The role of neuroimaging phenotypes in ecogenetic studies of psychotic disorder

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The role of neuroimaging phenotypes in ecogenetic studies of psychotic disorder

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The role of neuroimaging phenotypes in ecogenetic studies of psychotic disorder

PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de Universiteit Maastricht,

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To the key players in my memories

Paranimfen

Ilse Pipeleers
Kim Habets

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1

General introduction

PSYCHOTIC DISORDER

“And then one voice says that I have an easy life, another that I complain too much, and again another voice says that I have a difficult life and they fight and scream to each other and it’s like my body isn’t my own anymore, like different persons are intertwining with my body and develop and rise against each other, it is dreadful. I do not know what I do at night, this is so scary. There is still fear. Even in my own room. The learning process of a person who does not know what he wants to learn.

My world. Fast. When you say one word, a thousand pass my mind.

So many worlds. Of which I am ashamed of. My body. A home for different people, whom each have a different identity and world. They all have different ways to deal with things. One is better with dealing and then that one will do the talking.

My mind,

A mind that is afraid to show what it feels.”

(Scheeren, 2006, p. 23)(1)

Psychotic disorder is a collective term for a group of serious mental illnesses characterised by abnormalities in perception of reality and disruption of thought processes. The most known psychotic disorder is schizophrenia with a lifetime prevalence of 1% (2) and with the highest disability rating when compared to other mental illnesses (3). According to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) classification system (4), a psychotic disorder is diagnosed when symptomatology is severe enough to cause social and occupational dysfunction over a period of at least six months (5).

Psychotic disorder is often characterised by both positive and negative symptoms in the long term; however, disorganised behaviour and affective symptoms may be present. Positive symptoms involve impaired reality testing and include delusions and hallucinations. A delusion is a fixed false belief, such as the incorrect belief that one is being followed by government organisations. A variety of delusions can occur, and the specific delusional content is influenced by the person’s life and/or socio-cultural setting. Hallucinations are sensory experiences without an external stimulus and can occur in any of the five sensory modalities although auditory hallucinations are the most common (6). Negative symptoms involve blunted affect, poverty of speech, anhedonia, and reduced motivational drive and will (7, 8).

Although psychotic disorder is still diagnosed in the DSM-IV (9) as a categorical

defined set of symptoms, thus suggesting clear cut-off points between ill and healthy individuals, epidemiological research in the general population has shown that (subclinical) psychotic symptoms are present at a prevalence rate of 4%, whereas an even larger proportion (8%) report subclinical psychotic experiences (10). This finding suggests the existence of quantitative differences between “ill” and “healthy” individuals or a continuum of psychotic phenomena. Also, the marked heterogeneity of psychotic disorder and its pathophysiology and aetiology has become increasingly evident (11). In an attempt to reduce the heterogeneity, several dichotomous approaches have been developed. One of these dichotomous approaches distinguishes between type 1 and type 2 schizophrenia. Type 1 schizophrenia, also called the positive syndrome or non-developmental type, is characterised by high levels of positive symptoms, sudden onset, an episodic course with a good outcome and good response to antipsychotics. In contrast, type 2 schizophrenia, also called the negative syndrome or developmental type, is characterised by high levels of negative symptoms and cognitive impairments, a gradual onset, a chronic course with a poor outcome and resistance to drug treatment (12-17). However, the proposed dichotomous approaches have been found to be insufficient in explaining the heterogeneity of the disorder (18). More recently, the dimensional approach, in which psychopathology is rated on several symptom dimensions (i.e., groups of symptoms that occur together more often than would be expected by chance alone), has gained support (19-22). Using exploratory factor analyses, individuals can be defined by level scores on different dimensions that might co-exist. The initial work in this area examined the factor structure of the diagnostic category of schizophrenia and found evidence for three symptom dimensions: positive, negative and disorganised symptoms. When measures of affective symptomatology are considered, a five-factor model seems to apply with depressive and manic dimensions added to the list (19).

AETIOLOGY: GENE-ENVIRONMENT INTERACTIONS

Using family, adoption and twin studies, researchers have found that 80% of the liability to psychotic disorder has a genetic origin (23, 24). However, the search for candidate-risk genes for psychotic disorder is complicated due to the high phenotypic variability, diagnostic uncertainty, small effect of the identified genetic

risk and gene-gene interactions (24-27). Moreover, gene-environment interaction (GxE), which is genetically mediated sensitivity to the environment or vice versa, genetic expression that is conditional on the environment is also implicated in psychotic disorder. The influence of genetic effects could be misinterpreted if environmental effects are not considered (28). For example, simulations have shown that, of the 80% of the genetic liability, a proportion can be attributed to GxE (29). Further support for the influence of environmental factors has come from the finding that concordance rates in genetically identical monozygotic twins are approximately 50%.

The current view is that genetic, epigenetic and environmental factors combine via synergy or interaction in defining the risk for psychotic disorder (28). Research has identified several environmental risk factors for psychotic disorder such as living in an urban environment, social defeat, (childhood) trauma, daily life stress, cannabis use and obstetric complications. For the purpose of this thesis, we will focus on childhood trauma, cannabis use and daily life stress.

Childhood trauma

Studies have shown a relationship between the risk for developing a psychosis and exposure to traumatic events during childhood (30-33). A review by Read and colleagues (2005) found a 60.2% prevalence of sexual or physical abuse in patients with psychotic disorder. Moreover, there is evidence for a dose-response relationship between childhood trauma levels and psychotic symptoms, suggesting causality (34). More importantly, longitudinal (31, 35) population-based studies found that the risk of experiencing psychotic symptoms is increased in those exposed to early trauma compared to those not exposed.

During childhood, the brain is still fully developing and, therefore, is susceptible to changes. Childhood trauma may, consequently, cause neurodevelopmental abnormalities such as hippocampal volume reductions (36), contributing to an increased risk for psychotic disorder (37). However, whether brain alterations in psychotic disorder are associated with childhood trauma has not been investigated.

Cannabis

Another environmental factor linked to the development of psychotic disorder is cannabis use (39). In 2007, approximately 24% of the general Dutch population had tried cannabis at least once in their life. This frequency is even higher in the psychotic disorder population (51% in first-episode psychosis) (38). Patients who use cannabis have an earlier age of onset (39-41), worse outcome (42, 43) and more positive symptoms (40) than patients who have never used cannabis. Furthermore, when healthy individuals with no history of cannabis abuse were injected with 5 mg δ -9-tetrahydrocannabinol (δ -9-THC), the active ingredient of cannabis, they experienced psychotic-like positive and negative symptoms (44). In contrast, a large case-register study investigated the effect of cannabis on the symptomatology in patients with and without cannabis use prior to first symptomatology and found no differences between groups (45).

The mechanisms underlying the association between cannabis use and psychotic symptoms have not yet been determined, but it has been proposed that Val allele carriers of the COMT Val¹⁵⁸Met gene are more prone to develop psychotic symptoms than carriers of the Met allele when using cannabis (46, 47). THC is thought to increase mesolimbic dopamine signalling, and its acute effects on the emergence of psychotic symptoms may be most prominent in individuals with the Val/Val genotype, as the COMT Val¹⁵⁸Met Val allele is associated with higher levels of mesolimbic dopamine signalling compared to the Met allele (48). Furthermore, a recent study found evidence for the AKT1 gene influencing the association between cannabis use and risk for psychosis: genetic variation in AKT1 moderated the relationship between recent cannabis use and the level of schizotypy in siblings of patients with psychotic disorder. In patients, increased risk allele loading was associated with increased sensitivity to lifetime cannabis use, influencing the risk for psychotic disorder.

Cannabis may have an effect on synaptic plasticity, which is believed to be impaired in psychotic disorder (49) and is found to influence brain maturation during adolescent development (50). Because important brain maturational events, such as synaptic pruning, elaboration of dendritic arborisation and increased myelination, continue into early adulthood, sensitive periods for exposure to environmental risk factors such as cannabis use are not confined to the period between gestation and childhood (51). Furthermore, frontal and temporal cortical abnormalities, which have been replicated many times in psychotic disorder, mature last and could thus be most vulnerable for environmental (and genetic) influences (52, 53).

To date, only a few studies have investigated the effect of cannabis on brain structure in healthy individuals. These studies have been reviewed by Quickfall and co-workers (2006), who concluded that there is only minimal evidence for an effect of cannabis use on the human brain (54-56). With regard to psychotic disorder, studies investigating grey matter volume have produced mixed findings. Most studies did not find an effect of cannabis use in psychotic disorder (57), but there has been some suggestion for grey matter volume changes from cross-sectional (58) and longitudinal (59) studies. Clearly, more research is needed to further elucidate the relationship between brain changes in psychotic disorder and cannabis use.

Stress-reactivity

An important trait marker for psychosis is increased stress-reactivity. This marker does not only apply to the major stressors in one's life (60) but also to the more frequently occurring, small, daily-life events (61, 62). With the experience sampling method (ESM), a structured diary technique, current context, mood, and psychotic symptoms in daily life can be assessed. This method, applied to patients with psychotic disorder and first-degree relatives of patients with psychotic disorder, has yielded evidence for increased sensitivity to daily life stressors resulting in heightened negative emotions (62) and psychotic experiences (63). Furthermore, in a general population twin sample, increased psychometric risk for psychosis (i.e., high scores on a schizotypy questionnaire) was associated with increased emotional reactivity to stress (64). Additionally, a cross-trait, cross-twin association between stress-reactivity and subclinical psychosis was found, providing evidence for an unconfounded intermediate phenotype associated with genetic risk for psychosis (65). This increased stress-reactivity is associated with increased cortisol levels and has also been found in people at higher than average genetic risk for psychotic disorder with first-degree relatives displaying intermediate scores (62, 63). The underlying neural mechanisms of this augmented stress-sensitivity have not yet been identified. It has been hypothesised that this increased stress-sensitivity is associated with hyperactivity of the body's stress regulator: the hypothalamic-pituitary-adrenal (HPA) axis. However, the association between sensitivity to stress and proxy measures for HPA axis functioning, such as pituitary volume, hippocampal volume and cortisol levels, has not been examined in relation to genetic risk for psychotic disorder.

BRAIN ALTERATIONS

Even at the first description of schizophrenia by Kraepelin, he stated that the pathogenesis of schizophrenia, or “dementia praecox” as he named it, has a neurobiological underpinning (66). The technological advances of the last four decades have resulted in the development of non-invasive imaging tools, such as Magnetic Resonance Imaging (MRI), making it possible to investigate the brain in vivo. These tools have been used to discover and unravel the neural basis of psychotic disorder. Grey matter brain alterations in psychotic disorder have been extensively researched cross-sectionally (67, 68) and longitudinally (69-71) and have been associated with clinical measures, such as outcome (72, 73), cognitive functioning (74, 75) and positive symptoms (76, 77). Numerous studies have found evidence for widespread brain alterations, suggesting that psychotic disorder may not be a disorder of a single brain area but rather a disorder of brain networks (78-83). Diffusion tensor imaging (DTI) and resting state functional imaging data studying the connectivity between brain regions lend additional support to this theory (84, 85). The most consistent regional grey matter changes in psychotic disorder are volume reductions in the superior temporal gyrus (STG) and the parahippocampal gyrus (67, 68). Although some of these brain alterations have been found to be present near disease onset (86-88), it is not clear when these changes occur and whether or not these changes have a progressive nature. Several studies have found evidence for progressive brain volume loss (69, 89, 90) whereas other studies have not (91-93).

Cortical thickness

Measurements of grey matter volume may be too coarse to detect anatomical differences because volume is a combination of a region’s surface area and cortical thickness, and changes in either measure can be missed unless they are measured independently. Furthermore, variations in regional folding patterns of the cortex may explain variations in grey matter volumes. Fornito and co-workers (2008) examined cortical thickness and grey matter volume of the anterior cingulate in first-episode patients (FEP). They did not find differences in grey matter volume between patients and controls, but they did find a bilateral reduction in cortical thickness in the anterior cingulate (94). Their study demonstrated that microstructural changes in cortical thickness can be detected when macrostructural brain measurements,

such as grey matter volume, do not indicate noticeable differences.

Cortical thickness is a proxy measure for cytoarchitectural integrity. Diffuse alteration in cortical thickness may be an index of differences in the number of dendrites, dendritic spines, synapses (95, 96) or in reduced neuropil (97), which could result in disruptions of the neural circuits (98). Investigating cortical thickness may ultimately provide better insight into the causes and mechanisms contributing to the emergence and persistence of psychotic disorder.

Studies that have examined cortical thickness in psychotic disorder have made use of Region of Interest (ROI) and surface-based volumetry approaches. Because of the different ROIs that have been used, direct comparisons are difficult and findings are neither robust nor consistent. Main findings of ROI studies on chronic patients include reductions in frontal and temporal regions, in the cingulate (93, 99-102), in the parietal (93) and in the occipital regions (103). ROI studies on FEP suggest cortical thinning in the insula (101), cingulate (94), temporal and frontal regions (104-107) though some studies did not detect frontal alterations in this group (108, 109). Surface-based studies on FEP have reported reductions in cortical thickness in the frontal, temporal, parietal, occipital and cingulate regions (105, 106, 110-113) with one study reporting no differences between groups (104). In chronic patients, frontal and temporal thinning (93, 100, 103, 114), together with some evidence for parietal (93) and occipital thinning (103), have been described.

Intermediate phenotypes

The concept endophenotype or, the more accurate term, intermediate phenotype, was first proposed by Gottesman and Gould (2003) and can be defined as a biological marker of illness with a genetic underpinning. A candidate intermediate phenotype may be of neurophysiological, biochemical, neuroanatomical, or cognitive origin. Gottesman and Gould also offered a set of definitions for such biological markers. The intermediate phenotype (1) is associated with psychotic disorder, (2) is heritable, (3) is present even when the illness is not active (e.g., is state independent), (4) co-segregates with psychotic disorder within families, (5) and is present at higher rates than in unaffected family members when compared to the general population (115).

The search for intermediate phenotypes, such as shared structural brain alterations in patients and siblings, provides a means to circumvent the problems related to genetic studies (115). Genes predisposing to the clinical phenotype may be

transmitted without the expression of the clinical phenotype, thereby providing a more liable index of vulnerability than the illness itself. Furthermore, clinical heterogeneity can be further reduced by examining these quantitative biological markers as they are believed to be more proximal to the pathophysiological processes than the qualitative clinical diagnostic categories. Also, it is possible that several intermediate phenotypes contribute to the development of psychosis and that these are independent from each other. Identification of such intermediate phenotypes may, thus, lead to an improvement in diagnostic practices and to an increased knowledge on the causes of the illness.

Grey matter volume deficits as candidate intermediate phenotypes have been studied using family (116-118) and twin studies (119, 120). Overall, the studies investigating grey matter volume in non-psychotic relatives have provided mixed results, but a recent meta-analysis examining volumetric differences in total brain, intracranial ventricles, total grey matter, total white matter, amygdala, hippocampus and total cerebrospinal fluid concluded that reduced hippocampal volume appears to be the most affected area (121).

Only a few studies have examined cortical thickness in healthy relatives. Goghari and colleagues (2007) examined the relationship between cortical thickness/grey matter volume and genetic liability for psychotic disorder in 19 first-degree relatives of psychotic disorder patients and 22 controls (122, 123). They found bilaterally reduced cortical thickness in the cingulate gyrus and in the superior temporal sulcus. In another small study (28 patients, 28 siblings, 38 healthy controls), Calabrese and co-workers (2008) found that grey matter volume in the posterior cingulate gyrus in subjects with psychotic disorder and their non-psychotic siblings is reduced compared to healthy control subjects. Their analysis on cortical thickness and surface area reflect trend-level significant effects (102). In addition, a large study investigating 115 patients, 192 siblings and 196 controls found widespread cortical thinning in patients (predominantly in the frontal and temporal regions), but concluded that there were no significant differences in siblings compared to controls (82). This lack of clear evidence for cortical thickness alterations in siblings is consistent with other recent studies (103, 109, 124).

In sum, volumetric imaging studies have provided some evidence for brain alterations in siblings compared to controls with the strongest evidence for hippocampal volume reductions. However, the few studies on cortical thickness to date have failed to support the existence of a structural intermediate phenotype.

Importantly, MRI studies on psychotic disorder have rarely taken into account the effect of environmental factors which, in addition to presenting methodological issues, may well have affected the interpretation of some of the results from the neuroimaging literature.

Taken together, several environmental factors such as trauma, cannabis use and stress are thought to play a role in psychotic disorder. Hypotheses about underlying brain alterations and biological mechanisms through which environmental factors influence this risk are increasingly being put forward, but not many clinical studies in psychotic disorder have, as yet, tested these hypotheses. In other words, integrative studies wherein genetic-epidemiology is, for example, combined with the field of neuroimaging, are still scarce. Thus, a more translational view is needed to identify biological consequences of environmental risk factors that may impact the risk of psychosis (125). In this thesis, the association between brain alterations and environmental risk factors in psychotic disorder were investigated using a genetically sensitive sample, attempting to bridge neuroscientific and genetic-epidemiologic research on psychotic disorder.

GROUP PROJECT

Subjects were recruited from a multi-site longitudinal cohort study: Genetic Risk and Outcome of Psychosis (GROUP). This national study aimed at investigating risk and protective factors involved in the aetiology of psychotic disorder. The GROUP consortium consists of the academic psychiatric centres of Amsterdam, Groningen, Maastricht and Utrecht and their affiliated mental health care institutions (including some in Belgium). Data have been collected for a population-based cohort including 1120 patients with non-affective psychotic disorder, 1057 siblings of these 1120 patients, 919 parents of the patients and 590 unrelated controls. Assessments included symptomatology measures, various risk and protective factors, blood and urine samples for DNA analyses and drug screenings (126). In addition to the multicenter GROUP data, MRI scans were obtained locally at Maastricht University. The MRI study sample consisted of 89 patients with psychotic disorder, 98 non-psychotic siblings of patients with psychotic disorder, and 87 controls. The studies described in this thesis were based on (subgroups of) this sample.

