontal cortex with smaller reductions in the prefrontal cortex. The hippocampus, the putamen and caudate nucleus showed moderate volume reductions. This is the first comprehensive meta-analysis in major depressive disorder demonstrating structural brain abnormalities, particularly in those brain areas that are involved in emotion processing and stress-regulation.

With an estimated life-time risk of at least 10% (Kessler et al. 2003; Weissman et al. 1996) major depressive disorder (MDD) is one of the most common psychiatric illnesses. Moreover, depressive symptoms are highly prevalent among other psychiatric disorders such as schizophrenia (Häfner et al. 2005b), alcohol and drug abuse (Kessler et al. 1996; Regier et al. 1990), and post-traumatic stress disorder (Kilpatrick et al. 2003). Although brain abnormalities have been identified in MDD, the number of neuroimaging studies in patients with this illness pale in comparison to those performed in schizophrenia (for meta-analyses see: (Boos et al. 2007; Honea et al. 2008; Steen et al. 2006; Wright et al. 2000)). Despite an incomplete understanding of the neural circuitry underlying MDD, there is growing consensus that several brain areas are involved in depression (Beyer and Krishnan, 2002; Campbell and MacQueen, 2006; Drevets, 2000; Sheline, 2003; Videbech, 1997). Although reviews have appeared summarizing the results of neuroimaging studies of the hippocampus (Campbell et al. 2004; Videbech and Ravnkilde, 2004) and anterior cingulate cortex (Hajek et al. 2008), there have been no attempts to integrate all volumetric neuroimaging studies in depression to date. Since such an effort could clarify the role of specific brain areas in the pathogenesis of MDD, we conducted a meta-analysis to determine the magnitude and extent of brain volume differences between patients with MDD and healthy individuals as measured with magnetic resonance imaging (MRI).

Methods

Data sources

Magnetic resonance imaging studies that examined differences in brain volumes between patients with MDD and healthy control subjects were obtained through computerized database searches, including PubMED, Embase, Medline, Psychinfo and the Cochrane library. The keywords used in the computerized search were: (major) depression/-ive, unipolar, mood (disorder), affective (disorder), MRI, imaging, (brain) volume(s), morphometry, limbic (system) and gray/white matter. Titles and abstracts of the articles were examined to see whether they fulfilled the inclusion criteria. Bibliographies of included articles were checked for primary studies that might be of relevance.

Study selection

One hundred and four studies were identified as potential candidates for the meta-analysis. Studies were included if they (1) were MRI studies of brain structures in MDD published before January 2008, (2) compared patients with MDD with a healthy control group, (3) were published in the English language, (4) reported sufficient data to obtain an effect size (means, standard deviations, exact *P*-values, or exact *F*-values for a 2-group comparison), (5) reported brain volumes (from multiple slices) rather than an area (from a single slice) and (6) if the mean age in either the MDD or comparison group was above 18 years. Twenty-one studies had to be excluded from the meta-analysis because they did not present sufficient data to compute the Cohen's *d*-values (Ballmaier et al. 2004a; Ballmaier et al. 2004b; Bilder et al. 1999; Buchsbaum, 1986; Chen et al. 2007a; Frodl et al. 2004b; Frodl et al. 2007; Greenwald et al. 1997; Inagaki et al. 2004; Janssen et al. 2007; Kumar et al. 2000b; Kumar et al. 2000a; Lacerda et al. 2005; Lampe et al. 2003; Lavretsky et al. 2007; Lewine et al. 1995; Munn et al. 2007; Rabins et al. 1991; Rabins et al. 2000; Salloway et al. 1996; Shah et al. 1998b). Five studies were excluded because no healthy control group was included or patients with MDD had a life threatening comorbid disease (Dahabra et al. 1998; Ebmeier et al. 1997; Kumar et al. 1999; Nakano et al. 2002; Simpson et al. 2001). Finally, ten studies were excluded because they examined brain volumes in children and/or adolescents (Caetano et al. 2007; Gabbay et al. 2007; MacMaster et al. 2006; MacMaster et al. 2007; MacMaster and Kusumakar, 2004; MacMillan et al. 2003; Nolan et al. 2002; Rosso et al. 2005; Steingard et al. 1996; Steingard et al. 2002). Brain structures were only evaluated when volumes were available in (three or more) independent studies, therefore four studies had to be excluded (overlapping samples: (Frodl et al. 2004a; Lai et al. 2000; Sheline et al. 1999); less than three studies: (Rubin et al. 1996)), resulting in 64 suitable studies that reported volumes of 130 brain structures in a total of 2418 patients and 1974 control subjects. Table 1 lists the included articles and the brain structures that were analyzed.

Table 1. Summary of 64 Studies Included in the Meta-analysis

Source	No of patients	No of controls	Included brain volumes
Almeida et al. 200	51	37	TB, PFC
Ashtari et al. 1999	40	46	TB, Hip
Axelson et al. 1993	19	30	TB, Hip
Ballmaier et al. 2004c	24	19	IC, TB, OFC, ACC
Ballmaier et al. 2007	14	10	Hip
Botteron et al. 2002	30	8	TB, ACC
Brambilla et al. 2002	18	38	ACC
Bremner et al. 2000	16	16	IC, PFC, Hip, Amyg, Caud
Bremner et al. 2002	15	20	TB, OFC, ACC
Caetano et al. 2001	17	39	Thal
Caetano et al. 2004	31	31	IC, Temp, Hip, Amyg
Caetano et al. 2006	31	31	ACC
Colla et al. 2007	24	14	IC, Hip
Coryell et al. 2005	10	10	ACC
Drevets et al. 1997	17	21	ACC
Dupont et al. 1995	30	26	Caud
Frodl et al. 2002b	30	30	IC, TB, Hip
Frodl et al. 2002a	30	30	Amyg
Frodl et al. 2003 [a]	27	27	Amyg
Frodl et al. 2006	34	34	IC, PFC, Hip
Hannestad et al. 2006	182	64	Caud
Hastings et al. 2004	18	18	ACC, Amyg
Hickie et al. 2005	66	20	IC, Hip
Hickie et al. 2007	45	16	Amyg, Caud
Husain et al. 1991	44	44	TB, Puta
Janssen et al. 2004	28	41	IC, TB, OFC, Hip
Krishnan et al. 1992	50	50	TB, Caud
Krishnan 1993	25	20	TB, Thal, Puta, Caud
Kumar et al. 1998	53	30	IC, TB, PFC, Temp
Lacerda et al. 2003	25	48	Puta, Caud
Lacerda et al. 2004	31	34	OFC
Lange and Irle 2004	17	17	TB, Hip, Amyg
Lavretsky et al. 2004	41	41	IC, PFC, OFC
Lee et al. 2003 [b]	41	41	IC, OFC
Lenze and Sheline 1999	24	24	Puta, Caud
Lloyd et al. 2004	51	39	Hip
MacQueen et al. 2003	37	37	Hip

Table 1 continued			
Maller et al. 2007	45	30	Hip
Mervaala et al. 2000	34	17	Hip, Amyg
Monkul et al. 2007	17	17	OFC, ACC, Hip, Amyg
Naismith et al. 2002	47	20	Caud
Neumeister et al. 2005	31	57	Hip
O'Brien et al. 2004	61	40	TB, Hip
Pantel et al. 1997	19	13	IC, TB, PFC, Temp
Parashos et al. 1998	32	32	TB, PFC, OFC,Thal, Puta, Caud
Pillay et al. 1997	38	20	TB
Pillay et al. 1998	38	20	Caud
Posener et al. 2003	27	42	TB, Hip
Rusch et al. 2001	25	15	Hip
Salokangas et al. 2002	37	19	PFC, Temp
Saylam et al. 2006	24	24	IC, Hip
Sheline et al. 1996	10	10	TB, Hip
Sheline et al. 1998	20	20	TB, Amyg
Sheline et al. 2003	38	38	Hip
Steffens et al. 2000	66	18	Hip
Steffens et al. 2003 b	30	40	TB, OFC
Taylor et al. 2005	135	83	Hip
Taylor et al. 2007	226	144	OFC
Vakili et al. 2000	38	20	Hip
Velakoulis et al. 2006	19	87	Hip, Amyg
von Gunten et al. 2000	14	14	Hip, Amyg
Vythilingam et al. 2002	32	14	Hip
Vythilingam et al. 2004	38	33	Hip
Weniger et al. 2006	21	23	IC, TB, Hip, Amyg

Abbreviations: IC, intracranial; TB, total brain; PFC, prefrontal cortex; ACC, anterior cingulate; Temp, temporal cortex; OFC, orbitofrontal cortex; Hip, hippocampus; Amyg, amygdala; Puta, putamen; Caud, caudate nucleus; Thal, thalamus.

[a] Only patients with recurrent MDD and their matched healthy controls were selected from this study, data regarding the first episode patients was not included

[b] OFC volumes were excluded in the analyses due to overlapping samples with Taylor et al. (Taylor et al. 2007)

Data extraction

The brain structures that were suitable for analysis included intracranium, total brain, prefrontal cortex, anterior cingulate, temporal cortex, orbitofrontal cortex, hippocampus, amygdala, caudate nucleus, putamen and thalamus. If sufficient data was present, analyses were extended to examine the effect in the two hemispheres separately. When brain volumes were only reported per hemisphere total volume was calculated by summarizing the left and right brain volumes. To obtain the total standard deviation (SD_{To}), the following formula was used:

$$SD_{To} = \sqrt{(((SD_{Le}^2) + (SD_{Ri}^2)) + 2 * (Cor_{Le,Ri}) * SD_{Le} * SD_{Ri}))}$$

with $SD_{Le/Ri}$ being the standard deviation of the volume of the particular left/right brain structure and $Cor_{Le,Ri}$ being the correlation between the left and right volume of the brain structure. Although it is reasonable to assume that left and right brain volumes are (highly) correlated, it is unlikely that correlations are exactly "1" (e.g., due to the influence of handedness on brain volume (Geschwind et al. 2002)). However, to correct for rounding off errors and to be conservative in our estimation, we set the correlation at "1" and hence allowed larger estimated SD's for total volumes.

The key to a meta-analysis is defining an effect size statistic capable of representing the quantitative findings of a set of research studies in a standardized form that permits meaningful comparison and analyses across the studies (Lipsey and Wilson, 2001). Therefore, for each study in this meta-analysis, the effect size statistic Cohen's *d* was calculated. In this analysis, the mean volume of a specific brain structure for patients with MDD was subtracted from the mean volume for comparison subjects and divided by the pooled standard deviation of both. When means and standard deviations were not available, *d*-values were calculated from exact *P*-values, *t*-values, or *F*-values. Meta-analytic methods were applied to obtain a combined effect size, which indicated the magnitude of the association across all studies (Hedges and Olkin, 1985). A *t*-test was subsequently performed on the null hypothesis that the *d*-value is 0.00, which we report together with the associated *P*-value. According to Cohen (Cohen, 1988), *d*-values of 0.2 represent small effects, values between 0.4 and 0.6 moderate effects, and *d*-values of 0.8 or higher large effects.

A second measure of effect size was used to calculate a percentage difference between both groups. We used the ratio of the mean volume in the depression group divided by the mean volume in the comparison group. Specifically, for each region in study i (i= 1,2,3,…k) we used the weighted ratio effect size (EffRW) defined as:

$$EffRW_i = \left(\frac{M_{Pt,i}}{M_{NC,i}} \right) * w_i$$

where pt refers to patients with MDD, nc to the control group, w to the weights per study and M to the group regional volume mean. To control for sample size differences, for each region the EffRW is multiplied with the specific weights per study derived from our meta-analyses. By adding the separate weights, an average weighted percentage volume difference is obtained.
Next to the effect size the variance between and within studies has to be explored. All analyses were performed with a random-effects model using the statistical package Comprehensive Meta-analysis V2 (Borenstein and Rothstein, 1999). If there is significant heterogeneity among the results of the included studies, random effects models will give wider confidence intervals than fixed effect models (DerSimonian and Kacker, 2007; DerSimonian and Laird, 1986). For each brain region, a test of homogeneity (Cochran's *Q* test) was performed to test whether the studies could be assumed to share a common population effect size. A significant *Q* statistic indicates heterogeneity of the individual study effect sizes, which poses a limitation to a reliable interpretation of the results. Additionally, we also calculated I^2 to provide a more interpretable measure of consistency between studies in this meta-analysis (Higgins et al. 2003; Huedo-Medina et al. 2006). The I^2 index can be interpreted as the percentage of the total variability in a set of effect sizes due to true heterogeneity, that is, to between-studies variability. The I^2 is placed between 0% and 100% where a value of 0% indicates no observed heterogeneity and larger values imply increasing heterogeneity. Since MDD is a heterogeneous psychiatric illness we expected a significant result in the homogeneity test. Therefore, a meta-regression analysis was planned to explore the effects of potential sources of heterogeneity, by regressing effect sizes against mean age, gender, duration of illness, illness onset, number of depressive episodes, medication intake or symptom scores. The random effects regression analysis was performed using the unrestricted maximum likelihood model in Comprehensive Meta-analysis (Borenstein and Rothstein, 1999).

To examine the possibility of publication bias, we computed a fail-safe number of studies (Orwin, 1983; Rosenthal, 1991). Publication bias implies that studies with no effect may not be published, posing a threat to the stability of the obtained effect size. The fail-safe number (NFS) of studies indicates the number of unpublished studies with null effects that must reside in file drawers to reduce the observed effect size to a negligible level. The statistic can be calculated with the use of the formula given by Orwin (Orwin, 1983) and Lipsey and Wilson (Lipsey and Wilson, 2001):

$$NFS = k * [(ESk / ESc) - 1]$$

with k being the number of studies, ESk, the mean weighted effect size; and ESc, the criterion effect size (which we set at a *d*-value of 0.10).

Results

Meta-analyses

As presented in Table 2, the results of the meta-analysis indicate brain volume decreases in patients with MDD as compared to healthy control subjects. The 95% confidence intervals were methodologically stringent for all significant findings. In addition, the fail-safe number of studies for all analyses was large enough to lend credence to our findings.

Table 2. Brain structures included in Meta-analysis and Results

Brain Structure	No of studies	No of patients	No of controls	Mean weighted effect size Cohen's *d* (95% CI)	*P*- value for *d*	Average weighted percentage difference in %	Within-Category Homogeneity statistic Q	*P* -value for *Q*	I^2 in %	NFS
Intracranium	14	452	364	0.03 (-0.13 to 0.20)	0.37	NA	17.69	0.17	26.53	10
Total Brain	22	703	622	-0.06 (-0.16 to 0.05)	0.29	NA	15.89	0.78	0.00	35
Amygdala										
Left	13	321	361	0.07 (-0.28 to 0.43)	0.68	NA	49.53	<0.001	75.72	4
Right	13	321	361	0.14 (-0.11 to 0.40)	0.27	NA	26.00	0.011	53.85	6
Total	14	366	377	0.05 (-0.25 to 0.35)	0.76	NA	42.58	<0.001	69.47	7
Anterior Cingulate cortex										
Left	8	183	171	-1.11 (-1.88 to -0.34)	0.005	-12.19	74.83	0.005	90.65	97
Right	7	166	150	-0.62 (-0.88 to -0.37)	<0.001	-10.71	7.29	0.30	17.65	52
Total	8	181	170	-0.769 (-1.32 to -0.22)	0.006	-11.91	44.58	<0.001	84.30	71
Caudate Nucleus										
Left	5	285	172	-0.04 (-0.24 to 0.16)	0.67	NA	0.33	0.52	0.00	8
Right	5	285	172	0.00 (-0.20 to 0.20)	0.99	NA	1.27	0.87	0.00	5
Total	10	467	316	-0.31 (-0.58 to -0.04)	0.024	-6.74	26.89	0.001	70.19	41
Hippocampus [a]										
Left	30	1083	914	-0.37 (-0.52 to -0.23)	<0.001	-4.71	65.43	<0.001	55.68	142
Right	30	1083	914	-0.41 (-0.54 to -0.28)	<0.001	-5.12	58.14	0.001	50.12	153
Total	31	1114	991	-0.41 (-0.54 to -0.28)	<0.001	-5.07	60.67	<0.001	50.55	158

Orbitofrontal cortex										
Left	5	326	255	-0.52 (-0.85 to -0.18)	0.002	-9.48	10.76	0.029	62.82	31
Right	5	326	255	-0.47 (-0.79 to -0.15)	0.004	-8.71	9.87	0.043	59.48	29
Total	7	373	204	-0.43 (-0.78 to -0.09)	0.014	-9.18	21.95	0.001	72.67	38
Prefrontal cortex										
Left	5	157	119	-0.22 (-0.44 to -0.01)	0.045	-2.1	1.55	0.82	0.00	17
Right	5	157	119	-0.22 (-0.44 to 0.00)	0.053	-1.21	1.94	0.75	0.00	16
Total	7	242	181	-0.34 (-0.52 to -0.16)	<0.001	-3.35	4.82	0.57	0.00	31
Putamen										
Left	3	90	116	-.32 (-0.88 to 0.23)	0.26	NA	7.53	0.023	73.42	13
Right	3	90	116	-.34 (-0.95 to 0.27)	0.27	NA	9.05	0.011	77.90	14
Total	6	192	184	-0.48 (-0.80 to -0.16)	0.003	-11.28	11.77	0.038	57.52	35
Temporal cortex										
Left	3	87	63	0.07 (-0.24 to 0.37)	0.67	NA	0.44	0.80	0.00	2
Right	3	87	63	0.28 (-0.03 to 0.58)	0.076	NA	0.81	0.67	0.00	6
Total	4	140	93	-0.03 (-0.40 to 0.34)	0.88	NA	6.85	0.077	56.20	6
Thalamus	3	74	91	-0.17 (-0.55 to 0.20)	0.37	NA	3.08	0.21	35.10	9

Abbreviations: CI, confidence intervals; NFS, fail-safe number: number of unpublished studies with null effects that must reside in file drawers to reduce the observed effect size to a negligible level; I^2: percentage of the total variability due to true heterogeneity; NA, Not Applicable

[a] Since exclusion of studies examining medication naive and medication free (at least six weeks of medication) patients did not change the results, the full sample results are displayed.

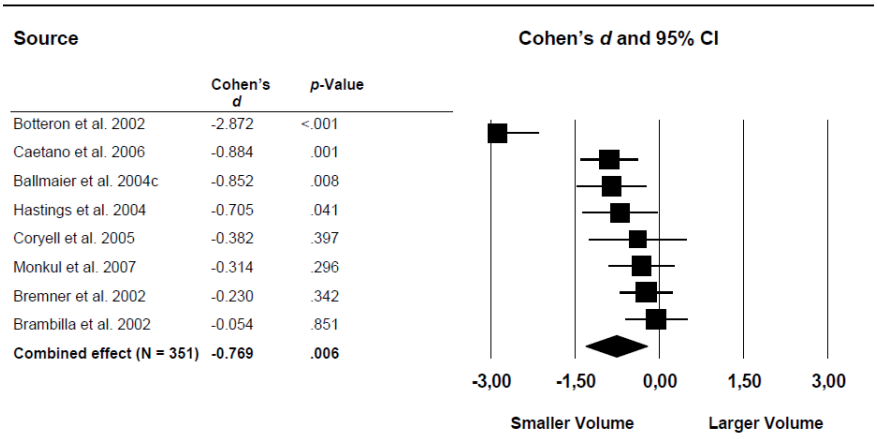

Figure 1. Mean total anterior cingulate cortex volume. Error bars indicate 95% confidence interval.

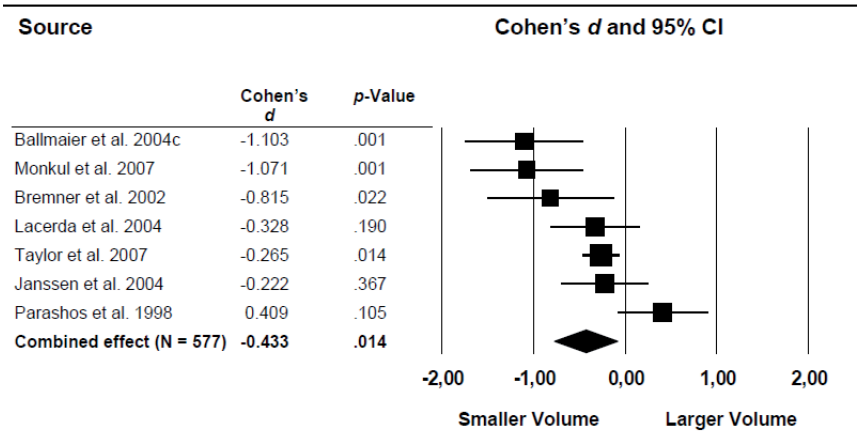

Figure 2. Mean total orbitofrontal cortex volume. Error bars indicate 95% confidence interval.

Areas in the frontal lobe

The largest effect was found for the anterior cingulate cortex, with smaller volumes in patients with MDD compared with healthy control subjects (Figure 1). Eight studies were included with a total group size of 181 patients with MDD and 170 healthy controls, resulting in a combined-effect Cohen's *d* of -0.769 (p=0.006). Excluding the studies that used specific sub-regions of the anterior cingulate cortex (Botteron et al. 2002; Brambilla et al. 2002; Bremner et al. 2002; Drevets et al. 1997; Hastings et al. 2004) did not change the results. The effect was most pronounced in the left anterior cingulate cortex volume (k=8; *d* =-1.11; p=0.005; right anterior cingulate cortex volume:

k=7; *d*=-0.624; p<0.001).

Other areas in the frontal lobe that showed reduced volumes in patients with MDD were the orbitofrontal cortex (total: k=7; *d*=-0.433; p=0.014; left: k=5; *d*=-0.516; p=0.002; right: k=5; *d*=-0.469; p=0.004; Figure 2) and the left (k=5) and total (k=7) prefrontal cortex (left: *d*=-0.223; p<0.005), total: *d*=-0.342; p<0.0001). The right prefrontal cortex volume difference between patients with MDD and healthy controls was significant at trend level (*d*= -0.216; p=0.053; Figure 3).

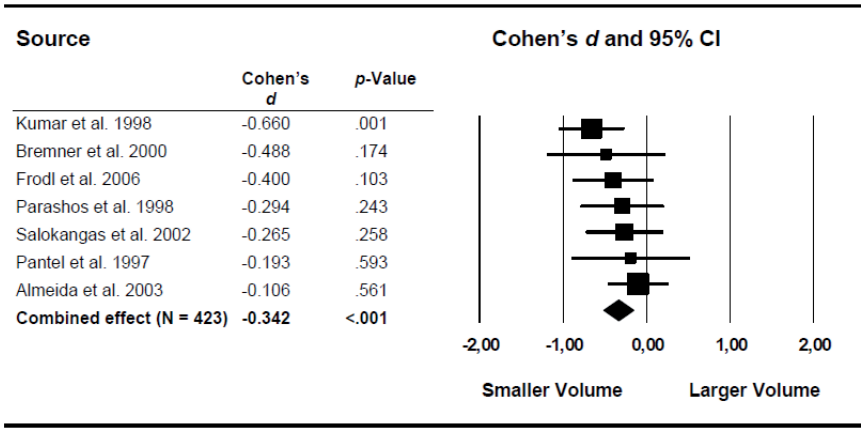

Figure 3. Mean total prefrontal cortex volume. Error bars indicate 95% confidence interval.