OUTLINE AND AIMS OF THE THESIS

The overall aim of this thesis was to examine potential determinants of structural brain alterations in psychotic disorder, using a family-based study design (as described above) and representing different levels of risk for psychotic disorder. First, it was examined whether cortical thickness may be a candidate intermediate phenotype for psychotic disorder. Secondly, it was tested whether there is evidence for GxE using two environmental exposures (childhood trauma and cannabis use) in models of cerebral cortical thickness. Finally, associations between specific brain volumes and stress-reactivity were investigated to provide more insight into the biological framework related to increased stress-reactivity in psychotic disorder.

Structural intermediate phenotypes

In **chapter 2**, cortical thickness was investigated as a function of the level of genetic risk for psychosis. Twin studies have provided evidence for a strong genetic component contributing to certain neuroanatomical phenotypes in psychotic disorder. Due to its sensitivity, cortical thickness measures may have greater potential than volumetric measures in identifying small anatomical differences in patients, especially in siblings at higher than average genetic risk for psychotic disorder. To test whether cortical thickness may be a marker of genetic risk, cortical thickness measurements were obtained from the patients with psychotic disorder, their non-affected siblings and the controls. Group differences in cortical thickness were analysed. Based on previous literature, the hypothesis was that patients would have a pattern of decreased cortical alterations in frontal and temporal areas and that siblings would share this pattern of cortical decrements with their ill family member to a certain extent. Furthermore, it was expected that environmental factors, such as antipsychotic medication and cannabis use, would explain some of the variations in brain alterations in individuals at high genetic risk.

Gene-environment interactions and cortical thickness

As mentioned earlier, differences in genetic liability can be explained by differences in genetic make-up, occasioning differential sensitivity to environmental risk factors. This GxE phenomenon is thought to play an important role in schizophrenia. Examining GxE is difficult because risk factors for developing psychotic disorder, such as cannabis use and childhood trauma, are susceptible to bias and reverse

causality. For example, patients may use cannabis and report more childhood trauma because of their illness. Experimental designs can overcome these issues, but this study design cannot always be conducted for ethical reasons. Alternatively, the illness phenotype (outcome variable) can be replaced with an objective biological measure that is only weakly associated with (risk for) psychotic disorder. In **chapter 3**, the aim was to examine whether cortical thickness alterations as an objective biological measure can be identified as an outcome of differential sensitivity to cannabis use and childhood trauma. Again, the family-based design was used in which patients and siblings represented the high genetic risk group and control subjects represented the low genetic risk group. The hypothesis was that a higher genetic risk would be associated with a greater impact of environmental exposures on the cortical phenotype.

Stress-reactivity, genetic risk and regional brain volumes

In **chapters 4 and 5**, the relationship between volumes of specific brain structures was investigated in relation to stress-reactivity and the genetic risk for psychosis. Patients with psychotic disorder and their siblings demonstrate increased stress-reactivity, that is, they experience heightened negative emotions and psychotic symptoms in response to daily life stressors. In addition, this increased emotional stress-reactivity is associated with increased cortisol levels in people at higher than average genetic risk for psychotic disorder. Furthermore, previous studies have suggested that pituitary volume is associated with (the vulnerability for) psychotic disorder. It could thus be hypothesised that the increased stress-reactivity in psychotic disorder is associated with increased pituitary volume, the latter being a proxy measure for HPA axis dysfunction. The study described in **chapter 4** tested this hypothesis by examining group (patients, siblings, controls) differences in the experience of negative emotions (emotional stress-reactivity) and cortisol levels (biological stress-reactivity) in the context of daily life stressors. Moreover, it was examined whether stress-reactivity is not only dependent on the level of genetic risk for psychotic disorder but also dependent on pituitary volume.

Reduced hippocampal size and increased sensitivity to stress are associated with a genetic risk for psychotic disorder. The role of hippocampal volume in daily life stress-reactivity in psychotic disorder has never been examined. Hippocampal volume may be appraised as a proxy for HPA axis dysfunction because the hippocampus plays a role in the negative feedback mechanism of the HPA axis. The hypothesis would

be that lower hippocampal volume would lead to diminished negative feedback, resulting in higher HPA axis functioning. In **chapter 5**, the association between hippocampal volume and the experience of negative emotions (emotional stress-reactivity) and cortisol levels (biological stress-reactivity) was examined in the context of daily life stressors, and it was examined whether genetic risk for psychotic disorder moderated this association.

In **chapter 6**, the results are discussed and implications for clinical practice as well as directions for future research are given. In **chapter 7**, a summary of the presented studies is provided.

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Cortical thickness alterations and risk for psychotic disorder: evidence for an intermediate phenotype

Submitted

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Abstract

Background: Brain structures show high heritability rates. However, it remains unclear to what extent heritable factors are involved in cortical alterations associated with psychotic disorder. The present family study examined to what degree cortical thickness in psychotic disorder may be an indicator of genetic risk.

Methods: MRI scans were acquired in 89 patients with psychotic disorder (highest genetic risk), 98 healthy siblings of patients (higher than average genetic risk) and 87 controls (average genetic risk). Cortical thickness was compared between groups using a surface based General Linear Model (GLM) analysis in Freesurfer.

Results: Analysis showed significant clusters of cortical thinning in both patients and siblings in frontal, temporal, parietal and occipital regions, compared to controls. In addition, a widespread pattern of patient-specific widespread cortical thinning was found, as well as some sibling-specific cortical thinning. After controlling for cannabis use, the number of significant clusters was reduced in both patients and siblings, but some shared regions of cortical thinning in patients and siblings and some patient-specific thinning remained significant.

Conclusions: Patients with psychotic disorder showed a more extensive pattern of reduced cortical thickness than non-affected siblings. In frontal, temporal, parietal and occipital regions, there was evidence for a cortical thickness intermediate phenotype. Although cannabis use could explain some of the variation in cortical thickness, the majority of the intermediate phenotype regions held suggesting that alterations in these areas represent indicators of genetic risk.

Introduction

In the general population, global brain measures such as total brain volume, cerebellar, gray and white matter volume have shown high heritability rates (70-95%), whereas regional volumes of the hippocampus or frontal lobe have shown moderate heritability rates (40-70%) (1). In addition, there is evidence that cortical thickness (CT) is influenced by regionally specific genetic factors (2), suggesting that different genes are involved in different neural circuits. Twin studies have provided evidence for a strong genetic component contributing to neuroanatomical phenotypes in monozygotic twins discordant for psychotic disorder whereas for dizygotic twins compared with healthy control twins, genetic effects were less strong (3, 4). The advantage of measuring CT instead of volume is that the latter may be too coarse to identify subtle anatomical differences. Volume is a combination of cortical surface area and thickness, possibly influenced by different sets of genetic factors (3), and changes in either measure can be missed unless they are measured independently. Furthermore, variations in regional folding pattern of the cortex may explain variation in grey matter volumes. CT is a proxy measure for cytoarchitectural integrity. Thus, diffuse alteration in CT may be an index of differences in number of dendrites, dendritic spines, synapses (5, 6) or reduced neuropil (7), which could result in disruptions in neural circuits (8).

To date, studies that have examined CT in psychotic disorder have made use of Region of Interest (ROI) and surface based volumetry approaches. Because of the different ROI's that have been used, direct comparisons are difficult and findings are neither robust nor consistent. Main findings of ROI studies on chronic patients with psychotic disorder include reductions in frontal and temporal regions and in the cingulate (9-13), as well as some evidence for reductions in parietal (11) and occipital regions (14). Similar results have been reported in surface based studies: frontal and temporal cortical thinning (10, 11, 14, 15), and some evidence for parietal (11) and occipital thinning (14). With regard to first-episode patients (FEP), ROI studies have shown cortical thinning in the insula (12), cingulate (16) and temporal and frontal regions (17-20), although there are also negative findings with regard to frontal cortical alterations (21, 22). Surface-based studies in FEP patients have reported reductions in CT in the frontal, temporal, parietal, occipital and cingulate regions (18, 19, 23-25) with one study reporting no differences between groups (17).

Only a few studies have examined CT in healthy relatives. The study by Goghari

and co-workers (2007) reported bilaterally reduced CT in the cingulate gyrus and in the superior temporal sulcus in siblings (26). However, other studies failed to find differences in CT between siblings and controls (14, 22). Also, two large, recent, studies on patients and siblings reported widespread cortical thinning in patients (predominantly in the frontal and temporal regions), but not in siblings (27, 28).

Over recent years, evidence for the effects of environmental exposures such as antipsychotic medication and cannabis use on brain measures has been growing and, beside methodological issues, this may have contributed to inconsistencies in the findings on cortical alterations in psychotic disorder (29-32).

In search for a CT intermediate phenotype, the present study examined CT in a relatively large genetically sensitive sample of patients with psychotic disorder, non-psychotic siblings and controls, while taking into account drug use and antipsychotic medication. It was expected that siblings would, to a certain extent, share a pattern of cortical decrements with their ill family member, but that environmental influence would also explain some of the brain alterations.

Materials and methods

Subjects

Data pertain to baseline measures of an ongoing longitudinal study in Maastricht, the Netherlands. In selected representative geographical areas in the Netherlands and Belgium, patients were identified through representative clinicians whose caseload was screened for inclusion criteria. Subsequently, a group of patients presenting consecutively at these services either as out- or in-patients were recruited for the study. First-degree relatives were sampled through participating patients. Control subjects were recruited from the same population as the patients using random mailings in the geographic region and through advertisements in newspapers. All interviews were conducted by trained psychology graduates. The resulting sample consisted of 89 patients with a diagnosis of psychotic disorder, 98 siblings of patients and 87 controls. The sample included 64 families, of which 40 families contributed one patient and one healthy sibling, 3 families contributed one patient and two healthy siblings and one family contributed one patient and three healthy siblings. Two families contributed two patients, 6 families contributed two healthy siblings, and 2 families contributed 3 healthy siblings. In the control group, 10 families contributed two siblings. In addition, 41 independent patients,

31 independent siblings, and 67 independent controls were included. Inclusion criteria were age range 16 to 50 years, diagnosis of non-affective psychotic disorder and good command of Dutch language. Diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria (33), assessed with the Comprehensive Assessment of Symptoms and History (CASH) interview (34). Patient diagnoses were: schizophrenia (n=65), schizophreniform disorder (n=3), brief psychotic disorder (n=2), psychotic disorder not otherwise specified (n=10) and schizoaffective disorder (n=9). In the sibling and control group, there were respectively 20 and 14 participants with a history of a major depressive disorder, but none presented with a current depressive episode.

Prior to MRI acquisition, participants were screened for the following exclusion criteria: (i) brain injury with unconsciousness for over 1 hour, (ii) meningitis or other neurological diseases that might have affected brain structure/function, (iii) cardiac arrhythmia requiring medical treatment (iv), severe claustrophobia. In addition, subjects with metal corpora aliena were excluded from the study, as well as women with intrauterine device status and (suspected) pregnancy. The study was approved by the standing ethics committee, and all the subjects gave written informed consent in accordance with the committee's guidelines.

Measures

The Positive and Negative Syndrome Scale (PANSS) (35) was used to measure psychotic symptoms over the last two weeks. Five subscales were computed: positive symptoms, negative symptoms, disorganization, excitement and emotional distress.

Educational level was defined as the highest accomplished level of education (0=No education; 1= Primary school; 2= Secondary school, lower level; 3= Secondary school, higher level; 4= High school, lower level; 5= High school, higher level; 6= Vocational education, lower level; 7= Vocational education, higher level; 8= University).

Antipsychotic medication was determined using reports of the participant's psychiatrist. Best estimate lifetime (cumulative) AP medication use was determined by multiplying the number of days of AP use with the corresponding haloperidol equivalents and summing up these scores for all periods of AP use (36).

Substance use was assessed using the Composite International Diagnostic Interview (CIDI) sections B-J-L (37). Cannabis use and other drug use (stimulants, sedatives,

opiates, cocaine, PCP, psychedelics, inhalants, or other (e.g. XTC, poppers)) was assessed as reported frequency of use (i) during the last 12 months, and (ii) lifetime. Alcohol intake was calculated as the average number of weekly consumptions over the last 12 months and tobacco use as the average number of daily cigarettes over the last 12 months.

MRI acquisition and processing

MRI scans were acquired using a 3T Siemens scanner. The following acquisition parameters were used for the anatomical scans: Modified Driven Equilibrium Fourier Transform (MDEFT) sequence: 176 slices, 1 mm isotropic voxel size, TE 2.4 ms, TR 7.92 ms, total acquisition time: 12 min 51s, and for the Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE: Alzheimer's Disease Neuroimaging Initiation (ADNI) sequence): 192 slices, 1mm isotropic voxel size, TE 2.6 ms, TR 2250 ms, total acquisition time 7 min 23 s. The matrix size was 256 x 256 and field of view was 256 x 256 mm. Two sequences were used because of a scanner update during data collection, precluding temporary use of the MDEFT sequence. The ADNI and MDEFT sequences are very similar, but in order to prevent any systematic bias, the total proportion of ADNI scans (around 1/3) was balanced between the groups.

MRI preprocessing

Scans were analyzed using Freesurfer stable release version 5.0.0. The technical details of these procedures are described in prior publications (38-49) and here only a brief description will be presented.

Cortex-based alignment

Variation between participants in regional folding pattern of the cortex may produce unreliable results. To improve inter-subject mapping, the reconstructed cortices were aligned using curvature information reflecting the individual cortical folding patterns to match cortical geometry across subjects (45). It has been shown that a cortical matching approach substantially improves statistical analysis across subjects by reducing anatomical variability (44). First, the reconstructed cortices of each subject of each hemisphere were morphed into a sphere. Each vertex on the sphere (spherical coordinate system) has a corresponding vertex of the folded cortex (Cartesian coordinate system). These spheres were aligned to a spherical atlas.

The cerebral cortex was parcellated into units based on gyral and sulcal structure (46, 50). Furthermore, a variety of surface-based data was created including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire three-dimensional MR volume in segmentation and deformation procedures to produce representations of CT, calculated as the closest distance from the grey/white boundary to the grey/CSF boundary at each vertex on the tessellated surface (40). The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The maps produced are not restricted to the voxel resolution of the original data thus are capable of detecting sub-millimeter differences between groups. Procedures have been validated against histological analysis (51) and manual measurements (9, 52). Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (47).

Statistical analyses

Main effects on total cortical thickness

The CT surface map was loaded, for each individual and for each hemisphere, in a group file, where individual CT values averaged over each predefined Region of Interest (ROI) (adapted from the Desikan atlas (50), 34 regions per hemisphere) were calculated and exported to STATA version 11 (53). Thus, for each individual 68 CT measurements were derived. Total CT was calculated by summing up the CT values of the 68 ROI's. Because patients and siblings were from the same family, compromising statistical independence of the observations, multilevel random regression models were fitted (54).

In order to investigate whether total CT differed between groups, multilevel regression analyses were conducted using the XTREG command in STATA with the variable "group" (patients, siblings, controls) as the independent variable, and total CT as the dependent variable. Age, gender, educational level, handedness and scan type were used as covariates in the models, based on their *a priori* hypothesized confounding effects. Effect sizes of explanatory variables were expressed as regression coefficients (B) from the multilevel models. In order to examine whether the effect of group on total CT was modified by gender, a gender x group interaction was incorporated in the model of CT and evaluated by Wald test.

Surface based regional cortical thickness analyses

The regional CT value at each vertex for each subject was mapped to the surface of the sphere. The cortical map of each subject was smoothed with a Gaussian kernel of 20-mm full-width at half-maximum (FWHM) for the entire cortex analyses.

A general linear model was estimated at each vertex across the cortical surface, with CT as dependent variable and group as the independent variable. Age, gender, educational level, handedness and scan type were used as covariates in the models, based on their *a priori* hypothesized confounding effects.

This procedure allows for generation of statistical parametric maps that were thresholded at P -values <0.05 and <0.01 . The left and right hemisphere cortical surfaces were analyzed separately. The maps show the distribution of $-\log_{10} P$ -values for pairwise comparisons between the groups, as defined by the following contrasts: patients versus controls and siblings versus controls. Neighboring significant vertices were grouped and presented as maps of clustered vertices.

Antipsychotic medication and drug use: associations with cortical thickness

In the model of total CT, main effects of alcohol and cannabis use on the one hand were assessed using multilevel regression and, when appropriate, additionally used as covariates in the surface based regional CT analyses.

In patients, the association between antipsychotic medication (present and lifetime exposure) and total CT was also evaluated.

Results

Demographic characteristics

Patients were younger than controls and had, on average, a lower educational level than controls and siblings. There were more men than women in the patient group, whereas the opposite held for the control group.

At the moment of the scan, 74 patients were receiving AP medication (second generation: $n=71$; first generation: $n=3$). The mean current dosage of AP medication in terms of standard haloperidol equivalents was 2.9 milligrams (mg) ($SD=2.7$). Lifetime AP use (total days of use times haloperidol equivalents) was 2331.6 ($SD=4352.5$). Furthermore, 17 patients used an antidepressant (selective serotonin reuptake inhibitor (SSRI)), 6 a benzodiazepine, 5 an anti-epileptic drug, and 2 lithium. In the control group, 3 participants received an SSRI and 1 also used a benzodiazepine. In

the sibling group, 3 participants received an SSRI. Siblings used more alcohol than controls. Patients displayed more drug use than controls and siblings, regardless of the type of drug (Table 1).

Main effects on total cortical thickness

Total CT did not differ between groups ($B = -0.3$, $CI = -1.0 - 0.39$, $P = 0.38$). In the combined group of patients, siblings and controls, CT was negatively associated with lifetime cannabis use indicating that drug use was associated with lower CT. This was not the case for alcohol use in the past 12 months. Neither current dose nor lifetime exposure to AP medication was significantly associated with CT. There was no significant association between gender and total CT, and no interaction between group and gender in the model of total CT ($\chi^2 = 3.13$, $P = 0.21$), indicating that the association between group and CT was not modified by gender (Table 2).

Surface based regional cortical thickness alterations

In comparison to controls, both patients and siblings displayed a significant decrease in CT in the following regions: **superior frontal gyrus bilaterally, right fusiform, right superior parietal, right postcentral, right supramarginal gyrus and right lateral occipital cortex.**

At a more conservative threshold ($P < 0.01$), the right supramarginal gyrus remained significant in both patients and siblings. The right superior parietal and the postcentral gyrus remained significant in patients, but not in siblings (Table 3; Figure 1A and 1B).

Decreases in CT, uniquely present in patients, were found in the following regions: left precentral gyrus, right rostral middle frontal gyrus, right caudal middle frontal gyrus, right inferior frontal gyrus, right lateral orbitofrontal cortex, right temporal pole, superior temporal gyrus bilaterally, left fusiform, middle temporal gyrus bilaterally, left superior parietal, precuneus bilaterally, **left supramarginal gyrus, left lateral occipital, left pericalcarine and left posterior cingulate.** In the left inferior parietal gyrus, patients showed an increase in CT compared to controls.

At the $P < 0.01$ threshold, the left precentral gyrus and left posterior cingulate remained significant (Table 4; Figure 1A).

Sibling-specific decreases were found in the right medial orbitofrontal gyrus, right pars orbitalis, inferior parietal bilaterally, left lingual and right cuneus. A sibling-specific increase in CT was found in the right precentral gyrus.

At the $P < 0.01$ threshold the right medial orbitofrontal gyrus and the right inferior parietal gyrus remained significant (Table 4; Figure 1B).

Surface based regional cortical thickness alterations controlled for cannabis use

The number of significant clusters in patients and siblings was reduced after controlling for lifetime cannabis use, though most clusters with cortical thinning that were present in both patients and siblings remained significant: the right superior frontal gyrus, right fusiform, right superior parietal gyrus. At the $P < 0.01$ threshold, the right supramarginal remained significant for both patients and siblings, whereas for the left precentral, right superior parietal and left precuneus, clusters were significant at $P < 0.01$ and in siblings at $P < 0.05$. The left precentral gyrus and the right precuneus became significant in both patients and siblings at $P < 0.05$.

In the patients versus control comparison, the clusters in the right rostral middle frontal gyrus, right precentral gyrus, left fusiform gyrus, left superior parietal cortex and left supramarginal gyrus, lateral occipital cortex bilaterally and left posterior cingulate remained significant. At the $P < 0.01$ threshold, the right caudal middle frontal gyrus right postcentral gyrus, remained significant.

In the siblings versus control comparison, the left superior frontal gyrus and right inferior parietal gyrus remained significant (Figure 1C and 1D).

Discussion

Using a family study design to examine the influence of different levels of genetic risk for psychotic disorder on surface based CT measures, some decreases in overlapping regions were found in patients with psychotic disorder and their healthy siblings. Shared cortical decrements were found in frontal, temporal, parietal and occipital regions in the two groups at higher than average genetic risk for psychosis. In addition, widespread patient-specific cortical thinning was found and some sibling-specific cortical thinning. In addition, cannabis use was associated with CT and explained some, but not all, of the variations.

Cortical thickness alterations in the higher than average genetic risk-groups

Because structural brain traits are under substantial genetic control (2, 55), investigating its potential as an intermediate phenotype for psychotic disorder may provide structural brain phenotypes that could narrow the search for genes influencing brain structure (1) and risk of psychotic disorder. The present study showed CT reductions in both patients and siblings in frontal, temporal, parietal and occipital regions, suggesting that regional cortical alterations can potentially be considered as intermediate phenotypes. This is not in accordance with two recent studies of large sample sizes, which did not find strong evidence for CT intermediate phenotypes (27, 28). It should be noted, however, that Goldman and colleagues (2009) did find CT alterations in siblings, but their finding did not survive the stringent False Discovery Rate (FDR) correction for multiple comparisons. In addition, using the Risch λ as an index for heritability, they found evidence for widespread regions under genetic control.

The most obvious reductions in both patients and siblings were the temporal and occipital regions and the regions that are part of the heteromodal association cortex (i.e., superior frontal gyrus, superior parietal, supramarginal gyrus). The heteromodal association cortex is a highly organised and interconnected neocortical brain network involving the superior and middle temporal gyrus, the dorsolateral prefrontal cortex, Broca's area and the inferior parietal lobe (56). Furthermore, the heteromodal association cortex has extensive connections with the cingulate gyrus (57). Studies have indicated that the heteromodal association cortex is disrupted in patients with schizophrenia (58, 59) and in their siblings (60). In addition, some of the clusters that were found (e.g., superior frontal gyrus, postcentral gyrus and superior parietal) are involved in sensory gating (61). Moreover, the P50 gating deficit has been described as the most robust physiological finding in schizophrenia research (62, 63). Sensory gating is thought to reflect the filtering of redundant and/or distracting sensory information, to protect from the processing of the first stimulus. Delusions and hallucinations could consequently arise from an individual's response to erroneous attribution of salience to external and internal stimuli (64).

Group-specific cortical alterations

The present study found widespread patient-specific cortical thinning as well as some sibling-specific alterations. The results in patients have face validity as they are consistent with findings in several other studies (18, 19, 23-25). The sibling-specific alterations that were found in the present study are not in accordance with the studies of Boos and co-workers (28) and Goldman and colleagues (27), although extensive correction in these studies could have led to false negatives. Nevertheless, a smaller study did find some evidence of cortical thinning in siblings of patients with psychotic disorder in frontal and temporal regions (14).

The brain alterations of schizophrenia are not well understood in relation to the clinical phenotype (65). Psychotic disorder may not be a disorder of a single brain area but rather a disorder of brain networks (27, 66). Diffusion tensor imaging (DTI) and resting state functional imaging data addressing the connectivity between brain regions have lent some support to this theory (67, 68), as do the present findings. In the group at highest genetic risk, alterations were much more pronounced and widespread than in the sibling group, which may be associated with more severe dysfunctional brain networks. Siblings shared some of the CT decrements with their ill family members and additionally showed some specific alterations while not expressing the clinical phenotype. It could be hypothesised that brain networks are flexible and able to compensate for certain failures until a certain threshold is reached. When the threshold is exceeded, the dysfunction of the network could result in the expression of overt psychotic symptoms. However, small failures in the network might contribute to the development of symptoms at a subclinical level. Therefore, future research should investigate whether the cortical alterations found in siblings are related to schizotypal traits.

In summary, the genetic vulnerability for psychotic disorder may be associated with cortical thinning in regions of the heteromodal association cortex. Environmental factors, specific (epi)genetic effects or gene-environment interactions may contribute to additional patient or sibling-specific cortical thinning.

Cannabis use

Total CT was associated with cannabis, and cannabis use explained some of the variation in cortical thickness as the number of cortical thickness clusters was reduced after adjustment for cannabis use in the surface based analyses. Moreover, in a previous publication on the same study sample, we were able to show that

cannabis use partly mediated the association between risk for psychotic disorder and cortical alterations (32).

Although evidence suggests that cannabis use is implicated in the aetiology of psychotic disorder (69-77), most studies investigating the association of cannabis use and brain structure in psychotic disorder have failed to find an association between cannabis use and brain alterations. However, the majority of these studies used global brain measures such as total brain volume; therefore, regional differences could have remained undetected. For example, Cahn *et al.* (2004) investigated the association between cannabis use and total brain volume and total grey and white matter volume in FEP, and they did not find differences between cannabis-naïve FEP and cannabis-using FEP. Studies investigating specific ROIs, on the other hand, have found grey matter volume reductions in the posterior cingulate gyrus (78) and in the anterior cingulate (79) in cannabis using patients. Additionally, two longitudinal studies indicated evidence for additional brain alterations in cannabis-using patients compared to cannabis-naïve patients with regard to lateral and third ventricle volumes (80) and CT (29). Most studies investigating CT excluded subjects with a history of alcohol or other drug dependence (9, 10, 12-17, 20-22, 28) whereas other studies did not exclude subjects based on substance use. The studies, however, did not control for substance use either (18, 23-25, 27). Patients with a psychotic disorder have a high rate of co-morbid drug use (81), and therefore, exclusion of these subjects could have led to a selection bias influencing the representativeness of the study population and the type of brain alterations. In addition, previous research has shown that any use of cannabis is associated with clinical and subclinical psychosis outcomes (75). Thus, it is possible that drug use not meeting the criteria for drug dependence can still be associated with some of the brain alterations found in psychotic disorder. Therefore, environmental factors such as cannabis use should be considered when conducting neuroimaging studies in psychotic disorder.

Antipsychotic medication

Current and cumulative AP medication dose does not show a significant association with total CT, which is in accordance with earlier reports (10, 24, 28). Recent reviews (30, 82) concluded that antipsychotics may act regionally rather than globally on grey matter volume with a possible differential association according to the type of medication (first vs. second generation AP). Although an association between

AP medication and cortical alterations cannot be completely ruled out, the present findings suggest that AP medication use cannot explain the total variation in CT, which is consistent with the results of other studies in medication-naïve patients (24, 83) and in healthy, non-medicated siblings (13, 26).

Conclusion

In addition to group-specific alterations, patients with psychotic disorder and their non-psychotic relatives were found to have a similar pattern of CT alterations compared to controls, suggesting a genetic influence. These structural abnormalities may underlie the formation of symptoms (e.g., language problems, attention problems, hallucinations) that are seen in patients with schizophrenia and, in a more subsyndromal form, in siblings. In addition, cannabis use appeared to be associated with the CT outcome measure and explained some of the variation in cortical thickness.

Methodological considerations

There is a lot of debate as to which method is most appropriate to correct for multiple comparisons in surface-based analyses, as currently available methods are often too conservative. In the present study, only the right superior parietal lobe remained significant in the patient-control comparison after FDR correction and the significant findings in relatives (like in the study by Goldman and colleagues (27)) did not survive the correction (results available on request). As FDR-corrected studies often use a $P < 0.05$ threshold, the current (not FDR-corrected) results were not only presented using the $P < 0.05$ threshold, but also with a more conservative threshold (i.e., $P < 0.01$). Using this method, the number of significant clusters was reduced at this latter threshold but not nullified. In addition, a comparative ROI analysis on this data yielded 20 significant regions out of 68 regions in patients and siblings compared to controls, which is far above the number of expected (7) significant regions based on chance alone. This indeed suggests that the FDR correction may be a too stringent correction. Previous studies using high smoothing levels did not correct for multiple comparisons for this reason (52, 84).

The significant clusters presented in this study may have somewhat higher P -values than other studies investigating surface-based cortical thickness, which

could be explained by the larger number of covariates that were used in the model, resulting in a reduction of statistical power. Indeed, when only age and gender were incorporated in the model, similar to most cortical thickness studies to date, P -values were lower (results available upon request).

The surface-based GLM analyses that were used to investigate group differences in cortical thickness precluded the possibility to consider the fact that some subjects (siblings) were part of the same family. To examine whether genetic relatedness of the subjects (violation of independence) had any large or significant effect on the results, multilevel regression analyses were conducted using the XTREG command in STATA investigating group differences in regional cortical thickness (34 regions per hemisphere). Controlling for familial relationships did not influence the results (results available upon request).

In the controls and siblings, some participants had a DSM-IV diagnosis of past major depressive disorder. These participants were not excluded from the study, as exclusion would result in an extremely healthy comparison group, compromising the representativeness of the population under study. It is unlikely that differences between siblings and controls can be fully explained by the (small) group of siblings and controls with a history of depression.

There were more men than women in the patient group, whereas the opposite was the case in the controls. Sowell *et al.* (2007) investigated differences in CT in healthy subjects for 90 men and 86 women, and concluded that women had increased thickness in the right inferior parietal lobe and in the posterior temporal cortex (85), compared to men. The present study did not reveal confounding or effect modification by gender, suggesting that the differences in CT between the study groups are not likely the result of gender effects.

Table 1. Subject demographics

	Patients (n=89) Siblings (n=98) Controls (n=87)		
Age at scan (yrs)	28.1(6.9)*	29.5 (8.7)	30.6 (10.9)
Level of education	4.3 (2.0)*†	5.1 (2.1)	5.4 (1.8)
Gender n (%) male	60 (67%)	50 (51%)	33 (38%)
Mean number of times cannabis use lifetime	49.6 (46.0)*†	22.3 (4.2)	14.0 (28.3)
Mean number of times hard drug use lifetime	53.7 (99.4)*†	12.8 (52.8)	3.3 (14.1)
Alcohol use last year	4.7 (8.9)†	9.5 (16.9)*	4.6 (6.9)
PANSS Positive	12.3 (6.1)*†	7.6 (1.2)	7.4 (1.4)
PANSS negative	12.0(5.6)*†	8.4 (2.1)	8.2 (1.0)
PANSS Disorganization	13.3 (4.4)*†	10.3 (0.7)	10.3 (1.2)
PANSS Excitement	10.3 (2.8)*†	8.6 (1.4)	8.3 (1.1)
PANSS Emotional distress	13.9 (5.3)*†	10.1 (2.7)	9.4 (2.3)
Age of onset (yrs)	22.6 (6.4)		
Illness duration (yrs)	5.5 (3.6)		
Lifetime exposure to AP	2331.6 (4352.5)		

Means ± SDs are reported

Abbreviations: PANSS, Positive and Negative Syndrome Scale; Yrs, years

AP, antipsychotics (haloperidol equivalents)

(*) Significantly different from controls

(†) Significantly different from siblings

Table 2. Main effects on total cortical thickness

	B	P	Cortical thickness CI	Mean (S.D.)		
Group	-0.3	0.38	-1.0 -0.39	Patients 170.7 (0.6)	Siblings 170.8 (0.6)	Controls 170.8 (0.7)
Antipsychotics				Low	Medium	High
Lifetime use	0.00	0.47	-0.01 - 0.01	167.7 (1.1)		171.3 (0.7)
Present use	-0.06	0.77	-0.46 - 0.34	171.5 (1.1)	170.0 (1.2)	170.3 (1.0)
Alcohol	-0.03	0.20	-0.1 - 0.02	171.5 (0.6)	170.1 (0.7)	170.3 (0.7)
Cannabis	-0.03	0.00	-0.04 - -0.01	171.5 (0.6)	171.1 (0.8)	169.5 (0.6)
Gender	0.66	0.23	-0.42 - 1.74	Men 170.2 (0.5)	Women 171.4 (0.6)	

B's represent the effect sizes from the regression analyses investigating the association between cortical thickness and use of antipsychotics, drugs and the association with gender
Antipsychotics, alcohol and cannabis use have been divided into tertiles in order to report descriptive cortical thickness values per group
For lifetime antipsychotic use only two groups could be created

Table 3. Regional decreases in cortical thickness in patients and siblings compared to controls

	Patients vs. controls			Siblings vs. controls		
	Number of clusters	Max. log value	Weight	Number of clusters	Max. log value	Weight
Right superior frontal gyrus	332	1.70	563.80	103	1.44	148.60
Left superior frontal gyrus	270	1.50	406.11	71	1.36	96.26
Right fusiform	564	1.95	1101.16	139	1.43	198.60
Right superior parietal	2993	2.63	7866.60	985	1.76	1731.90
Right postcentral gyrus	2307	2.72	6280.83	902	1.95	1754.58
Right supramarginal gyrus	1403	2.64	3708.04	1175	3.17	3730.21
Right supramarginal gyrus				570	2.10	1198.62
Right lateral occipital cortex	22	1.33	29.35	306	1.86	567.67

Weighting was calculated as the product of the number of vertices and the maximum log-value (expressed as 10-x).

Regional definitions were based on proximity to neural labels (Desikan et al., 2006; Fischl et al., 2002).

Positive log values represent decreases in cortical thickness when compared to controls and negative log values increases in cortical thickness

All regions are significant at $p < 0.05$; regions in bold are significant at $p < 0.01$

Table 4. Group specific alterations in cortical thickness

Region of interest	Number of clusters	Max. log value	Weight
Patients compared to controls			
Left precentral gyrus	521	2.05	1067.22
Right rostral middle frontal gyrus	488	1.99	972.18
Right caudal middle frontal gyrus	458	1.99	909.57
Left precentral gyrus	298	1.81	538.31
Right inferior frontal gyrus	242	1.65	400.11
Right lateral orbitofrontal cortex	192	1.55	297.59
Right temporal pole	690	1.80	1239.05
Left superior temporal gyrus	668	1.79	1198.53
Right superior temporal gyrus	338	1.64	554.08
Left fusiform	250	1.62	405.33
Left middle temporal gyrus	173	1.45	251.51
Right middle temporal gyrus	23	1.32	30.43
Left superior parietal	1266	1.97	2496.43
Right precuneus	240	1.66	399.25
Left Precuneus	164	1.62	265.42
Left supramarginal gyrus	171	1.48	252.23
Left superior parietal	140	1.50	209.58
Right precuneus cortex	125	1.44	180.25
Left inferior parietal	66	-1.35	-89.36
Left supramarginal gyrus	19	1.32	25.01
Left lateral occipital cortex	566	1.66	940.24
Left pericalcarine cortex	30	1.33	40.02
Left posterior cingulate	627	2.14	1340.46
Siblings compared to controls			
Right medial orbitofrontal gyrus	572	2.07	1183.88
Right pars orbitalis	323	1.72	554.74
Right precentral gyrus	201	-1.49	-300.49
Right inferior parietal	1055	2.18	2296.68
Left inferior parietal	128	1.44	183.70
Right inferior parietal	87	1.40	122.09
Left lingual	277	1.61	444.62
Left lingual	189	1.57	296.82
Right cuneus	86	1.42	122.37

Note: Regions were ordered by region and by weighting, calculated as the product of the number of vertices and the maximum log-value (expressed as 10-x). Regional definitions were based on proximity to neural labels (Desikan et al., 2006). Positive log values represent decreases in cortical thickness when compared to controls and negative log values increases in cortical thickness

All regions are significant at $p < 0.05$; regions in bold are significant at $p < 0.01$

Figure 1. Group differences in cortical thickness. Thickness values for each subject were mapped to an average surface and smoothed using a 20-mm FWHM Gaussian filter. Group contrasts were performed, covarying for age and gender, level of education, handedness and scan type. Results were thresholded at $P < 0.05$: A) Patients versus controls; B) siblings versus controls; C) Patients versus controls, with additional covariate cannabis use; D) siblings versus controls with additional covariate cannabis use.