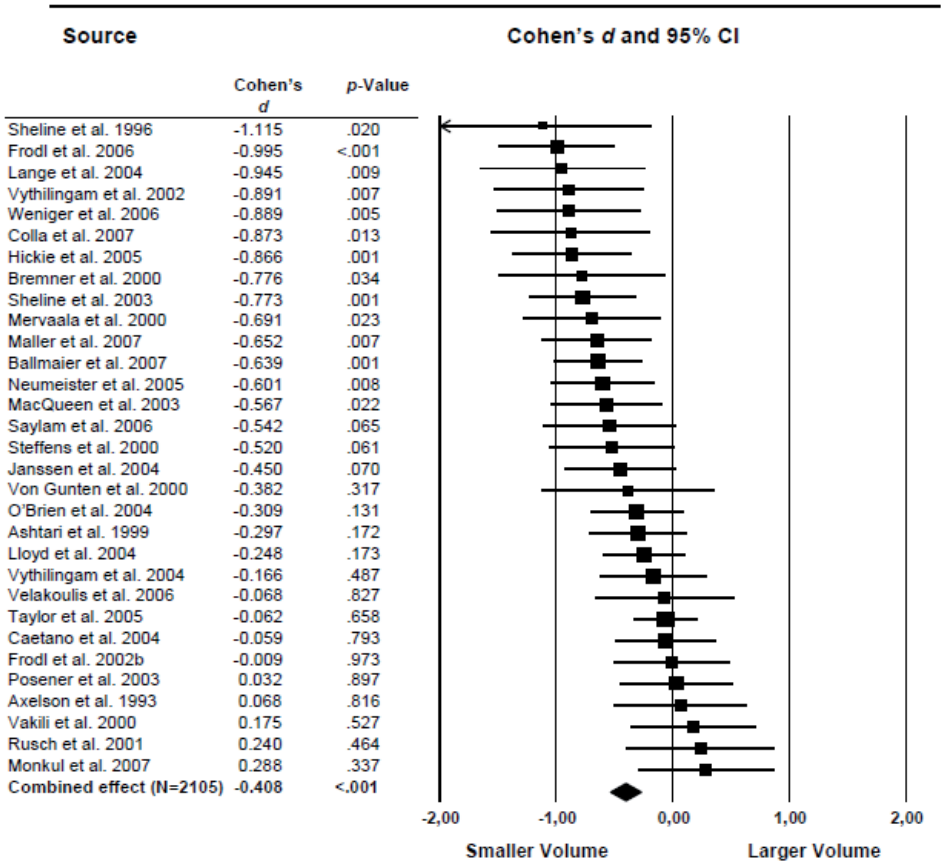

Figure 4. Mean total hippocampal volume. Error bars indicate 95% confidence interval.

Hippocampus

The hippocampus was the brain structure studied most often (k>30), with a total group size of 1114 (left/right: 1083) patients with MDD and 991 (left/right: 914) control subjects. Reduced hippocampal volumes were found in patients with combined-effect sizes of -0.373 ($p<0.0001$), -0.408 ($p<0.0001$) and -0.408 ($p<0.0001$) for the left, right and total hippocampus volume respectively (Figure 4). Excluding the studies that examined medication naive or medication free (at least six weeks free of medication) patients (MacQueen et al. 2003; Neumeister et al. 2005; Saylam et al. 2006; Vythilingam et al. 2004) did not change the results (total: k=27; d=-0.409; $p<0.001$). We also examined hippocampal volume of these studies with untreated patients to elucidate differences as compared to the whole sample with treated patients, but findings in this group are comparable (k=4; d=-0.362; $p=0.008$).

Striatum

Significant volume reductions of the striatum were found in the patient group compared to healthy individuals (Figures 5 & 6). Specifically, a moderate effect was found for the total putamen volume (k=6; *d*=-0.48; p=0.003) and a small effect was found in the total caudate nucleus volume, (*d*=-0.31, p=0.024).

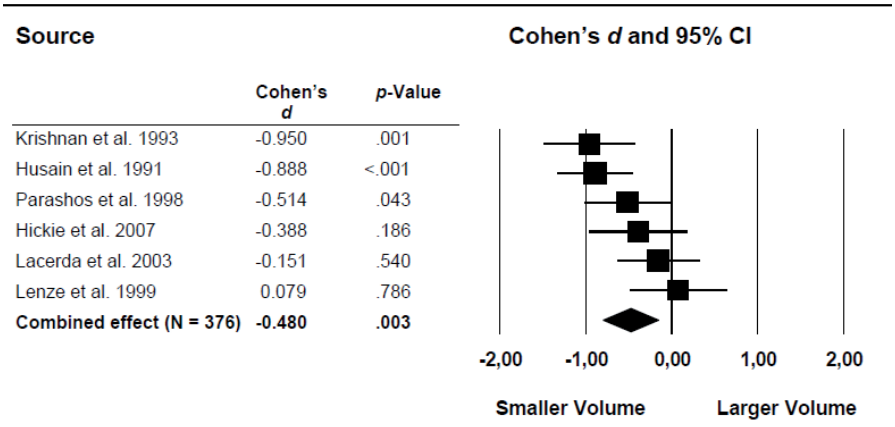

Figure 5. Mean total putamen volume. Error bars indicate 95% confidence interval.

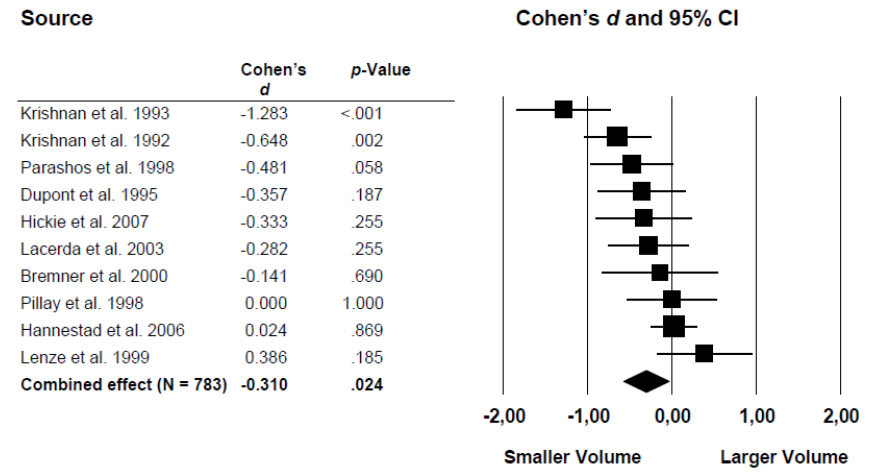

Figure 6. Mean total caudate nucleus volume. Error bars indicate 95% confidence interval.

Other brain areas

Analyses of volumes of the intracranial space, total brain, temporal cortex, (bilateral) amygdala, and thalamus did not show significant differences between the groups.

Meta-regression

Significant heterogeneity among studies was detected for several structures that showed significant differences between the groups (Table 2 lists the Cochran's *Q*-coefficients and I^2 for the homogeneity tests). A priori it was assumed that inter-study differences in age, gender, age of onset, duration of illness or symptom scores could explain some of the surplus in variance. Unfortunately, most studies provided insufficient data regarding medication intake or number of episodes; therefore we were unable to perform the meta-regression with these variables. A significant effect of age (z=-2.38; p=0.017) was found on total putamen volume (Q_{model}=5.33; p=0.02 vs. $Q_{residual}$=6.73; p=0.15). None of the other moderator effects reached significance.

Discussion

This meta-analysis integrated the results of 64 magnetic resonance imaging studies that compared the volumes of various brain structures in patients with MDD (N=2418) with those of healthy control subjects (N=1974). The results indicate pronounced brain volume reductions in specific brain areas in patients with MDD.

Frontal lobe

Several lines of evidence suggest the presence of specific neural circuits within the limbic-cortical system that mediate stress-responsiveness, mood and emotional regulation (Brody et al. 2001; Seminowicz et al. 2004; Tekin and Cummings, 2002). Interestingly, the results of this meta-analysis revealed the presence of structural brain abnormalities in patients with MDD in many of the regions involved in emotional processing and stress-responsiveness. Of particular interest are the prominent volume reductions in frontal regions (anterior cingulate, orbitofrontal and prefrontal cortex) which are known to control emotion regulation by inhibiting the activity of limbic regions such as the hippocampus and the amygdala (Beauregard et al. 2001; Hariri et al. 2000). Evidence from postmortem studies suggests cell loss and cell atrophy in subgenual prefrontal and orbitofrontal cortex in patients with MDD (Rajkowska, 2000). The results of this meta-analysis also showed a pronounced volume reduction of the left anterior cingulate cortex relative to the right side. This finding is consistent with previous studies reporting larger left than right subgenual anterior cingulate cortex volumes reductions (Chen et al. 2007b; Drevets et al. 1997).

Limbic system and frontal lobe

In addition to the decreased volumes of prefrontal regions, hippocampal volumes were also reduced in patients with MDD. Given the putative relationship between increased sensitivity to stress and affective disorders (Swaab et al. 2005), it is important to note that the hippocampus, amygdala and prefrontal cortex are also involved in Hypothalamus-Pituitary-Adrenal (HPA)-axis regulation. This is relevant, since MDD has been linked to disrupted HPA-axis activity and increased levels of cortisol (Bao et al. 2008; Swaab et al. 2005) which in turn has been postulated to effect hippocampal volume through the inhibition of neurogenesis in this brain structure (Czeh and Lucassen, 2007; Henn and Vollmayr, 2004; Sapolsky, 2000). Several mechanisms have been proposed to explain how prolonged stress can result in limbic and prefrontal abnormalities, such as decreased dendritic branching (Radley and Morrison, 2005), decreased neurogenesis (Duman, 2004), loss of neurons, or decreased expression of brain derived neurotrophic factor (Duman et al. 1997; Radley and Morrison, 2005). Evidence for stress-induced brain abnormalities in MDD is also provided by studies examining genetic variations in the glucocorticoid receptor gene. Especially functional polymorphisms of the NR3C1 gene (Nuclear Receptor Subfamily 3, Group C, Member 1) are associated with increased susceptibility to MDD (van Rossum et al. 2006; van West et al. 2006). Of particular interest are the findings of a recent study reporting an association of four illness-related polymorphisms of the NR3C1 gene with overall smaller hippocampal volumes in patients with MDD (Zobel et al. 2008). This suggests that "at-risk"-alleles of the NR3C1 gene influence hippocampal volume.