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Reduced Cortical Thickness as an Outcome of Differential Sensitivity to Environmental Risks in Schizophrenia

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Abstract

Background. The aetiology of schizophrenia is thought to involve differential – likely genetically mediated – sensitivity to environmental exposures. However, examination of differential sensitivity in models of psychopathological constructs is subject to bias as psychopathology itself may distort exposure assessment. The use of neuroimaging phenotypes, conversely, may provide unbiased evidence for differential sensitivity to environmental exposures. The present study examined the impact of two environmental exposures associated with both schizophrenia and MRI cerebral alterations, in models of cerebral cortical thickness.

Methods. T1-weighted MRI scans were acquired from 88 patients with schizophrenia, 98 healthy siblings at higher than average genetic risk for schizophrenia and 87 controls. Freesurfer was used to measure cortical thickness for 68 brain regions. Associations between cortical thickness on the one hand, and cannabis use and developmental trauma on the other were examined.

Results. A significant group x developmental trauma interaction ($\chi^2=9.65$, $p=0.01$), as well as a significant group x cannabis interaction ($\chi^2=6.04$, $p=0.05$) was apparent, indicating differential sensitivity of the patient group who displayed stronger reductions of cortical thickness for both exposures. A similar pattern was found in the sibling-control comparison for cannabis. For developmental trauma, siblings did not differ from controls, displaying an increase in cortical thickness with higher levels of trauma.

Conclusions. The findings suggest that schizophrenia and its genetic liability are associated with differential cerebral cortical sensitivity to developmental environmental exposures such as cannabis. Gene-environment interactions may underlie some of the brain alterations observed in patients with schizophrenia and their relatives.

Introduction

It is thought that most of the differences in liability to schizophrenia can be explained by genetic variation (1-3) occasioning differential sensitivity to environmental risk factors impacting during critical phases of development (4-7), a phenomenon known as gene-environment interaction (GxE) (8-10). Examining GxE is difficult because for ethical reasons people cannot be assigned randomly to, for example, heavy cannabis use or childhood trauma. The problems related to GxE research can be circumvented to a degree by (i) replacing the dependent variable in GxE analyses with an objective biological measure that is only weakly associated with (risk for) schizophrenia and (ii) by including siblings of patients as a group at high genetic risk but without the illness phenotype (Fig. 1). This method was pioneered by Cannon and colleagues (2002), who showed that obstetric complications (an environmental risk factor for schizophrenia (11)) was related to grey matter reductions and increased cerebrospinal fluid in patients and siblings (12). Thus, patients may over-report childhood trauma and start using cannabis because of their illness (reverse causality). However, such over-reporting cannot readily explain, for example, why a difference in cortical grey volume matter between exposed and unexposed patients and particularly siblings is greater than between exposed and non-exposed controls. Thus, although only random assignment can overcome reporting bias and reverse causality, several alternatives exist in observational research that, in combination, have complementary value in the interpretation of GxE.

As schizophrenia is associated with cortical grey matter alterations that to a lesser degree are also observed in healthy siblings (13, 14), and two environmental exposures associated with schizophrenia, cannabis use (15-18) and developmental trauma (19-21), are also associated with cerebral alterations (22-30), the environmentally sensitive (31) measure of cortical thickness (CT) was used in the analyses. The analyses focused on differential sensitivity to two environmental risk factors as a function of risk for schizophrenia, comparing patients (high genetic risk), their siblings (intermediate genetic risk) and controls (low genetic risk) and using measures of CT as the outcome. The hypothesis was that higher genetic risk would be associated with greater impact of environmental exposures on the cortical phenotype.

Methods and analyses

Subjects

Data pertain to baseline measures of an ongoing longitudinal MRI study in Maastricht, the Netherlands. In selected representative geographical areas in the Netherlands and Belgium, patients were identified through representative clinicians whose caseload was screened for inclusion criteria. Subsequently, a group of patients presenting consecutively at these services either as out- or in-patients were recruited for the study. First-degree relatives were sampled through participating patients. Control subjects were recruited from the same population as the cases using random mailings in nearby municipalities and through advertisement in newspapers. All interviews were conducted by trained psychology graduates. The sample consisted of 88 schizophrenia patients, 98 siblings of schizophrenia patients and 87 controls. The sample included 62 families, of which 39 families contributed one patient and one discordant sibling, 3 families contributed one patient and two discordant siblings, one family contributed one patient and three discordant siblings. Two families contributed two patients, 7 families contributed two discordant siblings, and one family contributed 3 discordant siblings. In the control group, 9 families contributed two siblings. In addition, 41 independent patients, 33 independent siblings, and 69 independent controls were included. Inclusion criteria were: (i) age: 16 to 50 years, (ii) diagnosis of non-affective psychotic disorder and (iii) good command of Dutch language. In a few instances, the patient refused whereas the sibling wished to participate, in which case the sibling was included; the majority represented sib-pairs with at least one ill relative. Siblings had to be free of any lifetime non-affective psychotic disorder. For the control subjects, the occurrence of any psychotic disorder in either the subject or any first-degree family member, assessed using the Family Interview for Genetic Studies (FIGS) (32), constituted an exclusion criterion.

Diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria (33), assessed with the Comprehensive Assessment of Symptoms and History (CASH) interview (34). Patients were diagnosed with: schizophrenia (n=62), schizoaffective disorder (n=9), schizophreniform disorder (n=4), brief psychotic disorder (n=2), and psychotic disorder not otherwise specified (n=11). The CASH was also used to confirm the absence of a diagnosis of non-affective psychosis in the siblings, and absence of a lifetime diagnosis of any psychotic disorder or

any current affective disorder in the healthy controls. Twenty-one siblings and 14 controls had a history of major depressive disorder, but none presented in a current depressive episode.

Prior to MRI acquisition, participants were screened for exclusion criteria: (i) brain injury with unconsciousness of > 1 hour, (ii) meningitis or other neurological diseases that might have affected brain structure/function, (iii) cardiac arrhythmia requiring medical treatment and (iv) severe claustrophobia. In addition, subjects with metal corpora aliena were excluded from the study, as well as women with IUD status and (suspected) pregnancy.

The study was approved by the standing ethics committee, and all the subjects gave written informed consent in accordance with the committee's guidelines.

Measures

The Positive and Negative Syndrome Scale (PANSS) (35) was used to measure psychotic symptoms over the last two weeks.

Educational level was defined as highest accomplished level of education. Antipsychotic medication (AP) was determined using the reports of the participant's psychiatrist.

Best estimate lifetime (cumulative) AP use was determined by multiplying the number of days of AP use with the corresponding haloperidol equivalents and summing up these scores for all periods of AP use (36).

Substance use

Substance use was assessed using the Composite International Diagnostic Interview (CIDI) sections B-J-L (37).

Cannabis use was assessed as the reported lifetime number of instances of cannabis use, ranging from 1-5, 6-9, 10-19, 20-39, 40-59, 60-79, 80-99 or 100 times or more. In the analyses, three groups were created, allowing for the assessment of dose-response: (i) subjects who had never used cannabis, (ii) subjects who used cannabis between 1 and 39 times and (iii) subjects who used cannabis at least 40 times in their life, as previous research in the Dutch and German general populations has shown that severity of exposure, defined in terms of frequency of use, represents an excellent tradeoff between sensitivity and specificity with regard to clinical and subclinical psychosis outcomes (16, 38). CIDI cannabis lifetime frequency data was available for 235 subjects (14% missing data).

Hard drug use, such as stimulants, sedatives, opiates, cocaine, PCP, psychedelics, inhalants, or other (e.g. XTC, poppers) was assessed as reported frequency of use (i) during the last 12 months, and (ii) lifetime.

Alcohol use was defined as the reported number of weekly consumptions during the last 12 months.

Developmental trauma

Developmental trauma was assessed with the Dutch version of the Childhood Trauma Questionnaire Short Form (CTQ). The short CTQ consists of 25 items rated on a 5-point Likert scale (1=never true to 5=very often true) enquiring about traumatic experiences in childhood. Five types of childhood maltreatment were assessed: emotional, physical and sexual abuse, and emotional and physical neglect, with five questions covering each type of trauma (39). A general measure of developmental trauma was created by calculating the mean of the 25 items. CTQ data was missing for one person.

MRI acquisition and processing

MRI scans were acquired using a 3T Siemens scanner and the following acquisition parameters: MDEFT sequence: 176 slices, 1 mm, TE 2.4 ms, TR 7.92 ms, TI 910 ms, flip angle 15°, total acquisition time: 12 min 51s, and for the MP-RAGE (ADNI) sequence: 192 slices, 1mm, TE 2.6 ms, TR 2250 ms, TI 900 ms, flip angle 9°, total acquisition time: 7 min 23s. The matrix size and field of view was 256x256. The number of excitations was 1. Two sequences were used because of a scanner update during data collection. The MP-RAGE and MDEFT are very similar, but to prevent any systematic bias, the total proportion of MP-RAGE scans (around 1/3) was balanced between the groups.

MRI preprocessing

Scans were processed and analyzed using Freesurfer stable release v4.5.0. Technical details of these procedures are described in prior publications (40-46). Data was automatically transformed into Talairach standard space.

Cortical thickness measurement

Before CT measurement, the cerebral cortex was parcellated into units based on gyral and sulcal structure (47, 48). Furthermore, a variety of surface-based data was created including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire three dimensional MR volume

in segmentation and deformation procedures to produce representations of CT, calculated as the closest distance from the grey/white boundary to the grey/CSF boundary at each vertex on the tessellated surface (41). The maps are created using spatial intensity gradients across tissue classes and are not restricted to the voxel resolution of the original data, thus are capable of detecting sub-millimeter differences between groups. CT measurement procedures have been validated against histological analysis (49) and manual measurements (50, 51).

Statistical analyses

The CT surface map was loaded, for each individual and for each hemisphere, in a group file, where individual CT values for each predefined region of interest (hereafter: region; adapted from the Desikan atlas (47), 34 regions per hemisphere) were calculated and exported to STATA version 11 (52). The dataset was transformed from a wide format to a long format, resulting in a hierarchically structured dataset, with 68 regional CT measures (level 1) nested in subjects (level 2) who were part of the same families (level 3). Because of the three-level grouping structure of the data, compromising statistical independence of the observations, multilevel random regression models were fitted (53) using the `XTMIXED` command in STATA with CT measures as the dependent variable and subject number and family number modeled as random effects. Mixed models contain both fixed and random effects, the fixed effects being analogous to standard regression coefficients (β).

In order to test the hypothesis that groups differed in their sensitivity to developmental trauma, analyses were conducted with trauma and group (patients, siblings and controls) as well as their interaction term as independent variables and CT as the dependent variable ($CT = \beta_0 + \beta_1 (\text{trauma}) + \beta_2 (\text{group}) + \beta_3 (\text{trauma} \times \text{group})$). The trauma $\times$ group interaction was fitted with the control group as the reference category. The trauma exposure was entered both as a linear variable and as dummy variables representing the distribution of the trauma score divided by its quartiles, allowing visualization of dose-response (0=no trauma, 1=low trauma, 2=medium trauma, 3=high trauma).

Interaction terms were evaluated by Wald test (54). Stratified effect sizes for all trauma levels were assessed by calculating the appropriate linear combinations from the model containing the interaction, using the `LINCOM` procedure in STATA. Analyses were adjusted for the *a priori* hypothesized confounders age, sex and level

of education.

Similar multilevel random regression analyses were conducted with the cannabis exposure as independent variable ($CT = \beta_0 + \beta_1 (\text{cannabis use}) + \beta_2 (\text{group}) + \beta_3 (\text{cannabis use} \times \text{group})$), cannabis exposure entered both as a linear variable and as dummy variables (0= no cannabis use, 1= moderate cannabis use, 2= heavy cannabis use).

Power analysis

The power of the 2-way and 3-way interaction analyses was calculated by empirical statistical simulation in STATA (www.stata.com/support/faqs/stat/power.html), as described previously (55). Effect sizes used were based on published work in this area. These power simulations showed that the 2-way interactions in the study had a power of 75% to find a significant effect at an alpha of 0.05.

The study was not powered to examine whether the differential impact of trauma and cannabis varied not only with group status, but also with brain region. Thus, the three-way interaction (trauma/cannabis x group x region) modeling these effects had only 10% power. Therefore, results of these three-way interactions will only be described exploratively in order to generate hypotheses for future research.

Results

Descriptive analyses

Patients had lower educational level than controls and siblings (Table 1). There were more men than women in the patient group, whereas the opposite held for the control group. Siblings used more alcohol than controls and patients. Patients reported more cannabis use than siblings and controls, and more lifetime and present (last 12 months) hard drug use than siblings and controls, with no large or significant differences between the latter two groups. For all groups, the age of first cannabis use was in adolescence, with a mean age of 16.9 years in patients, 16.8 years in siblings and 16.5 years in controls (d.f. (2,106), $F = 0.10$, $P = 0.90$). Patients reported more developmental trauma than siblings and controls, the latter groups having similar levels of reported trauma.

Seventy-three patients were receiving AP (second generation: $n = 70$; first generation: $n = 3$). Furthermore, 17 patients used antidepressants, 6 benzodiazepines, 5 anti-epileptic drugs and 2 lithium. Three siblings and 3 controls used antidepressants,

and 1 control used benzodiazepines. Lifetime AP use was associated with neither CT ($\beta=0.00$, $P=0.39$) nor cannabis use ($\beta=0.00$, $P=0.50$) (Table 1).

Main and interaction effects

Main effects of environmental exposure and group on cortical thickness

In the model without interaction terms, there was no significant association between developmental trauma and CT in the total group of patients, siblings and controls ($\beta=-0.001$, $P=0.78$). Heavy cannabis use was significantly associated with CT ($\beta=-0.05$, $P=0.00$) indicating that heavy cannabis users had lower CT values. There was no significant association between group and CT (Table 2).

Interaction between group status and developmental trauma

There was a significant group x trauma (linear variable) interaction ($\chi^2=9.65$, $p=0.01$), indicating that the effect of trauma on CT differed between groups. Stratified analyses revealed that patients had significantly lower CT values when exposed to higher levels of developmental trauma ($\beta=-0.02$, $P=0.03$), which was not found in controls ($\beta=0.003$, $P=0.72$). The opposite pattern was found in siblings ($\beta=0.02$, $P=0.05$), indicating higher CT values when exposed to higher levels of developmental trauma. Visualizing the effect over the four quartile groups of trauma exposure revealed progressively lower values of CT with progressively higher levels of trauma in exposed patients and exposed controls, whereas an opposite trend was apparent in the sibling group (Table 3, Fig. 2A).

Interaction between group status and cannabis

There was a significant group x cannabis interaction (linear variable) ($\chi^2=6.04$, $P=0.05$), indicating that the effect of using cannabis on CT varied as a function of group. Stratified analyses showed that patients with heavy cannabis use had significantly lower CT values compared to patients with no cannabis use ($\beta=-0.07$, $P=0.002$) and a similar pattern of results was found for siblings ($\beta=-0.06$, $P=0.01$), but not for controls ($\beta=0.01$, $P=0.65$) (Table 3, Fig. 2B). The p-values were only marginally affected after adjustment for hard drug use in patients ($\beta=-0.06$, $P=0.01$) and siblings ($\beta=-0.06$, $P=0.01$). Patients with moderate cannabis exposure had CT values that were intermediate to those with no or heavy use, whereas for siblings the effect on CT was confined mainly to the group of heavy use (Table 3, Fig. 2B).

Explorative group x exposure x region interactions

Explorative analyses of three-way interactions examining regional variation of differential sensitivity revealed no significant interactions for trauma. There was some suggestion for regional group interactions with cannabis in that in patients (compared to controls), cortical thinning in relation to heavy cannabis use, compared to non users, was apparent in the left frontal pole ($\chi^2=3.75$, $p=0.05$) and left ($\chi^2=4.65$, $p=0.03$) and right ($\chi^2=9.67$, $p=0.00$) parahippocampal gyrus. In siblings, findings were partly similar in that cortical thinning for heavy cannabis users compared to non users was apparent in the left frontal pole ($\chi^2=5.32$, $p=0.02$), right entorhinal cortex ($\chi^2=3.72$, $p=0.05$) and the right parahippocampal gyrus ($\chi^2=3.70$, $p=0.05$).

Discussion

The results showed that exposure to cannabis, as well as exposure to trauma in childhood, was associated with cerebral cortical thinning in individuals at high genetic risk for schizophrenia (patients), whereas this was not the case in those at low genetic risk (controls). For the cannabis exposure, the pattern of results in individuals with intermediate genetic risk (siblings) was similar to that of the patient group. Furthermore, there was a suggestion of a similar pattern of regional thinning in patient and sibling cannabis users in frontal and parahippocampal regions. For the developmental trauma exposure, siblings revealed CT increases with higher trauma levels, although this pattern was not significantly different from controls.

Differential impact of environmental exposures on the brain

No previous studies on the relationship between trauma and brain alterations in schizophrenia are available. However, childhood trauma has been associated with structural brain changes, such as volume loss in the hippocampus, corpus callosum, and prefrontal cortex (22, 24, 29, 30, 56, 57).

The finding that cannabis use had a differential impact on CT in not only patients but also siblings, extend earlier findings indicating that gene-environment interactions may underlie the association between cannabis and psychosis (5, 58-60). Furthermore, the findings are suggestive of a dose-response relationship (higher cannabis exposure associated with more severe CT alteration) in the group most at risk (patients). Studies investigating the effect of cannabis on the brain in healthy subjects have reported only minimal evidence for cannabis-induced brain

alterations (23, 25, 26, 61). In schizophrenia, studies are scarce and have produced inconsistent findings ranging from no effect of cannabis on brain morphology (62), to decreased grey matter volume (27). The fact that similar findings were apparent in the sibling-control comparison for the cannabis exposure is in line with the initial hypothesis: siblings, at higher than average genetic risk, also displayed differential sensitivity.