Amygdala

The meta-analysis revealed no significant volumetric abnormalities in the amygdala. However, the amygdala findings are highly inconsistent with a broad range of effect sizes. Although the anatomical boundaries of the amygdala are difficult to outline on MRI images, all studies reported high intrarater correlation coefficients suggesting reliably measured volumes. Also, no association was found between choice of segmentation protocol and amygdala volume increases or decreases. An explanation for the discrepancy between studies may relate to genetic differences between subjects and patients samples. For instance, reduced gray matter volume in the amygdala is more pronounced in those subjects carrying the s-allele of the serotonin transporter promoter polymorphism (Heinz et al. 2005; Pezawas et al. 2005). Indeed, individuals carrying the s-allele tend to have increased anxiety-related temperamental traits, which in turn are related to increased risk for developing

depression (Lotrich and Pollock, 2004). Only a few studies examined the association between the serotonin transporter polymorphism and brain structures in patients with MDD, showing mixed results with respect to amygdala, hippocampus and caudate nucleus volumes (Frodl et al. 2004b; Hickie et al. 2007; O'Hara et al. 2007; Taylor et al. 2005). Finally, it is unknown if other factors, such as whether a patient is in a current episode or in remission as well as duration of illness contributes to differences in amygdala volume. In addition, it is unclear to what extent the amygdala is affected by antidepressant medication.

Striatum

The findings from this meta-analysis support the presence of volume reductions in the striatum, primarily in total putamen volume. The striatum has been associated with mood, cognitive processes, motivation and regulation of movement and is also part of several neuroanatomic circuits that are involved in mood regulation (Alexander et al. 1986; Drevets, 2001; Rogers et al. 1998; Tekin and Cummings, 2002). Further evidence of striatal involvement in MDD is provided by a post-mortem study showing reduced putamen and pallidum volumes in patients with MDD compared to non-psychiatric subjects (Baumann et al. 1999). Interestingly, lesions of the putamen and of the caudate nucleus have been associated with a higher prevalence of MDD and/or depressive symptoms in post-stroke depression (Starkstein and Robinson, 1989; Vataja et al. 2004), Huntington disease (Slaughter et al. 2001a) and Parkinson disease (Slaughter et al. 2001b).

Antidepressant medication

Most studies included in this meta-analysis examined brain volumes in patients on antidepressant treatment; this hampered our ability to analyze effects of antidepressant medication on brain volumes in depression. Moreover, definition of medication free status varied greatly among studies. Although the effect of antidepressants on the brain is obviously important, so far only a few studies evaluated this in patients with MDD. In geriatric patients with depression, antidepressant exposure was associated with larger orbitofrontal gray matter volume compared with medication naïve patients (Lavretsky et al. 2005). In contrast, other studies have reported improved memory and decreased symptom severity, but failed to find an association with hippocampal or orbitofrontal cortex volume (Janssen et al. 2007; MacQueen et al. 2003; Vythilingam et al. 2004). However, one must bear in mind that with the exception of one study (Vythilingam et al. 2004), all studies were cross-sectional, and none of these studies corrected for cumulative or

life-time medication intake.

Overlap and differences with schizophrenia and bipolar disorder

Major depressive disorder, schizophrenia and bipolar disorder share important clinical features, i.e. depressive symptoms, anhedonia, memory deficits, and lack of motivation (Häfner et al. 2005b; Häfner et al. 2005a; Lake, 2008). Moreover, during the course of schizophrenia the prevalence of depression ranges widely, from 6% to 75% (Siris and Bench, 2003). While there is considerable overlap in risk factors and precursors in these disorders, the overlap in brain volume abnormalities is less clear. Recent meta-analyses indicate mild ventricular enlargement in bipolar disorder (Kempton et al. 2008; McDonald et al. 2004) and reduced cerebral, temporal lobe and amygdala volumes, and enlarged lateral and third ventricles, and basal ganglia volumes in schizophrenia (Boos et al. 2007; Wright et al. 2000) compared to healthy controls. Interestingly, based on our results and the previous meta-analyses in schizophrenia, patients with MDD and schizophrenia both show reduced hippocampal (Nelson et al. 1998), prefrontal and anterior cingulate cortex volumes (Baiano et al. 2007; Wright et al. 2000), suggesting that brain regions regulating stress response are affected in both disorders. Indeed, psychosocial stress is a well-established precipitant of depressive episodes as well as psychotic relapses (Walker, 2008). Moreover, stressors such as life events and high expressed emotion have been found to precede the onset and recurrence of depression (Kessler, 1997) and psychotic disorder (Bebbington et al. 1996). Therefore, it may well be that the phenotypic overlap of reduced brain volumes (on the basis of the previous mentioned meta-analyses) in MDD and schizophrenia could be explained by a common genetic vulnerability to stress. Indeed, this vulnerability may be phenotypically expressed as a decrease in hippocampal volume. In fact, evidence that schizophrenia and MDD share common genetic background is found in the original DISC1-gene study (DISC1=Disrupted in Schizophrenia-1 family)(Millar et al. 2000). Usually, DISC1 is considered to be a major risk gene for schizophrenia but affected individuals in this family displayed a range of diagnoses, with the majority being diagnosed with either schizophrenia or MDD (Blackwood et al. 2001; Hashimoto et al. 2006). In addition, the DISC1-gene is involved in structural and functional alterations in hippocampal formation and probably adult neurogenesis in the dentate gyrus (Callicott et al. 2005).

Limitations

Some limitations of this meta-analysis should be noted. First, structures other than those that have been evaluated in this meta-analysis may also be affected in patients with MDD. For instance, separated gray and white matter volumes of the cerebrum are scarcely reported. Moreover, only two studies (Parashos et al. 1998; Salokangas et al. 2002) examined ventricular volumes, while increased ventricular volume is the most replicated finding in schizophrenia (interestingly, both studies reported volume increases in MDD patients) (Wright et al. 2000). Second, although we found significant decreases in brain volumes, almost all analyses resulted in significant heterogeneity which hampers a reliable interpretation and may have influenced the results (Hedges and Vevea, 1998). However, all significant findings indicate brain volume reductions with reliable confidence intervals in patients with MDD relative to healthy control subjects. In addition, the meta-regression analyses did not show differences between studies, except for a small effect of age on the putamen. Unfortunately, many studies included in this meta-analysis did not provide sufficient data to examine the effects of antidepressant medication. Moreover, most studies lack information on number of depressive episodes and scores on symptom scales. Thus, the possibility that some of the significant effects are confounded by these factors cannot be ruled out.

Future directions

So far, most studies used a region of interest approach, however, the use of voxel-based morphometry allows comprehensive and global assessment of brain structures without a priori identification of regions of interest (Ashburner and Friston, 2000). Although, voxel-based morphometry studies are sparse in MDD (Bell-McGinty et al. 2002; Chen et al. 2007b; Shah et al. 1998a; Tang et al. 2007; Vasic et al. 2008), findings are in line with those found in our meta-analysis. Of interest is also the measurement of cortical thickness, i.e. a measure to determine the thickness of the gray matter of the human cerebral cortex (Davatzikos and Bryan, 1996; Fischl and Dale, 2000; Kabani et al. 2001; Thompson and Toga, 1996). Cortical thinning is frequently regionally specific and can therefore provide important additional information for characterizing disease-specific neuroanatomical changes. Thus far, there have been no published studies that measured cortical thickness in MDD.

Relatively new MRI techniques such as diffusion tensor imaging and magnetization transfer imaging are used to study the orientation of white matter tracts in vivo and yield an index of microstructural integrity (Basser et al. 1994; Wolff and Balaban, 1989). Interestingly, findings from these studies in MDD indicate microstructural white matter abnormalities in widespread prefrontal

and limbic regions (Alexopoulos et al. 2008; Bae et al. 2006; Gunning-Dixon et al. 2008; Li et al. 2007; Murphy et al. 2007; Nobuhara et al. 2004; Nobuhara et al. 2006; Taylor et al. 2004; Yang et al. 2007). Integration of white matter volume measurements and these MRI techniques may improve our understanding of the neural circuitry involved in MDD.

In addition, longitudinal imaging studies can clarify whether the volume reductions are static or progressive over time and to what extent the volume (change) is affected by the effects of antidepressant medication, illness severity or age. Future studies should also include relatives of patients with MDD and (discordant) monozygotic and dizygotic twin-pairs to examine the relationship between genetic vulnerability to develop the illness and brain morphology. Such studies have proven to be valuable in schizophrenia and bipolar disorder in clarifying some of the underlying mechanisms of the brain volume abnormalities in probands (Baaré et al. 2001; Boos et al. 2007; Brans et al. 2008; Lawrie et al. 2008; van der Schot et al. 2009; Whalley et al. 2007). Finally, future studies ought to focus on the search for susceptibility genes in relation to brain abnormalities by using linkage and association methods.

Conclusion

In summary, our results show that structural brain abnormalities are present in patients with MDD. Since MDD is characterized by abnormalities in emotion regulation and stress-responsiveness, the majority of studies in our meta-analysis focused on investigating those areas that are involved in these processes. Our findings indeed provide evidence that many, but not all, of those areas, show volume reductions in patients with MDD. Some of the brain abnormalities in depression are similar to those reported in patients with schizophrenia, such as the declines in hippocampal and frontal volumes with comparable effect sizes. Finally, this meta-analysis confirms the preservation of global cerebral and temporal cortex volume in MDD patients, which is in line with findings in patients with bipolar disorder, but in contrast to the slight but significant reduction found in schizophrenia patients. Our results strongly suggest that studying brain structure in MDD will contribute to understanding the pathogenesis of this disease.

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8 Summary & concluding words

Summary

The aim of this final chapter is to provide a summary and discussion of the main findings of this thesis. In this thesis we described a variety of studies that explore structural brain abnormalities in schizophrenia and major depressive disorder. To create order in this brainstorm called thesis, a division was made between genetics (**chapters 2, 3**), confounders in neuroimaging (**chapters 4-6**) and meta-analysis (**chapter 7**). In the sections that follow, an overview of the main findings and conclusions of each of the studies will be presented.

Genetics

In **chapter 2** we set out to investigate hypothalamus volume in monozygotic (MZ) and same-sex dizygotic (DZ) twin pairs discordant for schizophrenia with those in healthy MZ and same-sex DZ twin pairs in order to examine whether these volume alterations are due to genetic or environmental factors. Hypothalamus volumes were found to be decreased in patients as well as their unaffected co-twins compared to healthy twin pairs. However, after correction for total brain volume, these findings were no longer significant. This suggests that the decrease of hypothalamus volume is not specific and can be attributed to a reduction in total brain volume. Other studies that have examined the hypothalamus have found larger volumes in patients as well as their nonpsychotic relatives (Goldstein et al. 2007), or smaller volumes in poor outcome patients as compared to healthy controls (Hulshoff Pol et al. 2005). Recently, our group investigated hypothalamus and pituitary volumes in 154 patients with schizophrenia and 156 healthy comparison subjects (van Haren et al. 2009). No differences in volumetric measurements were found between patients and controls in both structures. The discrepancy between all these studies can be explained by differences in scan and segmentation protocols. Another possible explanation can be the intake of antipsychotic medication. Since the hypothalamus contains many dopamine receptors (Ben-Jonathan and Hnasko, 2001; Kienast and Heinz, 2006) and antipsychotics exert their effect, among others, on the dopamine system, this may effect hypothalamus volume. However, the decreased hypothalamic volumes in unaffected co-twins compared with healthy control twin pairs who never had taken antipsychotic medications suggest that it is unlikely that our findings can be explained by antipsychotic medication intake.

We also reported higher within-twin pair similarities in monozygotic compared to dizygotic twin pairs, suggesting that hypothalamus volume might be partly genetically controlled. Although no significant differences were found between patients, their unaffected co-twins and healthy control

twin pairs, the twin model remains a powerful approach for determining the relative contributions of genetic influences and common and unique environmental influences on variation in brain volumes and their common origin with disease liability (Rijsdijk et al. 2005).

It is still unknown which and how many genes are responsible for the onset of schizophrenia. Many studies have focused on testing specific genetic markers in known candidate genes for association with the disease. However, results have been disappointing, which has been attributed in part to the difficulty relating genes to a phenotype as complex as schizophrenia. Consequently, it has been suggested that linking genes to more straightforward phenotypes that are both heritable and related to the illness under study, may facilitate detection of these genes (Glahn et al. 2007). Indeed, brain structure may serve as such an intermediate (or endo)phenotype since some of the brain abnormalities associated with schizophrenia have been found to be related to the genetic risk to develop the disease. This approach, also referred to as "imaging genetics" is a rapidly developing field in schizophrenia and other psychiatric disorders. The aim of the study described in **chapter 3** was to investigate the relationship between hippocampal volume change over time and BDNF genotype in patients with schizophrenia and healthy control subjects. In contrast to our hypothesis, we did not find an effect of BDNF genotype on hippocampal volume change over time irrespective of diagnosis nor could we replicate earlier findings suggesting smaller hippocampal volume in Met-carriers. However, we did find a genotype-by-diagnosis interaction demonstrating smaller baseline hippocampal volumes in patients homozygous for the Val-allele relative to healthy Val-homozygotes. Also, an effect of diagnosis in general was found at baseline, reflecting smaller hippocampal volumes in patients relative to healthy controls irrespective of genotype. In the full baseline sample, our study confirms previous findings from cross-sectional studies reporting on decreased hippocampal volumes in schizophrenia patients relative to healthy controls (Nelson et al. 1998; Wright et al. 2000). In the present study, hippocampal volume decreased over time in both patients and controls. In the healthy control group, the hippocampal volume loss over time was found to be significantly larger than that of the patient group. Due to considerable overlap in our population compared with our previous study described in **chapter 4**, these findings are not surprising (Koolschijn et al. 2009a). The current finding underscores the excessive hippocampal volume loss with normal aging and is in line with earlier findings in normal aging demonstrating accelerated hippocampal volume loss in later life (Kennedy et al. 2008; Walhovd et al. 2005).

Our findings from the baseline analyses suggest that a smaller hippocampal volume is specific for patients with schizophrenia who are homozygous for the Val-allele and is not present in Met-carrier patients. This could indicate that Val-homozygous patients are more susceptible for decline in hippocampal volumes compared with patients who are Met-carrier. Given the susceptibility of the hippocampus to extraneous influences, one could speculate that having this particular genotype makes a patient more vulnerable to the effects of environment or the illness itself. As no genotype effect was found on hippocampal volume change over time, this genetic susceptibility possibly oc-curs early in the illness or before illness onset. Indeed smaller hippocampal volumes are already present in first episode patients (Steen et al. 2006; Vita et al. 2006). Less is known about the period of transition to illness, since both smaller and larger hippocampal volumes have been reported (Pantelis et al. 2007). It would be of interest to investigate the effect of BNDF in these samples.

Our results suggest that the BDNF Val66Met polymorphism is not associated with hippocampal volume change over time. Nevertheless, our findings may support the possibility that BDNF affects brain morphology differently in schizophrenia patients and healthy subjects.

Confounders in neuroimaging

In **chapter 4** we provided evidence for differences in the trajectory of hippocampal volume change in a five year follow-up study between patients with schizophrenia and healthy individuals. Before the age of 26, patients demonstrated a pattern of larger hippocampal volume loss relative to healthy controls, but thereafter patients did not show excessive volume loss compared with healthy controls. In fact, after age 40 healthy individuals showed a larger volume loss relative to the patients, suggesting that progressive brain abnormalities are present (only) in the early course of the disease. The linearly increasing hippocampal loss with increasing age in the healthy control group is in line with earlier findings in normal aging demonstrating accelerated hippocampal volume loss in later life (Kennedy et al. 2008; Walhovd et al. 2005).

The second goal of this study was to examine the influence of antipsychotic medication when investigating hippocampal volume over time. We found a significant positive association between cumulative intake of atypical antipsychotics, in particular olanzapine, and hippocampal volume change. Patients who were exposed for a longer period or received a higher dose of atypical antipsychotics over time showed less decrease or even small increases in hippocampal volume. In contrast, a negative correlation (although only

significant at trend level) was found between cumulative intake of typical antipsychotics and hippocampal volume change, suggesting that patients who received more typical antipsychotic medication during the scan interval showed larger decreases in hippocampal volume. Although our findings indicate a positive association between atypical antipsychotic medication intake and hippocampal volume change, suggesting possible neuroprotective properties of atypical antipsychotics similar with previous reports (Dazzan et al. 2005; Lieberman et al. 2005), these findings should be interpreted with caution since many of the patients currently receiving atypical medication may have been prescribed typical medication in an earlier stage of their illness.

In **chapter 5** we presented the first study to examine concurrently cortical thickness and voxel-based morphometric abnormalities in elderly female patients with major depressive disorder (MDD) in comparison with matched healthy subjects. Two principal findings emerge from this study. First, in contrast to our hypothesis, we did not find an effect of illness on cortical thickness or gray matter density measurements. The lack of illness-related cortical thinning or gray matter (GM) density decreases seems surprising, since we recently showed in our meta-analysis that especially frontal and temporal regions were decreased in volume in patients compared with healthy controls (Koolschijn et al. 2009b).
It is difficult to compare our findings with previous voxel-based morphometry (VBM) studies, because of differences in patient samples. Three VBM studies reported reduced gray matter density in frontal and temporal regions (Egger et al. 2008; Yuan et al. 2008) and in the right hippocampus (Bell-McGinty et al. 2002; Egger et al. 2008) in elderly depressed patients. However, in these studies patients were diagnosed with late onset depression in contrast to our early onset subject sample. It has been suggested that late-onset depression is often a precursor for dementia or other neurodegenerative processes. Furthermore, late onset depression is associated with cerebrovascular risk factors or disease and this is thought to be the dominant contributor to the pathogenesis of late onset depression (Krishnan et al. 2004). Early onset depression on the other hand is thought to be associated with stress-related neurotoxic factors (Mcewen, 2005; Pittenger and Duman, 2008). Therefore, it could be speculated that the suggested underlying mechanisms in late-onset depression lead to more tissue loss in later age than those underlying early onset depression.
Second, a diffuse pattern of highly significant age effects on cortical thickness and GM density were found, irrespective of diagnosis. Age-associated cortical

thinning was found throughout the whole cortex irrespective of diagnosis. In particular superior and transverse temporal (Heschl's) gyri and the parahippocampal gyrus showed excessive thinning with increasing age. These regions encompass the primary auditory cortex and are believed to be a major anatomical substrate for speech, language and communication. Furthermore, superior, middle and inferior frontal gyri showed strong age-related thinning. The thinning of the frontal cortex supports the selective vulnerability of the prefrontal cortex in aging, suggesting that brain areas that are latest to develop are most affected in later life (Raz, 2000). Recently, cortical thickness was associated with aging in a large sample of healthy individuals (Fjell et al. 2009). Our results confirm their findings indicating robust cortical thinning in frontal and temporal regions in this age range. Interestingly, Fjell and colleagues also reported the most pronounced excessive thinning in superior and transverse temporal gyri with increasing age. On the gray matter density maps, diffuse reductions of gray matter density were seen with age in the frontal and temporal cortex, and basal ganglia. These findings are comparable with other VBM and volumetric data in older healthy individuals showing normal age-related decreases in gray matter density in these areas (Raz and Rodrigue, 2006; Resnick et al. 2003; Salat et al. 2004; Smith et al. 2007). In summary, in this study we did not find evidence for differences in cortical thinning or gray matter density in elderly female patients with early onset MDD. However, widespread highly significant age effects on cortical thickness and gray matter density were found in frontal and temporal regions, irrespective of diagnosis.

In **chapter 6** the effect of cigarette smoking on brain volume changes over time in healthy subjects and patients with schizophrenia was investigated. Our main finding is that cigarette smoking does not explain the excessive brain tissue loss over time that we found in the patients relative to the healthy controls, despite a significant overrepresentation of smokers among the patients (56.25%) as compared to the healthy individuals (30.97%). Interestingly, excessive smoking (> 35 cigarettes per day) that was only present in the patients did accelerate brain loss over time, although it did not explain the excessive brain volume loss in schizophrenia patients as a whole.
As far as we know there is one other study investigating the effect of smoking on the structure of the brain in patients with schizophrenia (Tregellas et al. 2007). This cross-sectional study, using a voxel-based morphometry approach, reported increased lateral prefrontal and superior temporal gyrus gray matter volumes in 14 patients who smoked cigarettes relative to 18 patients who did not. The effect of smoking was not investigated in the control group as none

of them smoked cigarettes at the time of measurement. These findings contradict to the findings of our cross-sectional analyses performed at follow-up measurement. Smoking and non-smoking patients did not differ in brain volume at the time their smoking status was actually assessed.
Interestingly, we found no evidence for smoking to affect brain volume change in healthy subjects in this study. So far, no longitudinal studies in healthy individuals have been done investigating the effect of smoking on brain volume change over time. Previous cross-sectional studies showed smaller gray matter volumes and/or densities in the prefrontal, anterior cingulate, occipital, and temporal cortices (including parahippocampal gyrus), thalamus, substantia nigra and cerebellum in smokers compared to non-smokers (Brody et al. 2004; Gallinat et al. 2006). By comparing smoking and non-smoking healthy comparison subjects at follow-up measurement only, we confirmed earlier findings of a smaller gray matter volume in the frontal cortex, but not the temporal cortex, in smokers. The direction of this effect remains unclear; are the smaller frontal gray matter volume a consequence of smoking cigarettes or are subjects with smaller frontal gray matter volume more prone to start smoking cigarettes (or have more problems quitting)? Our data suggest that although smokers tend to have smaller frontal gray matter volumes there is no evidence to suggest that smoking leads to excessive loss over time in healthy individuals.
In conclusion, we showed that, despite the higher incidence of smoking and the higher number of cigarettes consumed per day in the patient sample, cigarette smoking did not explain the excessive decreases in cerebral (gray matter) volume in the patients. However, extremely heavy smoking may contribute to excessive gray matter volume in schizophrenia.

Meta-analysis

In **Chapter 7** we conducted a meta-analysis to determine the magnitude and extent of regional brain volume differences, as measured with magnetic resonance imaging (MRI) between patients with MDD and healthy control subjects. Our results show that structural brain abnormalities are present in patients with MDD. Since MDD is characterized by abnormalities in emotion regulation and stress-responsiveness, the majority of studies in our meta-analysis focused on investigating those areas that are involved in these processes. We found heavily reduced anterior cingulate (-12%) and orbitofrontal cortex (-9%) volumes as well as putamen (-11%) volume. Moderate volume decreases were found in the caudate nucleus (-7%), hippocampus (-5%) and prefrontal cortex (-3%). Our findings indeed provide evidence that many, but not all, of those areas show volume reductions in patients with MDD.

Unsuspected, we did not find differences between patients with MDD and healthy comparison subjects in amygdala volume. However, it must be noted that the amygdala findings were highly inconsistent between studies with a broad range of effect sizes. Possibly, other factors, such as whether a patient is in a current episode or in remission as well as duration of illness may contribute to differences in amygdala volume. Some of the brain abnormalities found in patients with depression are similar to those reported in patients with schizophrenia, such as the declines in hippocampal and frontal volumes, with comparable effect sizes. Finally, this meta-analysis confirms the preservation of global cerebral and temporal cortex volume in MDD patients, which is in line with findings in patients with bipolar disorder, but in contrast to the slight but significant reduction found in schizophrenia patients.