For the trauma exposure, there was evidence for an opposite effect in patients and siblings, whereas the pattern of results in siblings did not significantly differ from the pattern in controls. The directionally opposite finding in the siblings may represent chance or noise. Alternatively, siblings and controls may differ from patients in that the effect of exposure to traumatic experiences may be neutral (controls) or even trophic (siblings). To the degree that the effects of trauma on the brain are mediated by long-term psychological adaptation, resilience and coping may moderate the outcome of traumatic experiences over time, possibly mitigating the risk for later psychotic outcomes. In other words, not only sensitivity may be an important moderator determining the psychiatric outcome, but also individuals' resilience and coping. An individual's resilience is thought to be mediated by adaptive functioning of distinct molecular machineries and brain circuits that allow the individual to experience positive emotions rather than negative, and search positive ways to reframe stressful events (63). Given the fact that siblings only share some of their genes and environments with patients, the opposite effect in patients and siblings observed for childhood trauma may be explained by (i) genetic variants not shared between the sibs, (ii) non-shared environmental exposures, (iii) illness effects. In addition, evidence has emerged that this type of contrast in the findings may be indicative of genetic plasticity (64): the same gene may confer positive sensitivity to an exposure in some environments, but negative sensitivity in others. To the degree that differential negative sensitivity to childhood trauma may be contingent on illness expression, underlying illness-related brain changes or treatment factors such as antipsychotic medication may play a role. For the cannabis exposure, however, genes contributing to differential sensitivity to the environment may be shared between patients and their siblings and not be illness-dependent.

Final common pathway

The effects of cannabis or traumatic stress may impact along a final common pathway. Prefrontal cortical alterations could have an impact on the stress-buffering system, resulting in mesolimbic hyperdopaminergia. Repeated exposure to stress or cannabis may lead to prolonged changes in dopaminergic signaling, and eventually to dopamine sensitization (65-68). There was some evidence that cannabis-related regional thinning in patients and siblings was anatomically congruent with the hypothesis of impact on dopamine projections, as there was a suggestion of differential impact of cannabis on the frontal pole, the entorhinal cortex and the parahippocampal gyrus.

Possible underlying mechanisms

During childhood, the brain is still in full development and thus vulnerable to environmental exposures. Studies suggest that developmental trauma alters hypothalamic-pituitary-adrenal (HPA) axis functioning (69). These neurobiological abnormalities (70) could, in concert with an already existing genetic liability contribute to an increased risk for schizophrenia. The changes in the HPA axis could be the result of a hypersensitive glucocorticoid release or abnormalities in glucocorticoid receptors consequently upon (chronic) stress exposure (71). Due to synergism between the activity of the HPA axis and the dopaminergic circuits, glucocorticoid secretion may increase dopamine activity in the mesolimbic system (71). Some brain regions, such as the hippocampus or the prefrontal lobe, may be particularly sensitive to stressors (57, 72).

Delta-9-tetrahydrocannabinol (δ -9-THC), the active psychotropic ingredient of cannabis, activates the cannabinoid-1 (CB1) receptor, the primary binding site of endogenous cannabinoids. THC may influence dopamine firing in the ventral tegmental area (VTA), resulting in increased striatal DA levels; in addition, THC is also believed to impact synaptic plasticity, which is thought to be impaired in schizophrenia (73). Furthermore, THC-related glutamatergic effects may affect grey matter volume (78), and possibly CT, through a mechanism of neurotoxicity (74, 75). However, the exact influence of THC on endocannabinoid, dopamine, GABA and glutamate signaling remains to be elucidated, as is the relationship with neuronal network alterations and psychosis.

Limitations

Gene-environment interaction studies assume that the interacting variables are independent. In some instances, this assumption did not hold as patients reported more cannabis use and trauma than both controls and siblings. The consequence of this is that the interpretation of interaction cannot distinguish between moderation (genetic risk influences sensitivity to the environment) and mediation (genetic risk influences exposure to the environment). The fact that the violation of independence in the case of the cannabis exposure does not apply to the siblings, suggests that moderation rather than mediation is the underlying mechanism, as siblings displayed similar differences with the controls as the patients. Although for the trauma exposure we cannot exclude mediation in the case-control comparison, it is unlikely that mediation is the only underlying mechanism as for most exposures in psychiatry both moderation and mediation usually apply (76).

Assessments of childhood trauma in patients with psychotic disorder may be biased. However, recent work suggests that patient reports of environmental exposures such as childhood trauma have good reliability and validity and are not subject to reverse causality (77).

Although our design including a biological measure as dependent variable reduces the risk of reporting bias, and thus represents an alternative to previous studies using this paradigm, it cannot be ruled out that brain alterations, that are weakly associated with the illness, still have a minor impact on the reporting of childhood trauma or cannabis use. This, of course, applies to all neuroimaging studies that are observational in nature and cannot experimentally assign individuals to cannabis use or childhood trauma.

Although the effect of heavy cannabis use on mean CT was larger in patients (standardized effect size: -0.18) relative to controls (standardized effect size 0.03), giving rise to the reported significant two-way interaction, absolute effect sizes were small and difficult to interpret in terms of biological and clinical relevance. Effects were analyzed across 68 CT measures, hierarchically clustered within persons, so that larger effect sizes (as for example hypothesized in frontal areas and temporal areas) were averaged with smaller effect sizes. Future studies with larger sample sizes may provide more precise estimates of regional CT effect sizes associated with cannabis use.

Patients more often used cannabis than controls, which may distort interaction analysis given more precise estimates of cannabis effects on gray matter in the

patients; however, siblings and controls did not differ in cannabis exposure and a similar interaction was apparent in these groups.

Freesurfer CT measurements appear to be relatively robust to differences in MRI protocols and scanners (41). Thus, in our data, no large or significant interaction was found between scan type and group on CT ($\chi^2=0.99$ $p=0.61$). Similarly, adjusting for scanning sequence did not affect direction or significance of the results (results available upon request).

Table 1. Subject demographics

	Patients (n=88)	Siblings (n=98)	Controls (n=87)	F/chi ²	P
Age at scan	28.2 ± 6.9	29.5 ± 8.7	30.7 ± 10.8	1.8	0.17
Level of education	4.3 ± 2.0 ^{††}	5.1 ± 2.1	5.4 ± 1.8	7.8	0.00
Sex n (%) male	59 (67%)	50 (51%)	33 (38%)	14.9	0.00
Cannabis use n (%)					
None	28 (35%)	53 (62%)	48 (70%)		
Moderate	14 (17.5%)	15 (17%)	11 (16%)		
Heavy	38 (47.5%)	18 (21%)	10 (14%)	26.1	0.00
Mean number of times harddrug use last year	10.9 ± 30.4 ^{††}	0.90 ± 8.3	1.2 ± 7.8	8.6	0.00
Mean number of times harddrug use lifetime	54.2 ± 99.9 ^{††}	12.8 ± 42.8	3.3 ± 14.1	13.9	0.00
Alcohol use	4.8 ± 9.0 [†]	9.5 ± 16.9 [*]	4.6 ± 6.9	4.5	0.01
PANSS Positive	12.3 ± 6.1 ^{††}	7.6 ± 1.2	7.4 ± 1.4	45.4	0.00
PANSS negative	12.1 ± 5.8 ^{††}	8.4 ± 2.1	8.2 ± 1.0	29.2	0.00
PANSS Disorganization	13.3 ± 4.5 ^{††}	10.3 ± 0.7	10.3 ± 1.2	31.7	0.00
PANSS Excitement	10.3 ± 2.8 ^{††}	8.6 ± 1.4	8.3 ± 1.1	25.9	0.00
PANSS Emotional distress	13.9 ± 5.4 ^{††}	10.1 ± 2.7	9.4 ± 2.3	33.7	0.00
Emotional abuse	1.9 ± 0.9 ^{††}	1.4 ± 0.6	1.5 ± 0.7	11.3	0.00
Physical abuse	1.3 ± 0.7 [*]	1.1 ± 0.4	1.1 ± 0.3	3.9	0.02
Sexual abuse	1.3 ± 0.7 ^{††}	1.1 ± 0.2	1.1 ± 0.3	9.6	0.00
Emotional neglect	2.3 ± 0.9 [*]	2.0 ± 0.8	1.9 ± 0.8	5.5	0.00
Physical neglect	1.5 ± 0.6 ^{††}	1.2 ± 0.3	1.2 ± 0.4	12.2	0.00
CTQ total	7.2 ± 2.9 ^{††}	5.9 ± 1.6	5.7 ± 1.8	12.3	0.00
Age of onset	22.7 ± 6.4				
Illness duration	5.5 ± 3.7				
Lifetime exposure to AP	2743.4 ± 4625.7				

Means ± SDs are reported

Abbreviations: PANSS, Positive and Negative Syndrome Scale; CTQ, Childhood trauma questionnaire; AP, antipsychotics

F/Chi² and P-values refer to between-group differences

(*) Significantly different from controls

(†) Significantly different from siblings

Table 2. Comparisons of cortical thickness by group and environmental exposure

	No. of observations [†] (n) [‡]	Cortical thickness Mean ± SD	β [#]	P
Controls [*]	5916 (87)	2.59 ± 0.37		
Siblings	6664 (98)	2.58 ± 0.37	0.00	0.94
Patients	5984 (88)	2.57 ± 0.37	-0.02	0.17
No cannabis users [*]	8772 (129)	2.59 ± 0.37		
Medium cannabis users	2720 (40)	2.59 ± 0.37	-0.01	0.36
High cannabis users	4488 (66)	2.56 ± 0.36	-0.05	0.00
No childhood trauma [*]	4624 (68)	2.57 ± 0.37		
Low childhood trauma	4760 (70)	2.59 ± 0.36	0.02	0.29
Medium childhood trauma	4692 (69)	2.59 ± 0.37	0.02	0.29
High childhood trauma	4420 (65)	2.57 ± 0.37	-0.01	0.71

^{*} reference level

[†] no. of observations = number of subjects*number of region's ± 68

[‡] (n) = number of subjects

[#] the β's represent the regression coefficients from multilevel linear regression analyses, adjusted for age, gender and level of education

Table 3. Cortical thickness as a function of group status and environmental exposure

Environmental exposure	Patients			Siblings			Controls			Wald test	χ^2	P
	no. of obs (n) [†]	Cortical thickness Mean $\pm$ SD	β	P	no. of obs (n) [†]	Cortical thickness Mean $\pm$ SD	β	P	no. of obs (n) [†]	Cortical thickness Mean $\pm$ SD	β	P
No childhood trauma [*]	816 (12)	2.60 $\pm$ 0.37			1,564 (23)	2.57 $\pm$ 0.37			2,244 (33)	2.57 $\pm$ 0.36		
Low childhood trauma	1,156 (17)	2.62 $\pm$ 0.37	0.02	0.65	2,244 (33)	2.56 $\pm$ 0.36	0.01	0.69	1,360 (20)	2.61 $\pm$ 0.35	0.03	0.17
Medium childhood trauma	1,768 (26)	2.57 $\pm$ 0.37	-0.01	0.81	1,632 (24)	2.61 $\pm$ 0.37	0.04	0.13	1,292 (19)	2.60 $\pm$ 0.38	0.02	0.55
High childhood trauma	2,244 (33)	2.55 $\pm$ 0.37	-0.05	0.12	1,224 (18)	2.60 $\pm$ 0.36	0.05	0.09	952 (14)	2.59 $\pm$ 0.38	0.01	0.84
β linear trend [†]			-0.02	0.03			0.02	0.05			0.00	0.72
No cannabis use [*]	1,904 (28)	2.61 $\pm$ 0.38			3,604 (53)	2.59 $\pm$ 0.37			3,264 (48)	2.58 $\pm$ 0.37		
Moderate cannabis use	952 (14)	2.57 $\pm$ 0.38	-0.04	0.18	1,020 (15)	2.59 $\pm$ 0.36	-0.02	0.52	748 (11)	2.62 $\pm$ 0.36	0.01	0.69
Heavy cannabis use	2,584 (38)	2.54 $\pm$ 0.36	-0.07	0.00	1,224 (18)	2.55 $\pm$ 0.34	-0.06	0.01	680 (10)	2.62 $\pm$ 0.36	0.01	0.65
β linear trend [†]			-0.03	0.00			-0.03	0.01			0.01	0.59

* reference level

[†] summary change in CT with one unit change in exposure level[†] no. of observations = number of subjects*number of region's (68); (n) = number of subjects

P vs. C = patients versus controls; S vs. C = siblings versus controls

The χ^2 and P-values represent the results of the Wald test, testing the significance of stratified effects calculated from the model with the group x environment interaction term; the β 's represent the effect sizes of level of environmental exposure compared to the reference level

Figure 1. Environment to genotype design using biological alterations

Figure 1. Environment to genotype design using biological alterations. G+, high genetic risk; G–, low genetic risk; E+, exposed to environmental risk; E–, nonexposed to environmental risk. A common problem in gene–environment interaction (GE) studies is that the illness phenotype under investigation, and the dependent variable in the statistical model, influences assessment of the environmental exposure, giving rise to risk of bias (left-side figure of case– control study of GE). Although only experimental designs can overcome this issue, an alternative that reduces the risk of bias is to replace the outcome under investigation by a biological phenotype that is weakly associated with (risk of) the disease and examine differential impact of the environmental exposure in groups at high and low genetic risk with and without the illness phenotype (right-side figure). This study used a family-based design in which cases and siblings represent high genetic risk groups (with and without the illness phenotype respectively) and control subjects the low genetic risk group.

Figure 2. Interaction between environmental risk factors and group on cortical thickness

Figure 2. (A) Interaction between childhood trauma and group (linear trauma group interaction, p .01). (B) Interaction between cannabis use and group (cannabis $\times$ group interaction, p .05).

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4

Pituitary volume, stress-reactivity and genetic risk for schizophrenia

Submitted

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Abstract

Background: HPA axis hyperactivity, associated with increased pituitary volume, may mediate observed alterations in stress-sensitivity in patients with schizophrenia. We examined, for the first time, the association between pituitary volume, real-life stress-reactivity and genetic liability for schizophrenia.

Methods: Pituitary volumes were derived from MRI scans of 20 patients with schizophrenia, 37 non-psychotic siblings of these patients, and 32 controls. The Experience Sampling Method (ESM) was used to measure stress-reactivity (changes in negative affect [NA] and in cortisol levels associated with daily life stress). Associations between group status and pituitary volume were investigated, as were interactions between group, stress and pituitary volume in models of NA and cortisol.

Results: Groups did not differ in pituitary volume. There was a significant group x stress interaction in the model of NA, in that patients showed significantly higher stress-reactivity than both siblings and controls. In addition, there was a significant group x stress x pituitary volume interaction in the model of NA, indicating that stress-reactivity increased with increasing pituitary volume to a greater degree in patients than in controls and in siblings, whereas the latter two groups did not differ. Siblings had higher cortisol levels than controls, but showed no increased cortisol reactivity to stress. There was no interaction between pituitary volume, cortisol reactivity and group status.

Conclusion: Higher pituitary volume was associated with increased stress-reactivity in patients with schizophrenia, siblings and controls. The association was significantly stronger in the patient group, suggesting a process of progressive sensitization mediating clinical outcome.

Introduction

Altered volumes of the pituitary gland have been associated with increased vulnerability for schizophrenia (1). Patients with a first episode (FE) of schizophrenia display increased pituitary volume, compared to controls (2, 3), as do individuals with early prodromal signs and subsequent transition to schizophrenia, compared to those who do not make the transition (1). In samples diagnosed with schizophrenia, an inverse association has been reported between illness duration and pituitary volume (4), although not consistently so (5). In addition, there is evidence for larger pituitary volume in first-degree relatives of patients with schizophrenia (6).

The occurrence of pituitary enlargement in prodromal and antipsychotic-naïve patients as well as in relatives, suggests the enlargement is not due to antipsychotic (AP) medication. On the other hand, cross-sectional studies show positive associations between first-generation (FGA) or prolactin-elevating second-generation antipsychotics (SGA) (e.g., risperidone, amisulpiride) and pituitary volume (2, 7) in FE patients, although not all studies agree (3). In addition, a longitudinal study reported a dose-response effect of prolactin-sparing SGA (e.g., olanzapine, quetiapine, clozapine) on pituitary volume, higher doses predicting reduced volume (8). The mixed findings possibly reflect the fact that the pituitary gland is a highly dynamic organ, with many factors impacting on its volume over time. For example, studies have shown that women have larger pituitary volumes than men (9), and that both endogenous and exogenous estrogens are associated with pituitary volume (10).

The pituitary gland is a crucial organ involved in the hypothalamus-pituitary-adrenal (HPA)-axis stress response. In depression research, increased pituitary volume is thought to reflect HPA axis hyperfunctioning. Exposure to stress activates the HPA axis through the release of corticotropin releasing hormone (CRH) in the hypothalamus, resulting in secretion of adrenocorticotrophic hormone (ACTH) in the pituitary, stimulating the release of cortisol in the adrenal cortex. Cortisol, through a negative feedback mechanism, regulates HPA axis activity. Persistently elevated cortisol levels can impair the negative feedback mechanism (11), resulting in HPA axis hyperactivity. As a result of HPA axis hyperactivity, patients become more sensitive to stressors, referred to as stress sensitization (12).

Increased cortisol levels have been found in FE patients (13) as well as in siblings of patients with schizophrenia (14), and may predict transition to a clinical psychotic

outcome in young individuals at-risk (15). FE patients in the acute phase also show non-suppression of cortisol secretion in the dexamethasone suppression test and in the dexamethasone/corticotrophin releasing factor test (16, 17). Furthermore, there is evidence that patients with schizophrenia are more sensitive to daily life stressors resulting in increased levels of stress-related negative emotions (18) and psychotic experiences (19). Evidence of increased cortisol reactivity to stress has also been detected in people at higher than average genetic risk for schizophrenia (14). However, there is also evidence for a blunted cortisol response to metabolic and psychosocial stressors in patients with schizophrenia (20, 21) and in medication-naïve patients (22).