Methodological considerations & future directions

A number of methodological limitations must be considered when interpreting morphometry studies. First, bearing in mind that loss of neuronal bodies, decrease in neuronal size, and decreases in dendritic arborisation are all potential sources of volume reductions on T1- weighted images, the specific neurohistologic processes that account for the observed volume reductions cannot be distinguished. Second, the use of different methodological approaches (voxel-based morphometry vs. volumetry), may account for discrepancies between studies.

To partly overcome this, it is suggested to use multimodal MRI protocols to provide a "new" strategy for the detection and characterization of subtle structural (and functional) alterations in defined regions of the brain. This can be accomplished by combining different MRI methods, such as volumetric or voxel-based morphometry approaches with diffusion tensor imaging and magnetization transfer imaging which are used to study the orientation of white matter tracts in vivo and yield an index of microstructural integrity (Basser et al. 1994; Wolff and Balaban, 1989).

One may speculate that brain volume changes over time, rather than cross-sectional study designs, are better suited to predict outcome and progression of the disorders. In schizophrenia, different lines of evidence suggest that some structures appear abnormal before the first sign of any symptom and therefore during a first episode, while some of these same structures and others show a higher than expected volume loss over time and thus appear different at follow-up when compared with healthy controls (Pantelis et al. 2005). These progressive changes seem to be present during the initial years after the first episode and continue even in the more chronic phase of the illness (Hulshoff Pol and Kahn, 2008). Due to the lack of knowledge of disease

progression in major depressive disorder, longitudinal imaging studies can clarify whether the volume reductions we found are static or progress over time and to what extent the volume change is affected by the effects of antidepressant medication, illness severity, or age.

Third, the intake of psychotropic (and antidepressant) medication itself may modulate regional brain volumes. In **chapter 4** we showed that hippocampal volume change over time is differently affected by the intake of first or second generation antipsychotic medication. It is suggested that antipsychotic drugs act regionally rather than globally on the brain, with different effects on different brain structures. An estimate of the effect sizes of these volumetric changes suggests that they are of a greater magnitude in association with typical than with atypical antipsychotics (Navari and Dazzan, 2009). Fourth, as discussed in the introduction, it can be difficult to perfectly match patients and healthy controls. This was obviously made clear in **chapter 6** were patients smoked excessively more cigarettes per day compared with healthy controls.

Finally, in the relatively young discipline of imaging genetics, there is considerable room for methodological improvement. As whole-genome scans of hundreds of thousands of genetic variants have become feasible, the selection of variants to study has become a pressing, and so far unsolved, problem. Many variants that are statistically associated with psychiatric disease are of no known functional consequence. Furthermore, given the absence of reliable information on the heritability and reliability of most imaging phenotypes currently in use, a statistically significant result in neuroimaging is by itself not sufficient to establish that a given polymorphism is functional (Meyer-Lindenberg and Weinberger, 2006). Moreover, the complex nature of psychiatric disease suggests that gene-environment and gene-gene interactions may explain larger portions of variance than single candidate genes. However, it must be noted that brain volumes have been shown to be useful endophenotypes for studies in psychiatric illnesses because they are highly heritable, associated with the phenotype, and they cosegregate with the illness within families (Bearden et al. 2007; Braff et al. 2007; Glahn et al. 2007).

Concluding words

In this thesis we described a variety of MRI studies in schizophrenia and major depressive disorder. We highlighted the importance of genetic research by means of twin models, or the search for candidate genes and relating them to brain morphometry. Furthermore, we have shown the importance of longitudinal studies when investigating age-related changes or the confounding influences of antipsychotic medication and smoking on brain morphometry. In addition, the combination of different MRI techniques such as cortical thickness measurements and VBM can provide a better understanding of what is going on in the brain, not only cortical but also subcortical. Finally, the application of meta-analytic methods in MRI research increases our knowledge of which brain structures are affected, and shows the path to which future research should be directed.

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Nederlandse samenvatting

Brainstorm.

Waarschijnlijk is de eerste associatie die u heeft bij “Brainstorm”, er een van een sessie waarin spontaan nieuwe ideeën of oplossingen worden geopperd om problemen op te lossen. Deze associatie komt geheel overeen met mijn hele promotietraject. Brainstorm betekent ook het tijdelijk disfunctioneren van het brein of “hersenkronkel”. Als u het woord letterlijk zou nemen, dus een storm in het brein, kunt u waarschijnlijk ook de associatie maken met bijvoorbeeld de hallucinaties in schizofrene patiënten of de wisselende stemmingen bij depressieve patiënten. De ondertitel “structurele hersen-“afwijkingen” in schizofrenie en depressie” zal een betere weergave zijn van de inhoud van dit proefschrift.

Schizofrenie en depressieve stoornis (MDD) delen belangrijke klinische kenmerken, zoals depressieve symptomen, anhedonie, geheugenproblemen en gebrek aan motivatie. Bovendien varieert de prevalentie van depressie sterk in het verloop van de ziekte schizofrenie namelijk: van 6% tot 75%. Voor de meeste patiënten, zowel voor schizofrenie als voor MDD is de ziekte een levenslange aandoening met meerdere episodes of terugvallen. De chronische en het terugkerende verloop van beide aandoeningen is ingrijpend en een groot klinisch probleem wat vaak leidt tot een langdurige preventieve behandeling.
Schizofrenie is een complexe en ernstige psychiatrische ziekte die zich meestal in de late adolescentie of de vroege volwassenheid openbaart. De prevalentie van schizofrenie is ongeveer 1%. De diagnose van schizofrenie betreft een combinatie van zogeheten positieve en negatieve symptomen en de verslechtering van sociaal en beroepsmatig functioneren. Positieve symptomen zijn wanen (valse overtuigingen), hallucinaties (valse perceptie), gedesorganiseerd of bizar gedrag en formele denkstoornissen. Negatieve symptomen hebben betrekking tot affectvervlakking, gebrek aan motivatie, spraakarmoede en sociale teruggetrokkenheid.
Het woord depressie wordt veel gebruikt in de volksmond om een gemoedstoestand te beschrijven die gekenmerkt wordt door neerslachtigheid, verdriet of droefheid. Op deze manier is depressie de normale menselijke reactie op een verlies van iets waardevols. Een depressieve stoornis verwijst naar een klinische toestand welke bestaat uit een aantal symptomen die een herkenbaar patroon hebben met een volledig of slechts gedeeltelijk herstel tussen de episodes. Deze symptomen omvatten emotionele (bv. gevoelens van dysforie, verdriet), somatische (bijvoorbeeld slaapproblemen, gebrek aan energie, gewichtsvermindering zonder duidelijk dieet), gedrag (bv. psychomotorische agitatie of retardatie) en cognitieve (bv. gevoelens van waardeloosheid of

ongepast schuldgevoel, terugkerende gedachten aan de dood of zelfmoord, verminderd vermogen tot nadenken of concentratieproblemen) kenmerken. Met een geschatte prevalentie van tenminste 10% is de depressieve stoornis een van de meest voorkomende psychiatrische aandoeningen. Bovendien komen depressieve symptomen vaak voor in andere veel voorkomende psychiatrische aandoeningen zoals schizofrenie, alcohol- en drugsmisbruik en posttraumatische stress stoornis.

Tot op heden is de oorzaak van beide stoornissen nog niet bekend. In de afgelopen jaren is door middel van familiestudies aangetoond dat zowel schizofrenie als depressieve stoornis beiden voor een groot deel worden veroorzaakt door genetische factoren. Familieleden van deze patiënten hebben een verhoogd risico om de ziekte te krijgen. Dat risico stijgt naarmate de graad van verwantschap groter is. Bijvoorbeeld bij eeneiige tweelingen (genetisch identiek) geldt dat wanneer een van de twee ziek wordt de ander een verhoogd risico heeft van ongeveer 48 % (in schizofrenie). Ook al is uit tweelingonderzoek en uit familiestudies aangetoond dat genetische factoren een grote rol spelen, we weten nog niet precies welke genen hiervoor verantwoordelijk zijn. Wel is bekend dat er meerdere genen betrokken zijn bij het ontstaan van beide ziektes, die al dan niet in combinatie met elkaar en/of omgevingsfactoren, het risico op de stoornis vergroten.

Wat ook vast staat is dat beide stoornissen een hersenziekte zijn. Onderzoek met behulp van magnetische resonantie imaging (MRI) heeft overtuigend aangetoond dat de hersenen van patiënten met schizofrenie kleiner zijn in vergelijking met gezonde controles. Er wordt onderscheid gemaakt in grijze en witte stof. Grijze stof bestaat voornamelijk uit cellen; in deze cellen (neuronen) vindt de verwerking van informatie plaats. De witte stof bestaat uit de verbindingen tussen deze cellen en heeft als functie de informatieoverdracht tussen de neuronen. In schizofrenie lijkt de verkleining van het brein vooral verklaard te worden door een verkleining van het volume van de grijze stof. Dit betekent dat het totale grijze stof volume verkleind kan zijn, maar ook dat bepaalde structuren sterker verkleind kunnen zijn. Daarnaast heeft onderzoek ook aangetoond dat bij patiënten de laterale en derde ventrikels (hersenholtes gevuld met vloeistof) vergroot zijn. Aangezien schizofrenie wordt gekarakteriseerd als een progressieve ziekte vanwege de verslechtering in het dagelijks functioneren van patiënten, wordt de nadruk steeds vaker gelegd op longitudinaal MRI onderzoek. Uit deze onderzoeken blijkt dat bepaalde hersenstructuren progressief in volume afnemen over de tijd heen zowel in chronisch zieken als in patiënten na de eerste episode.

In depressie heeft het meeste structurele MRI onderzoek zich gericht op structuren die betrokken zijn bij de emotie- en stressregulering. Vooral de

hippocampus, een structuur onder andere betrokken bij geheugen, leren, stress en emotieregulatie, is veelvuldig onderzocht en in de meeste studies is het volume hiervan verkleind. Later in deze samenvatting zal kort worden ingaan op welke hersenstructuren nog meer betrokken zijn bij deze ziekte.

Wanneer u terugdenkt aan mijn titel zal het duidelijk zijn dat er in dit proefschrift niet één centrale vraag is die beantwoord wordt. Om toch enige orde te scheppen in deze brainstorm, heb ik een onderscheid gemaakt tussen genetica (**hoofdstukken 2, 3**), confounders (factoren die (ongewild) resultaten kunnen beïnvloeden) in neuroimaging (**hoofdstukken 4-6**) en meta-analyse (**hoofdstuk 7**). In de volgende paragrafen zal een beknopte samenvatting gegeven worden van de hoofdresultaten uit mijn onderzoeken.

Genetica

Hoofdstuk 2 beschrijft een tweelingstudie naar hypothalamus volume in monozygote (MZ; eeneiig) en dizygote (DZ; twee-eiig) tweelingparen van hetzelfde geslacht en discordant voor de ziekte schizofrenie in vergelijking met gezonde MZ en DZ tweelingparen. We hebben dit onderzoek bij tweelingen uitgevoerd om te kijken wat de invloeden zijn van genetische en omgevingsfactoren. Dit is mogelijk, omdat binnen de discordante paren de een ziek wordt, maar de ander niet en dus niet alleen genetische factoren ten grondslag kunnen liggen aan de ziekte. Daarnaast wilden we weten of de hypothalamus, een structuur die betrokken is bij het autonome zenuwstelsel en het endocriene systeem, en een cruciale rol speelt bij de organisatie van gedragingen die zorgen voor de overleving van het individu zoals eten, vechten, vluchten en paren, betrokken is bij schizofrenie. We vonden dat hypothalamus volumes zowel bij de patiënten als bij hun gezonde co-twin verkleind waren vergeleken met gezonde tweeling paren. Echter, deze volumeverkleining was niet specifiek en kon worden toegeschreven aan de vermindering van het totale hersenenvolume. Om te bepalen of hypothalamus volume genetisch bepaald was, hebben we onderzocht of de eeneiige tweelingen meer op elkaar lijken qua hypothalamus volume dan DZ tweelingen. We vonden dat de gelijkenis tussen MZ tweelingen groter was dan die van DZ tweelingen, wat erop wijst dat hypothalamus volume deels genetisch bepaald is.