To date, the association between pituitary volumes and stress-reactivity in schizophrenia remains unclear. The present study is the first to examine pituitary volume in relation to cortisol (biological stress-reactivity) and negative affect (emotional stress-reactivity) in the context of daily life stressors, using a genetically sensitive sample (patients at highest genetic risk, siblings of patients at higher than average genetic risk, and controls at average genetic risk). The hypothesis was that patients and, to a lesser extent, siblings would show increased stress-reactivity compared to controls, accompanied by larger pituitary volumes.

Methods and Materials

Subjects

Data pertain to baseline measures of an ongoing longitudinal study in Maastricht, the Netherlands. Details on the selection procedures are described in a prior publication (23). The sample consisted of 20 patients with a diagnosis of schizophrenia or related psychotic disorder, 37 siblings of patients with schizophrenia and 32 controls. Inclusion criteria were: (i) age: 16 to 50 years, (ii) diagnosis of non-affective schizophrenia and (iii) good command of Dutch language.

Diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria (24), assessed with the Comprehensive Assessment of Symptoms and History (CASH) interview (25). Patients were diagnosed with: schizophrenia (n=11), schizoaffective disorder (n=2), schizophreniform disorder (n=1), brief psychotic disorder (n=2), and psychotic disorder not otherwise specified (n=4). The CASH was also used to confirm the absence of a diagnosis of non-affective schizophrenia in the siblings, and absence of a lifetime diagnosis of any psychotic

disorder or any current affective disorder in the healthy controls. Six siblings and seven controls had a history of major depressive disorder, but none presented in a current depressive episode. Prior to MRI acquisition, participants were screened for the following exclusion criteria: (i) brain injury with unconsciousness for over 1 hour, (ii) meningitis or other neurological diseases that might have affected brain structure/function, (iii) cardiac arrhythmia requiring medical treatment (iv), severe claustrophobia. In addition, subjects with metal corpora aliena were excluded from the study, as well as women with intrauterine device status and (suspected) pregnancy. The study was approved by the standing ethics committee, and all the subjects gave written informed consent in accordance with the committee's guidelines.

Measures

The Positive and Negative Syndrome Scale (PANSS) (26) was used to measure psychotic symptoms over the last two weeks.

Use of antipsychotic medication was determined using reports of the participant's psychiatrist. Best estimate lifetime (cumulative) AP medication use was determined by multiplying the number of days of AP use with the corresponding haloperidol equivalents and summing up these scores for all periods of AP use.

Estrogen exposure was determined by multiplying the number of months of contraceptive medication use with micrograms estrogen (of the corresponding contraceptive) per month.

Substance use was assessed with the Composite International Diagnostic Interview (CIDI) sections B-J-L (27). Use of cannabis and other drugs was assessed as reported frequency of use during the last 12 months, as well as lifetime. Alcohol intake was calculated as the average number of weekly consumptions during the last 12 months and tobacco use as the average number of daily cigarettes during the last 12 months.

Experience Sampling Method (ESM)

ESM is a random time-sampling self-assessment technique; studies have demonstrated the feasibility, validity, and reliability of ESM in general and patient populations (28, 29). Subjects received a digital wristwatch that emitted a signal ten times a day on six consecutive days, at unpredictable moments between 7:30 a.m. and 10:30 p.m. After each 'beep', subjects completed ESM self-assessment

forms concerning current context, thoughts, emotions, and psychotic experiences. Subjects were instructed to complete their reports immediately after the beep, thus minimizing memory distortions. Reports were considered valid when subjects responded within 15 min after the beep, as determined by comparing the actual beep time with the reported time of completion. For inclusion in the analyses, a valid response to at least one-third of the emitted beeps was required (30).

Stress-reactivity, as described in previous work, was conceptualized as emotional (18) and biological reactivity (14) to minor disturbances in daily life. Measures of stress, mood and cortisol were derived from the experience sampling data as described below.

Salivary cortisol sampling

After each ESM beep, participants collected a saliva sample with a cotton swab (Salivette; Sarstedt, Etten-Leur, the Netherlands), replaced the swab in the salivette tube, and recorded the exact collection time. Samples were stored in subjects' home freezers until transport to the lab, where uncentrifuged samples were kept at -20°C until analysis. Saliva samples collected more than 15 minutes after the beep were excluded from the analysis.

ESM measures

In accordance with previous work, two different stress measures were computed: event stress and social stress (18).

Event-stress was assessed as follows: after each beep, the most important event that had happened between the current and the previous report was reported. This event was subsequently rated on a bipolar Likert scale (-3=very unpleasant, 0=neutral, 3=very pleasant). The responses were recoded to allow high scores to reflect stress (-3=very pleasant, 0=neutral, 3=very unpleasant).

To measure social stress, subjects were asked to evaluate the social context when other persons were present, by rating on a 7-point Likert scale (1=not true, 7=very true) the item: "I don't like the company" and the subsequent item: "I would rather be alone". The mean of these two items constituted the social stress scale.

Assessment of negative affect

After each beep, participants were asked to answer questions regarding their mood on 7-point Likert scales (1=not at all, 7=very). ESM negative affect was assessed with

6 mood-related adjectives (down, guilty, insecure, lonely, anxious, angry/ irritated) that were reduced to a single measure of the mean Negative Affect [NA](Cronbach's $\alpha=0.86$).

Salivary cortisol

Salivary cortisol is a reliable and non-invasive measure of the free, unbound cortisol in blood, which is considered to be the biologically active hormone. Radioimmunoassays were run in duplicate, using a tracer solution of cortisol-3CMO coupled with 2-[125 I] histamine and specific antibodies raised against cortisol-3CMO-BSA (31) (Dr. J. Sulon, Department of Reproductive Physiology, School of Veterinary Medicine, University of Liège). The lower detection limit of the assay was 0.2 nmol/L. The intra- and inter-assay coefficients of variation were <5% and <12%, respectively. All samples from an individual were analyzed in the same assay to reduce sources of variability. Samples with cortisol >44nmol/L ($n=0$) were considered physiologically abnormal and were excluded from the statistical analysis. As antipsychotic medication may influence cortisol values, cortisol was only measured in siblings and controls.

MRI acquisition and processing

MRI scans were acquired with a 3T Siemens scanner using the following acquisition parameters: Modified Driven Equilibrium Fourier Transform (MDEFT) sequence: 176 slices, 1 mm isotropic voxel size, TE 2.4 ms, TR 7.92 ms, flip angle 15°, total acquisition time: 12 min 51s, and the Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE: Alzheimer's Disease Neuroimaging Initiation (ADNI)) sequence: 192 slices, 1 mm isotropic voxel size, TE 2.6 ms, TR 2250 ms, flip angle 9°, total acquisition time: 7 min 23s. For both sequence the matrix size was 256x256 and the field of view 256x256 mm. Two sequences were used because of a scanner update during data collection. The MP-RAGE and MDEFT are very similar, but in order to prevent any systematic bias, the total proportion of MP-RAGE scans (27%) was balanced between the groups.

MRI preprocessing

Scans were preprocessed with Freesurfer stable release v5.0. Technical details of these procedures are described in prior publications (32, 33).

Volume measures

Pituitary volumes were obtained after manual tracing using GIANT (General Image ANalysis Tools developed by EHBMG (34), a customised software program that allows tracing of regions of interest in triplanar view and calculation of volumes of interest. All tracings were carried out on the native images. Each pituitary was traced in all coronal slices where it could be visualized, after the protocol that has been described previously (35). Pituitary volume (in mm³) was calculated by summing the areas of the relevant slices and multiplying by the voxel size. All images were traced by the same rater (SB), who was blind to group assignment. The intra-class correlation coefficient of repeated measurements, based on a training set of 15 scans, was $r=0.92$.

Statistical analyses

ESM and cortisol data were analysed using multilevel regression techniques, thus taking into account the hierarchical structure of the data. Repeated momentary measurements (level 1) were nested in subjects (level 2) who were part of the same family (level 3). Given these three levels, data were analyzed using the XTMIXED multilevel random regression routine in STATA 11.0 (36). The B's are the fixed regression coefficients of the predictors in the multilevel model. Interaction terms were evaluated by Wald tests (37). In case of significant interaction effects, stratified analyses were conducted in order to quantify group differences, using the STATA MARGIN command to calculate the appropriate linear combinations from the model containing the interaction. All analyses were adjusted for the a priori hypothesized confounders age, sex, intracranial volume, estrogen exposure and scan type.

Group differences in pituitary volumes were examined using multiple regression procedures, with pituitary volume as dependent variable and group status (dummy variable: 0 = controls, 1 = siblings, 2 = patients) as independent variable.

Group differences in stress-reactivity were assessed by multilevel analyses, with NA as the dependent variable and group, stress (event stress and social stress) and their interactions as the independent variables.

To test the hypothesis that group status modified the association between pituitary volume and stress-reactivity, multilevel regression analyses were conducted with stress, pituitary volume and group as well as their interaction terms as independent variables and NA as the dependent variable: $NA = B_0 + B_1(\text{group}) + B_2(\text{stress})$

+ B3(volume)+ B4(group × stress) + B5(group × volume) + B6(stress × volume) + B7(group × stress × volume). The group × stress × volume interaction was fitted with the control group as the reference category. Pituitary volume was entered both as a linear variable and as dummy variables representing the distribution of volume calculated at the 33th and 66th percentiles of pituitary volume of the controls, allowing visualization of dose-response (1= $\leq$ 33th percentile: low pituitary volume, 2= $\geq$ 33th and $<$ 66th percentile: medium pituitary volume, 3= $\geq$ 66th percentile: high pituitary volume).

The same pattern of analyses as described above were applied to the biological stress measure, the log transformed cortisol values (Incort)) (=dependent variable). Additional confounders were: time of cortisol sample, time2, recent consumption of food or tobacco (i.e. in the approximately 90 minute interval since the previous beep). To test whether mean cortisol level differed between the two groups, a regression model was estimated with Incort as the dependent variable and group status (dummy variable: 0=controls, 1=siblings) as the independent variable.

The association between pituitary volume and both exogenous estrogen (in women) and lifetime AP (in patients) was investigated using multiple regression analysis.

Power analysis

Power calculations for the 3-way interaction analyses were carried out by empirical statistical simulation in STATA (www.stata.com/support/faqs/stat/power.html), as described previously (23). Effect sizes were based on previously published work in this area. The effect sizes for the association between group and NA, between stress and NA and the interaction between group and stress were adapted from Myin-Germeys *et al.*, (2001)(18). Similarly, effect sizes in the model of cortisol were adapted from Collip *et al.*, (in press)(14). The effect sizes for the other interactions were not known. Therefore, effect sizes of 0.2 SD (small) were used for the patient versus control simulations, and effect sizes of 0.1 SD were used for the sibling versus control simulations. At an alpha of 0.05, the 3-way interaction analysis had a power of 56% to detect a significant difference between patients and controls and a power of 18% to detect a significant difference between siblings and controls. Therefore, results of the three-way interactions measuring differences between siblings and controls will only be described exploratively in order to generate hypotheses for future research and help generate realistic power calculations based on published effect sizes in these groups.

Sensitivity analysis

Studies report an association between pituitary volume and illness duration ((4, 38), with increased pituitary volume for FE patients and decreased pituitary volume for established schizophrenia patients (ESP). Therefore, explorative analyses were carried out separately in patients with an illness duration of ≤ 5 years (FE; $n=10$) and >5 years (ES; $n=10$).

Results

Descriptive analyses

Groups were well matched on most demographic variables (Table 1). Patients smoked more cigarettes and cannabis and used more hard drugs (lifetime) than siblings and controls, with no difference between the latter two groups. Eighteen patients received AP (atypical: $n=2$; typical: $n=16$). Furthermore, 3 patients and 2 controls used antidepressants and 1 patient and 1 control used benzodiazepines. Patients reported higher PANSS scores than controls and siblings with no difference between the latter two groups (Table 1).

Group differences in pituitary volume and in emotional stress-reactivity

Mean pituitary volume did not differ between groups (patients: 602.6 mm³, SE 23.4; siblings: 624.5 mm³, SE 17.5; controls: 624.4 mm³, SE 18.0; $B=5.34$, $p=0.76$). There was a trend significant group $\times$ event stress interaction in the model of NA (Table 2), indicating that stress-reactivity differed between groups. Stratified analyses revealed that patients had significantly higher levels of stress-reactivity compared to both controls and siblings. For social stress, there was no significant interaction effect with group in the model of NA (Table 2).

Interaction between genetic risk, emotional stress-reactivity and pituitary volume

In the combined group of patients, siblings and controls, a significant association between volume (linear variable) and stress-reactivity (social stress) ($\chi^2=23.03$, $p=0.00$) was found. There was a significant group $\times$ social stress $\times$ volume (linear variable) interaction in the model of NA, indicating that the association between pituitary volume and stress-reactivity differed between groups ($\chi^2=20.13$, $p=0.00$). Stratified analyses showed that, with increasing pituitary volume, stress-reactivity was progressively greater in patients compared to both controls and siblings,

whereas no difference was apparent between the latter two groups. Visualizing the effect over the three tertile groups of pituitary volume revealed progressively higher stress-reactivity with progressively higher pituitary volume in all three groups, but much more pronounced in the patients compared to siblings and controls (Table 3) (Figure 1). Stress-reactivity was significantly higher in the high volume group when compared to the low volume group in controls ($\chi^2=7.17$, $p=0.01$), siblings ($\chi^2=5.34$, $p=0.02$) and in patients ($\chi^2=12.72$, $p=0.00$). In patients, stress-reactivity was also significantly increased in the medium volume group, compared to the low volume group ($\chi^2=30.62$, $p=0.00$).

The event stress x volume interaction effect in the model of NA was not significant, nor was the group x event stress x volume (linear variable) interaction ($\chi^2=1.15$, $p=0.56$) (Table 3), however effect sizes were directionally similar to those observed for social stress.

Group differences in cortisol levels and in biological stress-reactivity

Siblings had significantly higher cortisol levels than controls ($B=.30$, $P=0.00$). There was no significant association between pituitary volume and cortisol levels in the combined group of siblings and controls ($B=-0.001$, $P=0.25$).

The group x stress interaction in the model of cortisol was significant for neither social nor event-related stress (Table 2).

Interaction between genetic risk, biological stress-reactivity and pituitary volume

The stress x volume interaction and the group x stress x volume interaction in the model of cortisol were not significant for either social ($\chi^2=1.04$, $p=0.31$) or event stress ($\chi^2=1.49$, $p=0.22$) (Table 3).

AP medication use and estrogen exposure in relation to pituitary volume

Lifetime AP use did not predict pituitary volume (linear variable: $B=1.04$, $P=0.19$; tertiles: $B=29.32$, $P=0.51$), nor did estrogen exposure ($B=0.00$, $P=0.48$). There was no significant interaction between group and estrogen exposure in the model of pituitary volume ($\chi^2=0.00$, $p=0.99$).

Women had larger pituitary volumes than men ($B=63.94$, $P=0.04$), but no group x gender interaction on pituitary volume was apparent ($\chi^2=2.00$, $P=0.37$). The direction and significance of the results remained the same when estrogen exposure was removed as confounding factor from the analyses (results available upon request).

Sensitivity analysis

Although results were not statistically significant, FE patients had higher pituitary volumes (mean: 641.1 mm³, SE 33.79) than controls, and ES patients had lower pituitary volumes (mean: 564mm³, SE 91.08) than controls. Post-hoc analyses revealed that the group x social stress x volume (linear variable) interaction was attributable predominantly to the FE patients ($\chi^2=22.66$, $p=0.00$), whereas in the ES patients this interaction was no longer apparent, although effect sizes were directionally similar ($\chi^2=1.65$, $p=0.44$).

Discussion

There was no mean difference in pituitary volume among the three groups. Patients with schizophrenia showed higher levels of emotional stress-reactivity compared to siblings and controls. Emotional stress-reactivity was not only moderated by group, but also by pituitary volume. In all three groups, increased emotional stress-reactivity was associated with higher pituitary volume, but this association was much more pronounced in the patient group, suggesting a process of progressive sensitization mediating clinical outcome.

Emotional stress-reactivity

The finding of increased emotional stress-reactivity in the group at highest genetic risk group (patients) is in line with other studies that have shown that minor stressors in the flow of daily life were associated with an increase in negative emotions in individuals with a diagnosis of schizophrenia (18, 29). Although there was no difference in pituitary volume between the groups, emotional stress-reactivity was associated with larger pituitary volume in all three groups, with a much more pronounced increase in the patients than in the other two groups (Table 3). More specifically, each unit increase in stress-reactivity was associated with an increase of pituitary volume of 0.20 SD in the patients. This phenomenon may indicate a mechanism of stress sensitization in the patients and mediate the onset of schizophrenia.

Stress sensitization occurs when early and /or frequent exposures to stressors alter the stress response system, sensitizing individuals to later stress, leading to increased biological and behavioral response to that stressor, even when less severe than the original stressor. Given the fact that many environmental risk factors associated with

schizophrenia may be linked to stress, stress sensitization may represent a common mechanism linking multiple environmental exposures (12). For example, evidence suggests that patients with schizophrenia, who report more childhood trauma, show increased stress-reactivity (39). Furthermore, previous exposure to stressful life events has been associated with higher sensitivity to small stressors in daily life (40).

Higher pituitary volume may be the result of differential functional states (e.g., increased stress-sensitivity/hypercortisolism) or, vice versa, contribute causally to increased stress-sensitivity. Post-hoc analyses revealed that FE patients had higher and patients with ES lower pituitary volumes than controls, in line with previous studies (2, 5). In addition, the association between increased stress-reactivity and pituitary volume was confined to the FE group. These observations are in agreement with the hypothesis of two biologically distinct pathways resulting in mean group differences between (i) type 1 psychotic disorder, characterized by high levels of positive symptoms and an episodic course (41,42), as well as high stress-reactivity and associated larger pituitary volume, and (ii) type 2 psychotic disorder, characterized by high levels of negative symptoms, cognitive impairments and a chronic course (43-46), as well as low stress-sensitivity and smaller pituitary volumes. A larger study sample and a longitudinal design are needed to draw more definitive conclusions about underlying mechanisms and direction of effects.

Biological stress-reactivity

Mean cortisol levels were higher in siblings than in controls, consistent with another study from our group (14). It has been suggested that elevated cortisol levels in patients with a diagnosis of schizophrenia (13, 47) and their siblings reflect disturbances in the negative feedback regulation of the HPA axis (48). A comparable observation in depression is that HPA axis hyperactivity is thought to reflect a lack of negative inhibitory feedback by circulating cortisol on the HPA axis, particularly at the level of pituitary cells producing ACTH, leading to an increase in the size and number of these cells. Persistently elevated cortisol secretion could also lead to resistance of the glucocorticoid receptors for cortisol (49).

Cortisol reactivity to daily life stress, however, did not show a significant difference between siblings and controls and therefore does not agree with an earlier study (14). The present analyses, although based on the same cohort as described by Collip and colleagues, were carried out in individuals for whom MRI data were available,

resulting in a sample size reduction. Thus, statistical power was insufficient to detect small differences in cortisol reactivity to daily life stress between siblings and controls and/or as a function of pituitary volume.

In sum, although some evidence for HPA axis hyperactivity was found (increased mean cortisol values in siblings), cortisol reactivity was not mediated by group status or pituitary volume in this study. However, larger studies are needed before more definitive conclusions can be drawn. Nevertheless, the biological and emotional stress-reactivity pathways seemed to be associated, as similar patterns in both biological and emotional stress-reactivity were apparent in siblings and controls.

Methodological considerations

Some authors have cast doubt on the reliability and subject compliance in paper-and-pencil ESM studies, favouring the use of electronic devices (50). However, in a comparative study, Green *et al.* (2006) concluded that both methods yielded similar results (51).

Two different scanning sequences were used in this study, which could in theory have influenced the volume measurements. However, it has been shown that within-scanner measurements were reliable for subcortical volumes across scan sessions (52). Furthermore, in our data, no large or significant interaction was found between scan type and group on pituitary volume ($\chi^2=0.09$ $p=0.96$). Similarly, adjusting the analyses for scanning sequence did not affect direction or significance of the results.

Numerous external factors can influence pituitary volume, such as the use of AP. According to the study by Nicolo *et al.* (2010) in FE patients, SGA's reduce pituitary gland volume in a dose-dependent manner (8). However, some studies report that prolactin-elevating medication increases pituitary volume in comparison with prolactin-sparing medication (2, 6), whereas others do not (3, 4). The present study did not find an association between cumulative lifetime exposure to AP medication and pituitary volume, but the groups were too small to examine differential effects of AP group (SGA versus FGA). Furthermore adding lifetime AP as an additional confounder did not change the results (results available upon request). Exogenous estrogen exposure has also been associated with pituitary volume. Reduced pituitary volumes have been found in women taking oral contraceptives (Grams *et al.*, 2010) but, in contrast, elevated pituitary volumes have been found

in menopausal women using estrogens (53). The present study did not find an association between life-time estrogen exposure and pituitary volume. Larger studies investigating the association between estrogens and pituitary volume longitudinally are warranted. Nevertheless, there was a gender effect, i.e. pituitary volumes were higher in women than in men, possibly reflecting differences in endogenous estrogen levels (9). The role of estrogen in schizophrenia remains unknown. Evidence suggests that estrogens may exert a neuroprotective effect and possibly have antipsychotic properties (54). On the other hand, if estrogen could also lead to pituitary enlargement, this may potentially be less beneficial in terms of vulnerability for schizophrenia. It is advisable to take estrogen measures into account when examining pituitary volumes.

Table 1. Subject demographics

	Controls	Siblings	Patients	Test statistic +
	(n=32)	(n=37)	(n=20)	p-value
Age, years: mean (SD)	31.7 (11.4)	28.3 (7.8)	29.1 (8.0)	F= 1.95 p= 0.15
Gender (male:female)	10:22	14:23	11:09	$\chi^2= 2.97$ P= 0.23
Completed education	5.8 (1.8)	5.3 (2.2)	4.7 (1.8)	F= 5.49 P= 0.00
PANSS Scores				
Mean positive scale (SD)	7.4 (1.3)	7.5 (1.1)	12.4 (5.1)	F= 10.00 p= 0.00
Mean negative scale (SD)	8.0 (0.2)	8.2 (1.0)	10.8 (3.3)	F= 9.15 p=0.00
Mean disorganization scale (SD)	10.2 (0.4)	10.2 (0.5)	31.4 (4.1)	F= 8.65 p= 0.00
Mean excitement scale (SD)	8.3 (0.6)	8.4 (1.1)	9.9 (2.1)	F= 5.38 p= 0.00
Mean emotional distress scale (SD)	9.5 (2.5)	9.9 (2.3)	14.2 (5.0)	F= 7.56 p= 0.00
Mean alcohol use present state (SD)	6.1 (8.7)	7.6 (9.2)	4.9 (6.6)	F= 1.64 P= 0.19
Mean cigarettes use present state (SD)	1.1 (3.9)	1.8 (4.6)	12.1 (11.9)	F= 7.86 p= 0.00
Cannabis use				
Mean cannabis use last 12 months (SD)	1.6 (8.8)	3.4 (12.6)	34.3 (99.8)	F= 1.68 P= 0.18
Mean cannabis use lifetime (SD)	16.8 (34.4)	19.6 (35.7)	54.2 (48.0)	F= 4.49 p= 0.00
Hard drug use				
Mean hard drug use last 12 months (SD)	1.6 (9.2)	0.0 (0.0)	5.0 (18.2)	F= 0.78 p= 0.51
Mean hard drug use life time(SD)	3.4 (13.0)	5.1 (15.4)	40.5 (73.05)	F= 3.48 P= 0.02
Antipsychotics				
Type AP (typical:atypical)			2:16	
Mean haloperidolequivalent present state(SD)			2.4 (1.9)	
Mean lifetime AP use in haloperidolequivalent (SD)			48.2 (47.1)	
Mean lifetime estrogen exposure ¹ (SD)	17589 (31700)	18474 (29410)	672 (2013)	F= 9.23 P= 0.00
Scan type (MDEFT:ADNI)	23:09	26:11	16:04	$\chi^2= 0.7$ P= 0.72

Abbreviations: PANSS, Positive and Negative Syndrome Scale; AP, antipsychotic

¹ months use of oral contraceptives multiplied with micrograms estrogen (of the corresponding oral contraceptive) per month

F/ χ^2 and P-values refer to between-group differences

Table 2. Multilevel regression estimates of emotional- and biological stress reactivity

	χ^2	P	B
Biological stress reactivity (cortisol)			
Group*event stress	0.22	0.64	
Group*social stress	0.56	0.45	
Emotional stress reactivity (NA)			
Group*event stress	5.47	0.06	C=0.04 ¹ ; S=0.04 ¹ ; P = 0.07 ¹
Group*social stress	2.48	0.29	

¹ P < 0.05

The χ^2 and P-values represent the results of the Wald test, testing the significance of stratified effects calculated from the model with the group x stress interaction on respectively cortisol and negative affect

C= controls; S= siblings; P= patients

the B's represent the effect sizes of level of stress sensitivity compared to the reference level

Table 3. NA and cortisol as a function of daily life stress and pituitary volume, stratified by group

	Controls			Siblings			Patients		
	B	P	CI	B	P	CI	B	P	CI
Negative affect									
Event stress * pituitary volume	0.04	0.00	0.03-0.06	0.04	0.00	0.02-0.06	0.07	0.00	0.05-0.10
Social stress * pituitary volume	0.10	0.00	0.07-0.13	0.08	0.00	0.04-0.11	0.20	0.00	0.14-0.26
Low volume	0.06	0.03	0.01-0.11	0.05	0.04	0.00-0.09	0.02	0.49	-0.02-0.06
Medium volume	0.10	0.00	0.05-0.15	0.05	0.24	-0.03-0.12	0.38	0.00	0.26-0.50
High volume	0.16	0.00	0.11-0.22	0.14	0.00	0.08-0.20	0.42	0.00	0.20-0.62
Cortisol									
Event stress * pituitary volume	0.01	0.66	-0.02-0.04	-0.01	0.74	-0.04-0.03			1.49
Social stress * pituitary volume	0.00	0.97	-0.07-0.06	-0.04	0.31	-0.12-0.04			1.04
									0.31

the B's represent the effect sizes of stress reactivity compared to the reference level

The χ^2 and P-values represent the results of the Wald test; testing the significance of stratified effects calculated from the group x stress x volume interaction in the model of negative affect/cortisol;

CI= 95% Confidence interval

Figure 1. Stress-reactivity as a function of pituitary volume, stratified by group. Stress-reactivity was significantly higher in the high volume group when compared to the low volume in controls ($P=0.01$), siblings ($P=0.02$) and in patients ($P=0.00$). When comparing middle volume with low volume only patients showed increased stress-reactivity ($P=0.00$). Patients showed higher stress-reactivity when compared to controls in the medium pituitary volume group ($P=0.00$) and in the high pituitary volume group ($P=0.02$).

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Hippocampal volume as marker of daily life emotional and cortisol stress-sensitivity in psychosis

Submitted

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Abstract

Background: Reduced hippocampal size and increased stress-sensitivity are associated with genetic risk for psychotic disorder. However, to what degree the hippocampus is implicated in daily life stress-reactivity in psychotic disorder has never been examined. The current study investigated (i) whether hippocampal volume (HV) was associated with emotional and cortisol daily stress-reactivity, and (ii) whether genetic risk (the contrast between controls, patients and siblings of patients) moderated this relationship.

Methods: 1-weighted MRI scans were acquired from 20 patients with schizophrenia, 38 healthy siblings at higher than average genetic risk for schizophrenia and 33 controls. Freesurfer 5.0.0 was used to measure HV. The Experience Sampling Method (ESM; a structured momentary assessment diary technique) was used to assess emotional stress-reactivity (the effect of momentary stress on momentary negative affect (NA)). Cortisol stress-reactivity was assessed using momentary cortisol levels extracted from saliva in the control and sibling groups.

Results: Multilevel linear regression analyses revealed a significant three-way interaction between group, HV, and momentary stress in both the model of NA and the model of cortisol. Increased stress-reactivity was associated with smaller HV in patients and larger HV in controls. In line with the results in patients, siblings with small HV demonstrated increased cortisol stress-reactivity compared to those with large HV, although there was no association between HV and emotional stress-reactivity in siblings.

Conclusions: The findings suggest that HV may index risk and possibly disease-related mechanisms underlying daily life emotional and cortisol stress-reactivity in psychotic disorder.

Introduction

Patients with psychotic disorder generally display reduced hippocampal size [1-4]. Recent studies have shown that similar alterations in hippocampal volume (HV) may be present in first-degree relatives of patients with schizophrenia [5, 6], suggesting that HV alterations constitute part of the liability to psychosis. The hippocampus plays a pivotal role in regulating emotional responses to stressful stimuli and in the negative feedback mechanism controlling hypothalamic-pituitary-adrenal (HPA) axis activity [7-11]. Because the hippocampus has an inhibitory influence, a smaller HV might be associated with increased HPA axis reactivity to stress. Indeed, in clinical samples, studies have reported an inverse association between HV and cortisol levels. In two studies, hippocampal atrophy was accompanied by hypercortisolemia [12, 13]. Similarly, in patients with a psychotic disorder, smaller left HV was associated with higher salivary cortisol levels [14].

In contrast, findings in healthy volunteers show mixed results. One study, examining the association between HV and cortisol response to experimental psychological stress as well as awakening [15], found a positive association between HV and cortisol reactivity in young healthy volunteers, whereas a negative association between HV and the cortisol response to a physiological challenge was found in another sample [16]. These findings indicate that the association between HV and stress-reactivity may be different in healthy and clinical populations.

Familial vulnerability for psychosis has been associated with amplified emotional and cortisol responses to stress in daily life. Patients with a psychotic disorder and their unaffected first-degree relatives have been found to display increased negative emotions and an increase in psychotic experiences [17, 18]. Similarly, a recent study showed that siblings of patients compared to controls respond with increased cortisol secretion to minor everyday stressors (Collip et al., in press). The neural mechanisms underlying this augmented stress-sensitivity, however, have not yet been identified.

The current study investigates whether HV is associated with emotional and cortisol reactivity to daily life stress, as indexed by momentary variation in negative affect and momentary cortisol levels. Given the reported discrepancy between clinical and non-clinical samples in the association between HV and cortisol [19], we examined whether this association was moderated by genetic risk for psychosis. Because some earlier studies suggest that cortisol levels are primarily associated with left

HV [20 491], analyses also investigated hemispheric differentiation with regard to: differences in the HV stress-reactivity. Analyses thus examined to what degree (i) HV was associated with emotional and cortisol reactivity to daily stress, and (ii) group (control, sibling, patient), moderated the association between (emotional and cortisol) reactivity to stress and HV.

Methods and Materials

Subjects

The study sample consisted of patients with a diagnosis of non-affective psychotic disorder, their siblings, and controls from the general population, in the context of the Dutch national GROUP project [21]. In selected representative geographical areas in the Netherlands and Belgium, patients were identified through clinicians whose caseload was screened for inclusion criteria. Subsequently, a group of patients presenting consecutively at these services either as outpatients or inpatients, were recruited for the study. First-degree relatives were recruited through participating patients. Control subjects were recruited from the same population as the patients, through random mailings in the geographic region and advertisements in newspapers. All interviews were conducted by trained psychology graduates. From the wider sampling frame, the following subgroups were selected: 20 patients with a diagnosis of schizophrenia or related psychotic disorder, 37 siblings of patients with a diagnosis of schizophrenia and 32 controls. Inclusion criteria were: (i) age 16 to 50 years, (ii) diagnosis of non-affective psychotic disorder and (iii) good command of the Dutch language. Siblings had to be free of any lifetime non-affective psychotic disorder. For the control subjects, the occurrence of any psychotic disorder in either the subject or any first-degree family member, assessed using the Family Interview for Genetic Studies (FIGS) [22], constituted an exclusion criterion.

Diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria [23], assessed with the Comprehensive Assessment of Symptoms and History (CASH) interview [24]. Patients were diagnosed with: schizophrenia (n=11), schizoaffective disorder (n=2), schizophreniform disorder (n=1), brief psychotic disorder (n=2), and psychotic disorder not otherwise specified (n=4). The CASH was also used to confirm the absence of a diagnosis of non-affective psychosis in the siblings, and absence of a lifetime diagnosis of any psychotic disorder or any current affective disorder in the healthy controls. Six siblings and seven controls had

a history of major depressive disorder, but none presented in a current depressive episode.

Prior to MRI acquisition, participants were screened for the following exclusion criteria: (i) brain injury with unconsciousness for over 1 hour, (ii) meningitis or other neurological diseases that might have affected brain structure/function, (iii) cardiac arrhythmia requiring medical treatment and (iv) severe claustrophobia. In addition, subjects with metal corpora aliena were excluded from the study, as well as women with an intrauterine device or (suspected) pregnancy. The study was approved by the standing ethics committee, and all the subjects gave written informed consent in accordance with the committee's guidelines.

Measures

The Positive and Negative Syndrome Scale (PANSS) [25] was used to measure psychotic symptoms over the last two weeks.

Antipsychotic medication was determined from reports of the participant's psychiatrist. Best estimate lifetime (cumulative) antipsychotic medication (AP) use was determined by multiplying the number of days of AP use with the corresponding haloperidol equivalents and summing up these scores for all periods of AP use [26].

Estrogen exposure was estimated by multiplying the number of months of oral contraceptive use with micrograms estrogen (of the corresponding oral contraceptive) per month.

Substance use was assessed using the Composite International Diagnostic Interview (CIDI) sections B-J-L [27]. Cannabis use and other drug use (stimulants, sedatives, opiates, cocaine, PCP, psychedelics, inhalants, or other (e.g., XTC, poppers)) was assessed as reported frequency of use (i) during the last 12 months, and (ii) lifetime. Alcohol and tobacco use was defined as the reported number of weekly consumptions during the last 12 months.

Experience Sampling Method (ESM)

The Experience Sampling Method is a random time-sampling self-assessment technique; studies have demonstrated the feasibility, validity, and reliability of ESM in general and patient populations [28, 29]. Subjects received a digital wristwatch that emitted a signal ten times a day on six consecutive days, at unpredictable moments between 7:30 a.m. and 10:30 p.m. After each 'beep', subjects completed ESM self-

assessment forms concerning current context, thoughts, emotions, and psychotic experiences. Subjects were instructed to complete their reports immediately after the beep, thus minimizing memory distortions. Reports were considered valid when subjects responded within 15 min after the beep, as determined by comparing the actual beep time with the reported time of completion. For inclusion in the analyses, participants had to have provided valid responses to at least one-third of the emitted beeps [30].

Stress-reactivity, as described in previous work, was conceptualized as emotional [18] and cortisol reactivity (Collip et al., in press)[31] to minor disturbances in daily life. Momentary measures of stress, negative affect (NA) and cortisol were derived from the experience sampling data as described below.

ESM measures

Event stress. In accordance with previous work, stress was conceptualized as the subjectively appraised unpleasantness of distinctive events [18]. After each beep, participants were asked to report the most important event that had happened between the current and the previous report and then to rate this event on a 7-point scale (-3 = *very unpleasant*, 0 = *neutral*, 3 = *very pleasant*). The responses were recoded to allow high scores to reflect stress (-3=very pleasant, 0=neutral, 3=very unpleasant).

Negative affect. In line with previous reports [18] ESM NA was assessed as the mean score on 6 ESM items, rated on 7-point Likert scales (1 = *not at all* to 7 = *very*): “*I feel insecure*”, “*I feel lonely*”, “*I feel anxious*”, “*I feel down*”, “*I feel guilty*” and “*I feel angry/irritated*” (Cronbach’s $\alpha = .84$).