Zoals al eerder is besproken is het nog onbekend hoeveel en welke genen verantwoordelijk zijn voor het ontstaan van schizofrenie. Toch hebben verschillende onderzoeken zich gericht op het testen van specifieke genetische markers in bekende kandidaat-genen voor een associatie met een ziekte of ziektegerelateerde endophenotypes zoals hersenenmorfologie. Deze aanpak,

die ook wel bekend staat als “imaging genetica” is een zich snel ontwikkelend onderzoeksgebied in schizofrenie en andere psychiatrische stoornissen. Een van deze kandidaat genen is BDNF, brain-derived neurotrophic factor. Het BDNF gen speelt een grote rol in neuronale differentiatie tijdens de ontwikkeling, maar ook in synaptische plasticiteit en neuronale overleving in het volwassen brein welke mogelijk zijn aangedaan bij de ziekte schizofrenie. Tevens is de hippocampus een van de hersenstructuren waarin BDNF het meeste voorkomt. In **hoofdstuk 3** beschrijven we de relatie tussen volume verandering over tijd van de hippocampus en BDNF genotype in patiënten met schizofrenie en gezonde controlepersonen. Hierbij maakten we gebruik van een Single Nucleotide Polymorfisme; dit betreft een variatie in het DNA van een enkele nucleotide lang. Dit betekent dat bij verschillende mensen op precies dezelfde plek in het DNA er een andere nucleotide aangetroffen kan worden. Een veelvoorkomend polymorfisme in het BDNF gen welke zorgt voor een aminozuur substitutie in codon 66, is die Valine (Val) naar Methionine (Met). Hiervan is bekend dat deze substitutie de activiteit van de excretie van BDNF, en tevens het episodisch geheugen beïnvloedt.

Op basis van de literatuur hadden we verwacht dat de Val-variant een beschermende werking zou hebben op hippocampus volume verandering over tijd. Echter, in tegenstelling tot onze hypothese vonden we geen effect van BDNF genotype op hippocampus volumeverandering, ongeacht diagnose. Ook konden we eerdere bevindingen die kleinere hippocampus volumes vonden in Met-allel dragers niet repliceren. We vonden echter wel genotype-x-diagnose interactie, wat inhoudt dat op de baseline meting we kleinere baseline hippocampus volumes vonden bij patiënten die homozygoot zijn voor het Val-allel ten opzichte van gezonde Val-homozygoten. Een ander belangrijke bevinding uit dit onderzoek is dat hippocampus volume afnam in de loop van de tijd in zowel de patiënten en controles. Echter, bleek het volumeverlies in de gezonde controle groep aanzienlijk groter te zijn dan dat in de patiënt groep. Vanwege de grote overlap in onze studiepopulatie in vergelijking met onze eerdere studie beschreven in **hoofdstuk 4** zijn deze bevindingen niet verrassend. De huidige conclusie onderstreept het excessieve hippocampus volumeverlies bij normale veroudering en is congruent met eerdere bevindingen in de normale veroudering waarin versneld hippocampus volume verlies op latere leeftijd is aangetoond.

Samengevat, duiden onze resultaten erop dat het BDNF Val66Met polymorfisme niet geassocieerd is met hippocampus volumeverandering over tijd. Echter, onze bevindingen tonen aan dat dit BDNF genotype hersenmorfologie mogelijk anders beïnvloed bij patiënten met schizofrenie dan bij gezonde controles.

Confounders in neuroimaging

Confounding beschrijft een associatie tussen twee variabelen (bijv. blootstelling aan straling en een ziekte), wat zou duiden op een direct en causaal verband, maar in werkelijkheid bestaat vanwege een buiten beschouwing gelaten variabele(n). Dit leidt dan tot een valse interpretatie van de relatie tussen deze variabelen. Het is een type fout die voortvloeit uit het niet herkennen (d.w.z. identificeren, meten, en rekening houden met) van een factor die bijdraagt tot een effect, wat leidt tot de verkeerde conclusie dat een waargenomen associatie wordt weergegeven als een oorzaak-gevolg relatie. Doordat confounding in feite het werkelijke effect maskeert, dient dit voorkomen te worden. Er zijn verschillende manieren van aanpak om confounding te voorkomen of te beperken, bijvoorbeeld door zoveel mogelijke variabelen af te stemmen tussen groepen. In case-control studies betekent dit dat voor een eventuele confounder zoals leeftijd, voor elke patiënt met een bepaalde leeftijd een gezonde controle wordt uitgezocht met dezelfde leeftijd of door de gemiddelde leeftijd per groep gelijk te maken. Kenmerkende confounders in psychiatrisch en neuroimaging onderzoek zijn leeftijd, geslacht, sociale economische status, IQ en ras. Het is relatief eenvoudig om rekening te houden met deze kenmerken door te matchen op groeps- of individueel niveau. In de volgende sectie worden (mogelijke) confounders die van belang zijn, namelijk leeftijd, medicijngebruik en roken beschreven met betrekking tot verschillen in de hersenen van patiënten met schizofrenie en/of depressie.

Leeftijd & medicatie

In **hoofdstuk 4** hebben wij in een longitudinale studie met een interval van vijf jaar bewijs gevonden voor verschillen in het leeftijdsverloop van hippocampus volumeverandering in patiënten met schizofrenie en gezonde individuen. Tot 26 jaar laten patiënten een progressieve afname zien van hippocampus volume zien ten opzichte van gezonde controles. Na deze leeftijd is er geen sprake meer van buitensporig volumeverlies in patiënten vergeleken met gezonde controles. Echter, na de leeftijd van 40 jaar laten gezonde personen een groter volume verlies zien ten opzichte van de patiënten, wat erop wijst dat progressieve hersenafwijkingen (alleen) aanwezig lijken te zijn in het begin van de aandoening of op jongere leeftijd. Het lineaire verband van een toenemend hippocampus volumeverlies met toenemende leeftijd in de gezonde controlegroep komt overeen met eerdere bevindingen in het normale verouderingsproces op latere leeftijd. De tweede doelstelling van deze studie was te onderzoeken wat de invloed is van antipsychotische medicatie bij hippocampus volumeverandering. We vonden een significante positieve associatie tussen de cumulatieve inname van atypische antipsychotica, in het bij-

zonder olanzapine en hippocampus volumeverandering. Patiënten die voor een langere periode of een hogere dosis atypische antipsychotica gebruikten lieten in de loop van de tijd minder volumeverlies of zelfs een kleine toename in hippocampus volume zien. Aan de andere kant vonden we een negatieve correlatie (op trendniveau) tussen de cumulatieve inname van typische antipsychotica en hippocampus volumeverandering, wat erop wijst dat patiënten die meer typische antipsychotische medicatie tijdens het scaninterval kregen ook een groter volumeverlies lieten zien. De positieve relatie tussen atypische antipsychotica en hippocampus volumeverandering duidt mogelijk op de neuroprotectieve eigenschappen van deze medicatie en is vergelijkbaar met eerdere onderzoeken. Echter dienen deze resultaten met enige terughoudend te worden geïnterpreteerd, aangezien veel van de patiënten die op het moment van de tweede scan op atypische medicatie waren ingesteld mogelijk in een vroeger stadium van de ziekte typische medicatie voorgeschreven hebben gekregen.

In **hoofdstuk 5** hebben we voor het eerst gelijktijdig corticale dikte metingen en op voxel gebaseerde morfometrie (VBM) gebruikt om mogelijke hersenafwijkingen in oude vrouwelijke patiënten met MDD te onderzoeken in vergelijking met gezonde proefpersonen. Twee belangrijke bevindingen kwamen uit deze studie. Ten eerste, in tegenstelling tot onze hypothese, vonden we geen effect van ziekte op corticale dikte of grijze stof "dichtheid". Het ontbreken van ziektegerelateerde corticale dikte veranderingen of een afname van grijze stof dichtheid is verrassend, aangezien we onlangs in onze meta-analyse hebben laten zien dat vooral gebieden in de frontaal en temporaal kwabben een kleiner volume hebben in patiënten vergeleken met gezonde controles. Het is moeilijk om onze bevindingen te vergelijken met eerdere VBM studies, omdat er verschillen zijn in de diagnoses van deze patiëntenpopulaties. Ten tweede, vonden we een diffuus patroon van zeer significante leeftijdseffecten op de corticale dikte en grijze stof dichtheid. Leeftijdsgeassocieerde corticale dikte veranderingen werden gevonden in de gehele cortex ongeacht diagnose. In het bijzonder bleken delen van de temporaal kwab (o.a. Heschl's gyrus) en de parahippocampale gyrus excessief dunner te worden met het ouder worden. Deze hersengebieden omvatten de primaire auditieve cortex en gebieden die worden gezien als een belangrijke neuroanatomisch correlaat voor spraak, taal en communicatie. Bovendien, vonden we ook excessief dunnere superiore, midden en inferiore frontale gyri met toenemende leeftijd. Het dunner worden van de frontale cortex ondersteunt de selectieve gevoeligheid van de prefrontale cortex in de veroudering, wat suggereert dat de hersengebieden die het laatst ontwikkelen het meest worden

getroffen in het latere leven. Ook de afname van grijze stof dichtheid met toenemende leeftijd gedroeg zich als een diffuus patroon in de gehele cortex, met de grootste afnamen in frontale en temporale gebieden, maar ook in de basale ganglia. Deze bevindingen zijn vergelijkbaar met andere VBM en volumetrische onderzoeken in oudere gezonde individuen en komen overeen met de normale veroudering en leeftijdsgerelateerde grijze stof afname in deze gebieden.
Kortom, in deze studie hebben we geen bewijs vinden voor verschillen in corticale dikte of grijze stof dichtheid bij oude vrouwelijke patiënten met MDD. Echter, we vonden wel op grote schaal grote significante effecten van leeftijd op corticale dikte en grijze stof dichtheid in frontale en temporale hersengebieden, ongeacht de diagnose.

Roken
In **hoofdstuk 6** hebben we gekeken naar het effect van het roken van sigaretten op volumeverandering van het brein over tijd in gezonde proefpersonen en patiënten met schizofrenie. Onze voornaamste bevinding is dat het roken van sigaretten niet het buitensporige verlies van hersenweefsel over tijd in de patiënten verklaard ten opzichte van de gezonde controles, ondanks een aanzienlijke oververtegenwoordiging van rokers onder de patiënten (56,25%) ten opzichte van de gezonde personen (30,97 %). Een interessante bevinding was dat overmatig rookgedrag (> 35 sigaretten per dag) wat alleen in de patiënten voorkwam het volume verlies deed versnellen in de loop van de tijd, echter verklaarde deze factor slechts een klein deel van het buitensporige volumeverlies bij schizofrenie patiënten.
Een andere interessante bevinding was dat we geen bewijs vonden voor de van invloed van roken op hersenvolumeverandering in gezonde proefpersonen in deze studie. Tot op heden zijn er geen longitudinale studies bij gezonde personen verricht naar de gevolgen van roken op volumeveranderingen over tijd. Er zijn echter wel enkele kleine cross-sectionele onderzoeken geweest die kleinere grijze stof volumes en/of dichtheid in de prefrontale, anterior cingulate, occipitale en temporale cortices (inclusief parahippocampale gyrus), thalamus, substantia nigra en cerebellum hebben aangetoond bij rokers vergeleken met niet-rokers. Als we rokende en niet-rokende gezonde proefpersonen alleen vergeleken op de tweede meting, bevestigden onze resultaten eerdere bevindingen van een kleinere hoeveelheid grijze stof in de frontale cortex, maar niet in de temporale cortex bij rokers. De richting van dit effect blijft onduidelijk; zijn de kleinere frontale grijze stof volumes een gevolg van het roken van sigaretten of hebben personen met een kleiner frontale grijze stof volume meer kans om te beginnen met het roken van sigaretten

(of nog meer problemen met het stoppen met roken)? Onze gegevens doen vermoeden dat hoewel rokers meestal minder frontale grijze stof volume hebben, er geen aanwijzingen zijn dat roken leidt tot overmatig volumeverlies over tijd bij gezonde personen.
Kortom, wij toonden aan dat, ondanks de hogere incidentie van roken en het grotere aantal sigaretten per dag in de patiëntgroep, het roken van sigaretten geen verklaring geeft voor de excessieve afname van het cerebrale (grijze stof) volume in de patiënten. Echter buitensporig rookgedrag bij patiënten met schizofrenie kan bijdragen aan excessief verlies van corticale grijze stof.

Meta-analyse

De term meta-analyse wordt omschreven als' de statistische analyse van een grote collectie analyses die voortvloeit uit afzonderlijke studies ten behoeve van het integreren van bevindingen". Een meta-analyse gaat verder dan een literatuurstudie, waarin slechts een beschrijving van de resultaten van verschillende onderzoeken worden besproken, vergeleken en misschien in tabellen worden weergegeven, omdat het de resultaten van de afzonderlijke studies tot een geheel maakt. Het belangrijkste kenmerk van een meta-analyse is het definiëren van een effect size die de kwantitatieve bevindingen van verschillende individuele onderzoeken in een gestandaardiseerde vorm kan weergeven. Hierdoor kunnen er zinvolle vergelijkingen en analyses worden uitgevoerd over alle onderzoeken als geheel. In **hoofdstuk 7** beschrijven we een meta-analyse die we hebben uitgevoerd om een duidelijk beeld te krijgen of er daadwerkelijk verschillen zijn tussen focale en globale hersenmaten, zoals gemeten met MRI, in patiënten met depressie en gezonde controles, en hoe groot deze verschillen dan zijn. Aangezien MDD wordt gekenmerkt door afwijkingen in de emotie- en stressregulering, heeft het merendeel van de onderzoeken in onze meta-analyse zich ook gericht juist op die gebieden die betrokken zijn bij deze processen. We vonden dat de volgende hersenstructuren sterk verkleind zijn bij patiënten met MDD ten opzichte van gezonde controles: anteriore cingulate (-12%) en orbitofrontale cortex (-9%), evenals het putamen (-11%). Middelgrote volumeverkleiningen werden gevonden in de caudate nucleus (-7%), hippocampus (-5%) en de prefrontale cortex (-3%). Onze bevindingen tonen daadwerkelijk aan dat een groot aantal, maar niet alle, structuren die bij deze processen betrokken zijn, kleiner zijn bij patiënten met MDD. Echter, we hebben geen verschillen gevonden tussen patiënten met MDD en gezonde proefpersonen met betrekking tot amygdala volume. Hierbij moet wel worden opgemerkt dat de amygdala bevindingen zeer inconsistent waren tussen de verschillende onderzoeken met zeer uiteenlopende resultaten. Mogelijk hebben andere factoren, zoals de vraag of een

patiënt in een huidige episode verkeert of in remissie is, of ziekteduur invloed op amygdala volume.
Enkele resultaten uit ons onderzoek bij depressie zijn vergelijkbaar met die van patiënten met schizofrenie, zoals de verkleining van de hippocampus en frontale volumes, met vergelijkbare effect sizes. Ten slotte bevestigt deze meta-analyse het behoud van globale en temporale cerebrale cortex volumes in MDD patiënten, wat in overeenstemming is met bevindingen bij patiënten met een bipolaire stoornis, maar in tegenstelling tot de lichte maar significante verkleining die wordt gevonden in schizofrenie patiënten.

Hoe verder..?

Een aantal methodologische beperkingen moeten worden genomen bij het interpreteren van morfologische onderzoeken. Ten eerste zijn er verschillende potentiële oorzaken die ten grondslag kunnen liggen aan afnamen van breinvolumes, zoals het verlies van het aantal neuronen, afname van de neuronale grootte en afname van de dendritische vertakking. Met andere woorden, er kan geen onderscheid gemaakt worden tussen de specifieke neurohistologische processen die ten grondslag liggen aan deze volumeverschillen. Ten tweede, het toepassen van verschillende methodologische benaderingen (voxel gebaseerde morfometrie vs. volumetrische), kan mogelijk verschillen verklaren tussen de diverse onderzoeken met tegenstrijdige bevindingen. Dit probleem kan gedeeltelijk worden opgelost door gebruik te maken van multimodale MRI protocollen om een “nieuwe” strategie voor de detectie en typering van subtiele structurele (en functionele) veranderingen in bepaalde gebieden van de hersenen te verschaffen. Dit kan worden bereikt door het combineren van verschillende MRI methoden, zoals volumetrische of VBM benaderingen met diffusie tensor imaging en magnetisatie transfer ratio imaging die gebruikt kunnen worden om de richting van witte stof banen en de concentratie van macromoleculen (zoals myeline) in de witte stof te verkrijgen. Men kan speculeren dat volumeveranderingen in de hersenen over tijd, in plaats van cross-sectionele onderzoeken, beter geschikt zijn om het verloop van de ziekte en de voortgang van aandoeningen te voorspellen. In personen waarbij de eerste symptomen zichtbaar zijn, maar waar nog geen sprake is van een echte psychose, worden al verschillen in het brein gevonden ten opzichte van gezonde controles. Er is gebleken dat sommige hersenstructuren geleidelijk veranderen na de eerste episode, terwijl andere structuren progressieve veranderingen laten zien die zowel in de jaren na de eerste episode of zelfs in de meer chronische fase van de ziekte zichtbaar blijven. In het imaging onderzoek bij depressie is nog weinig bekend over eventuele progressieve veranderingen in het brein. Longitudinaal onderzoek is daarom van

groot belang om te kijken naar veranderingen in het brein, maar ook om de invloed van antidepressiva, ziekteduur en het aantal episodes, of leeftijd te onderzoeken.
Ten derde is gebleken (in **hoofdstuk 4**) dat de inname van (verschillende soorten) psychotrope (of bijv. antidepressiva) medicatie kan resulteren in veranderingen van breinvolumes. Het is zeer waarschijnlijk dat antipsychotische medicatie vooral aangrijpt op focale hersengebieden dan op de gehele cortex en dat de effecten verschillend zijn tussen de eerste en tweede generatie van antipsychotica.
Ten slotte, in de relatief jonge discipline van imaging genetics, is er veel ruimte voor methodologische verbetering. Nu het mogelijk is om het hele menselijk genoom bloot te leggen en er honderdduizenden genetische varianten onderzocht kunnen worden is de keuze welke variant bestudeerd moet worden een nog niet opgelost probleem. Daarbij geldt ook dat vele varianten die statistisch geassocieerd zijn met een psychiatrische ziekte niet noodzakelijkerwijs ook een functioneel gevolg hebben.

Conclusie

In dit proefschrift beschreven we een aantal MRI studies in schizofrenie en depressieve stoornis. Hierbij benadrukken we het belang van genetisch onderzoek met behulp van tweelingstudies, of het zoeken naar kandidaat-genen en de relatie met hersenmorfologie. Bovendien hebben we aangetoond dat het van belang is longitudinale onderzoeken uit te voeren om leeftijdsgerelateerde veranderingen of invloeden van confounders zoals medicatiegebruik of roken op hersenmorfologie te onderzoeken. Bovendien kan de combinatie van verschillende MRI technieken zoals corticale dikte metingen en VBM een beter begrip geven van wat er gaande is in de hersenen, niet alleen op corticaal maar ook subcorticaal niveau. Ten slotte kunnen we met behulp van meta-analytische methoden (in MRI onderzoek) een idee krijgen welke hersenstructuren zijn aangedaan in een stoornis, en waar toekomstig onderzoek zich op zou moeten richten.

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Dankwoord

Yeeehaaaaa! De storm is gaan liggen.
Na deze ***Brainstorm*** met als gevolg eindeloze lappen tekst en tabellen met resultaten over schizofrenie, depressie, (longitudinaal) MRI onderzoek en andere aanverwante zaken, is het nu tijd voor het meest gelezen, of het meest leesbare hoofdstuk van dit boekje. Ook al doet de F5 tornado op de omslag anders vermoeden (deze duurde slechts 40 minuten), een promotietraject duurt toch wat langer. In die 4 jaren sta je er ook niet alleen voor, daarom wil ik graag van de gelegenheid gebruik maken om alle mensen te bedanken die ieder op hun eigen manier hebben bijgedragen aan de totstandkoming van dit proefschrift.

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Zo, en nu is het tijd voor een sappie!
Cheers!
Cé

Curriculum Vitae

Curriculum Vitae

Cédric Koolschijn was born at February 26 1980 in ‘s Gravenhage, The Netherlands. He obtained his high school certificate at the Adelbert College in Wassenaar in 1998. After taking several foundation courses in Nature Sciences & Innovation Management from 1998-2001, he started to study psychology at Utrecht University in 2001. He graduated in 2005 with a specialization in biopsycho-logy. After working as a neuroimaging assistant at the structural neuroimaging lab, Cédric started his PhD program in Neuroscience at the Department of Psychiatry at the University Medical Center in Utrecht in 2005, supervised by Prof. Dr. René Kahn and Dr. Neeltje van Haren, and in the last year also by Prof. Dr. Hilleke Hulshoff Pol.
Cédric will continue his work in the field of neuroscience with the focus on neuroimaging research. He accepted a postdoc position starting in september 2009 at the Brain and Development lab of Prof. Dr. Eveline Crone in Leiden.

Cédric Koolschijn werd op 26 februari 1980 geboren te ’s Gravenhage, Nederland. In 1998 behaalde hij het VWO diploma aan het Adelbert College te Wassenaar. In datzelfde jaar startte hij met de opleiding Natuurwetenschappen en Bedrijf & Bestuur (later Natuurwetenschappen & Innovatiemanagement) aan de Universiteit Utrecht. Nadat de studie van curriculum veranderde heeft hij na het behalen van zijn propedeuse, gekozen voor de studie psychologie aan de Universiteit Utrecht. In 2005 voltooide hij deze opleiding met als specialisatierichting biopsychologie. Voor zijn afstuderen werkte hij al als onderzoeks-assistent bij de neuroimaging groep van de afdeling volwassenen psychiatrie van het Universitair Medisch Centrum Utrecht. In 2005 is hij bij het Rudolf Magnus Instituut voor Neurowetenschappen onder supervisie van Prof. Dr. René Kahn en Dr. Neeltje van Haren, en later ook Prof. Dr. Hilleke Hulshoff Pol, begonnen met zijn promotieonderzoek.
Cédric blijft werkzaam op het gebied van neuroimaging. In september vervolgt hij het MRI onderzoek in het Brain and Development lab van Prof. Dr. Eveline Crone in Leiden.