Salivary cortisol sampling

After each ESM beep, participants (siblings, controls) collected a saliva sample with a cotton swab (Salivette; Sarstedt, Etten-Leur, the Netherlands), replaced the swab in the salivette tube, and recorded the exact collection time. Samples were stored in subjects’ home freezers until transport to the lab, where uncentrifuged samples were kept at -20°C until analysis. Saliva samples collected more than 15 minutes after the beep were excluded from the analysis.

Salivary cortisol

Salivary cortisol is a reliable and non-invasive measure of the free, unbound cortisol in plasma, the biologically active hormone. Radioimmunoassays were run in duplicate, using a tracer solution of cortisol-3CMO coupled with 2-[¹²⁵I] histamine and specific antibodies raised against cortisol-3CMO-BSA [33] (Dr. J. Sulon, Department of Reproductive Physiology, School of Veterinary Medicine, University of Liège). The lower detection limit of the assay was 0.2 nmol/L. The intra- and inter-assay coefficients of variation were < 5% and < 12%, respectively. All samples from an individual were analyzed in the same assay to reduce sources of variability. All samples were within the normal physiological range (< 44nmol/L). Cortisol was not measured in the patients with a psychotic disorder, as they usually receive antipsychotic medication, which can affect cortisol levels.

MRI acquisition and processing

MRI scans were acquired using a 3T Siemens scanner and the following acquisition parameters: Modified Driven Equilibrium Fourier Transform (MDEFT) sequence: 176 slices, 1 mm isotropic voxel size, TE 2.4 ms, TR 7.92 ms, flip angle 15°, total acquisition time: 12 min 51s, and for the Magnetization Prepared Rapid Acquisition Gradient Echo(MP-RAGE): Alzheimer's Disease Neuroimaging Initiation (ADNI) sequence: 192 slices, 1mm isotropic voxel size, TE 2.6 ms, TR 2250 ms, flip angle 9°, total acquisition time: 7 min 23s. For both sequences the matrix size and field of view were 256x256. Two sequences were used because of a scanner update during data collection. The MP-RAGE and MDEFT are very similar, but to prevent any systematic bias, the total proportion of MP-RAGE scans (around 1/3) was balanced between the groups.

MRI preprocessing

Scans were processed and analyzed using Freesurfer stable release v5.0.0. Technical details of these procedures are described in prior publications [34-40]. Data were automatically transformed into Talairach standard space.

Volume measures

The automated procedures for volumetric measures of the different brain structures are described by Fischl et al. [36]. This procedure automatically assigns a neuroanatomical label to each voxel in an MRI volume based on probabilistic information automatically estimated from a manually labelled training set. The

training set included both healthy persons in the age range 18–87 years and a group of Alzheimer’s disease patients in the age range 60–87 years, and the classification technique employs a registration procedure that is robust to anatomical variability, including the ventricular enlargement typically associated with neurological diseases and aging. The technique has previously been shown to be comparable in accuracy to manual labelling. The segmentations were visually inspected for accuracy.

Statistical analyses

ESM and cortisol data were analysed using multilevel regression techniques, which take the hierarchical structure of the data into account. In the current study, repeated momentary measurements (level 1) were nested in subjects (level 2) who were part of the same family (level 3). Data were analyzed using the XTMIXED multilevel random regression routine in STATA 11.0 [41]. Effect sizes from predictors in the multilevel model were expressed as *B*, representing the unstandardized fixed regression coefficient. Interactions were assessed by Wald test. The size of the moderator effects was calculated by applying and testing the appropriate linear combinations using the STATA MARGIN command. Raw cortisol values were log transformed to reduce skewness of distribution, generating the variable *Incort*. The variable *time* was centered around the grand mean for all samples. To model the cortisol diurnal curve, the variable *time* was included as a predictor in all analyses with *Incort* as dependent variable; addition of higher order polynomial terms did not improve model fit. Analyses were adjusted for the *a priori* selected confounders age, sex, intracranial volume, and scan type. Moreover, analyses were re-run including estrogen exposure and AP [14, 42], respectively.

Group differences in hippocampal volume and cortisol

To test whether HV or mean cortisol level differed between groups, a regression was estimated with HV and *Incort* respectively as the dependent variable and the categorical variable *group* (0 = controls, 1 = siblings, 2 = patients; for cortisol, controls and siblings only) as independent variable.

Interaction between group, stress-reactivity and volume

In order to test whether HV was associated with emotional stress-reactivity, and whether this was moderated by group, regression analyses were conducted with *event stress*, *HV* and *group* (entered as linear three-level variable of patients, siblings and controls = reference) as well as their interaction terms as independent variables and *NA* as the dependent variable $NA = B_0 + B_1 (\text{group}) + B_2 (\text{stress}) + B_3 (\text{volume}) + B_4 (\text{group} \times \text{stress}) + B_5 (\text{group} \times \text{volume}) + B_6 (\text{stress} \times \text{volume}) + B_7 (\text{group} \times \text{stress} \times \text{volume})$.

In separate analyses, HV was entered either as a linear variable or as dummy variable representing the distribution of volume calculated at the 33rd and 66th percentiles of HV of the controls, allowing visualization of dose-response (1= <33th percentile: low HV, 2= >33th and <66th percentile: middle HV, 3= >66th percentile: high HV). To ensure that definitions for small, medium and large HV were the same for all groups, patients and siblings were allocated to hippocampal group on the basis of the criteria for the control group.

In order to test the hypothesis that controls and siblings differed in their association between HV and cortisol stress-reactivity, the same model as described above was applied to cortisol (*Incort*) as the dependent variable. Additional confounders for all cortisol analyses were: time of cortisol sample, estrogen exposure and recent consumption of food or tobacco (i.e., in the approximately 90 minutes interval since the previous beep).

Results

Descriptive analyses

Groups were well matched on most demographic variables (Table 1). Patients smoked more cigarettes and cannabis than siblings and controls and had more lifetime hard drug use than siblings and controls, with no significant differences between the latter two groups. Eighteen patients received AP (atypical: n=2; typical: n=16). Furthermore, three patients used an antidepressant and one patient used a benzodiazepine. Two controls used an antidepressant, and one control used a benzodiazepine. Patients reported higher total, positive, and negative PANSS scores than controls and siblings, with no differences between the latter two groups (Table1).

Group differences in hippocampal volume, cortisol and stress-reactivity

There was a significant association between group and HV. Patients ($B=-247.6$, 95% CI $-415.1, -80.01$; $p=0.01$) and siblings ($B=-148.6$, 95% CI $-292.66, -4.47$; $p=0.04$) had smaller HV than controls. Patients and siblings did not differ ($B=99.0$, 95% CI $-57.63, 255.61$; $p=0.22$).

Siblings had significantly higher cortisol levels than controls ($B=0.28$, 95% CI $0.11, 0.45$; $p=0.001$). However, no significant association between cortisol levels and total HV ($B=-186.2$, 95% CI $-398.16, 25.76$; $p=0.085$), left HV ($B=-157.1$, 95% CI $-393.72, 79.62$; $p=0.19$) or right HV ($B=-215.4$, 95% CI $-452.63, 21.94$; $p=0.075$) was found in the combined group of siblings and controls and no significant interaction between cortisol and group (siblings and controls) was found for total HV ($\chi^2(1) = 0.00$; $p=0.99$), left HV ($\chi^2(1) = 0.03$; $p=0.85$) or right HV ($\chi^2(1) = 0.03$; $p=0.86$).

There was a significant *group x event stress* interaction in the model *NA*, indicating that emotional stress-reactivity differed between groups ($\chi^2(2)=10.05$, $p=0.01$). Stratified analyses revealed that patients had significantly higher stress-reactivity ($B=0.08$, 95% CI $0.054, 0.097$; $p=0.00$) compared to both controls ($B=0.05$, 95% CI $0.054, 0.097$; $p=0.00$) and siblings ($B=0.04$, 95% CI $0.024, 0.056$; $p=0.00$). For cortisol, no significant interaction was found between group (siblings and controls) and event stress ($\chi^2(1)=0.24$; $p=0.62$).

Emotional stress-reactivity contingent on hippocampal volume and genetic risk

A significant *group x event stress x total HV* interaction was found in the model of *NA* ($\chi^2(2)=5.88$, $p=0.05$). Differentiation by hemisphere revealed that the interaction with *left HV* ($\chi^2(2)=8.04$, $p=0.02$), but not with *right HV* ($\chi^2(2)=3.24$, $p=0.20$), was significant. Thus, the association between *left HV* and stress-reactivity differed between groups (Figure 1). Stratified analyses revealed increased emotional stress-reactivity in controls with larger left HV in comparison to controls with medium ($\chi^2(1)=5.28$, $p=0.02$) or small left HV ($\chi^2(1)=9.85$, $p=0.01$). In siblings, no significant differences in stress-reactivity were found between siblings with small, medium or large HV (medium vs. high: $\chi^2(1)=2.93$, $p=0.09$; low vs. medium: $\chi^2(1)=2.95$, $p=0.09$; low vs. high: $\chi^2(1)=0.02$, $p=0.89$). Patients with small and medium HV were significantly more stress-reactive than patients with large HV ($\chi^2(1)=21.17$, $p<0.01$; $\chi^2(1)=19.65$, $p<0.01$, respectively). Emotional stress-reactivity in patients differed from that of controls and siblings at all three levels of left HV. There was a trend difference in emotional stress-reactivity between controls and siblings with large left HV ($\chi^2(1)=$

3.78, $p=0.052$).

Analysis controlled for antipsychotic medication & estrogen exposure

The association between group and left HV decreased slightly after controlling for AP use ($\chi^2(2) = 5.92$; $p = 0.05$), but remained after controlling for estrogen exposure ($\chi^2(2) = 6.80$; $p = 0.03$). The interaction between *group x event stress x left HV on NA* remained significant after controlling for use of *antipsychotic medication or estrogen levels*, ($\chi^2(2)=6.08$, $p=0.05$; $\chi^2(2)=6.89$, $p=0.03$, respectively).

Cortisol stress-reactivity contingent on hippocampal volume and genetic risk

There was a significant *group x event stress x total HV* interaction in the model of *cortisol* ($\chi^2(1)=3.77$, $p=0.05$). Differentiation by hemisphere revealed that the interaction was significant for *left HV* ($\chi^2(1)=4.11$, $p=0.04$), but not for *right HV* ($\chi^2(1)=2.65$, $p=0.10$), suggesting that the association between left HV and cortisol reactivity to stress differed between the control and sibling group. For the control group, stratified analyses revealed no significant differences in cortisol response to daily stress between the hippocampal groups. In the sibling group, however, left HV moderated the effect of stress on cortisol, with small compared to large HV being associated with increased cortisol responses to stress ($\chi^2(1)=9.66$, $p=0.002$). Regarding differences between the control and sibling group, a non-significant trend difference was found for large left HV only ($\chi^2(1)=2.89$, $p=0.09$).

Discussion

To our knowledge, this is the first study to examine the association between stress-sensitivity, indicators of HPA axis activity, and HV in a sample with different levels of genetic risk for psychosis. Results show that the immediate effect of daily stress on negative affect and cortisol was not only conditional on HV, but also on genetic risk for psychosis. Patients with a small and medium left HV reported a larger increase in emotional stress-reactivity compared to patients with large HV. In line with the results in patients, siblings with small HV demonstrated increased cortisol reactivity to stress compared to those with large HV, although there was no association between HV and emotional stress-reactivity in this group. In contrast, controls with a large HV were more emotionally stress reactive than controls with small HV,

although this was not the case for cortisol reactivity.

Hippocampal Volume and overall diurnal cortisol

In line with a substantial amount of (meta-analytic) evidence [3, 4], HV in the patient group was smaller than that of control participants. Similarly, decreased HV in the siblings of patients with a psychotic disorder was found, which corresponds with findings from a recent meta-analysis [5, 6], as does the finding of absence of differences in HV between patients and their non-psychotic relatives [43].

There was no association between overall diurnal cortisol levels and HV, which is in line with a previous study that found no association between HV and cortisol in first episode psychosis [44]. Another study, however, that differentiated between left and right HV, found that baseline cortisol levels were associated with smaller left HV in first-episode psychosis, but not in controls [20]. Although we investigated left and right HV separately, no association between HV and cortisol level was apparent in siblings and controls, combined or in interaction. Nevertheless, there was a non-significant trend in the direction of a negative association between HV and diurnal cortisol levels, which is in line with the results of Mondelli and colleagues (2010b).

Stress-reactivity and Hippocampal Volume in patients with psychotic disorder

In patients with a psychotic disorder, smaller HV was associated with increased emotional stress-reactivity, while larger HV was associated with a diminished emotional response to stress. Thus, patients with a psychotic disorder were not only more likely to have a small hippocampus, but those with a decreased HV were also more likely to experience augmented stress-reactivity. These findings support the evidence that the hippocampus has an inhibitory role on the human stress response, extending findings to the realm of daily life.

We found differences in stress-reactivity *within* the group of patients with psychotic disorder that could be traced to differences in HV, i.e. patients with smaller HV were most responsive to the environment. This finding underscores the notion that there are different pathways to psychotic disorder [45], stress-reactivity being associated particularly with positive symptoms and a better prognosis [45, 46]. For example, patients with smaller HV may have experienced childhood trauma, impacting on cumulative glucocorticoid exposure and HV. The HV in turn, as shown in the current study, influences stress-reactivity. On the other hand, lower stress-reactivity may predict more negative symptoms. Unfortunately, the current patient group was too

small to explore clinical differences as a function of HV.

It is important to note that the direction of the association between HV and stress-reactivity is unresolved. The question of whether heightened cortisol reactivity may ultimately cause hippocampal volume changes or whether alterations in HV, possibly through decreased inhibitory functioning of the hippocampus, are responsible for increased stress-reactivity, should be answered in longitudinal studies.

Stress-reactivity and Hippocampal Volume in subjects at increased genetic risk for psychotic disorder

Similar to the findings in patients, siblings with smaller HV exhibited increased cortisol stress-reactivity, while larger HV in the siblings was associated with decreased cortisol response to stress. Interestingly, however, this was not found at the emotional level. Buchanan and colleagues (2009) suggest that the hippocampus may be a crucial element of a network involved in producing an integrated response to psychosocial stress (indexed by behavior and HPA activity). We found a decreased cortisol response to stress in siblings with larger HV and in patients a large HV was associated with reduced emotional response to stress. It may be that these represent markers for reduced integration of the stress response in those at increased risk for psychosis. In other words, decreased cortisol concentrations in the siblings with larger HV reflect suboptimal HPA axis functioning, resulting in increased liability for psychosis. Another possibility is that the blunted cortisol stress response in siblings with larger HV represents a protective factor against illness expression, given increased background vulnerability. It may also indicate that, even given suboptimal cortisol reactivity, siblings manage to cope better with stress and/or have higher resilience and therefore show relatively “healthy” emotional stress-reactivity.

Stress-reactivity and Hippocampal Volume in healthy controls

In controls, we found the reverse pattern, with smaller HV associated with reduced emotional stress-reactivity, and larger HV associated with increased emotional stress-reactivity. Cortisol reactivity to small daily hassles, however, did not differ as a function of HV in the control group.

These findings contradict another study in healthy adults that report evidence for an association between stress level and smaller anterior HV. However, the stress measure used comprised retrospective summary information. In contrast, the current study measures emotional stress-reactivity in daily life [47]. Our findings at the emotional

level do correspond with a study by Pruessner and colleagues (2007), who found that larger HV in healthy young participants was associated with increased cortisol response to awakening (CAR) and to an experimental stressor [19]. Pruessner and co-workers (2007) speculated that a larger hippocampus may require increased cortisol concentrations for optimal functioning. However, we found no association between HV and cortisol stress-reactivity in the control group. Differences in cortisol measures between the current study and the study by Pruessner and colleagues may constitute one explanation for the discrepant findings. Cortisol responses to laboratory stress and to awakening may affect different aspects of HPA axis reactivity than the reactivity to everyday hassles, which are likely more subtle stimuli. Nevertheless, the control participants with larger HV reported elevated emotional stress-reactivity to naturally occurring stressors compared to those with smaller HV, which corresponds to the cortisol reactivity findings reported by Pruessner and colleagues (2007).

Strength and weaknesses

This study has several limitations. First, use of ESM booklets instead of electronic devices means that the exact timing of participants' self-reports and saliva samples cannot be firmly established [48]. However, results of a study comparing self-reported and electronically monitored saliva collection times, with the same intensive, semi-random time-sampling protocol used in the current study, indicated that saliva was generally collected very close to the prescribed time and that self-reported collection times corresponded well with the electronic time-stamps [49]. Another comparative study concluded that paper and electronic diaries yield similar results [50]. Second, no saliva samples were taken at the time of awakening, so that the current dataset does not allow examination of the CAR, a measure of HPA axis activity that appears to be blunted in first-episode psychosis [14] and may be associated with HV [19].

The current study also had some specific strengths. In particular, the repeated sampling of salivary cortisol over 6 days takes into account the well-known but often ignored unreliability of cortisol measures obtained at infrequent intervals [51]. Multiple cortisol measures per person were complemented by a relatively large number of participants. Use of multilevel modeling allowed assessment of within-person associations between cortisol and subjective experience in real time and real-life contexts, as moderated by HV. Although cortisol measures were within the normal range, intensive sampling revealed different patterns of HPA activity with

different HVs. Moreover, we combined measures of emotional and cortisol stress-sensitivity with HV size, allowing a more comprehensive examination of HPA axis functioning.

Table 1. Subject demographics

	Controls	Siblings	Patients	Test statistic +
	(n=32)	(n=37)	(n=20)	p-value
Age, years: mean (SD)	31.7 (11.4)	28.3 (7.8)	29.1 (8.0)	F= 1.95 p= 0.15
Gender (male:female)	10:22	14:23	11:09	$\chi^2= 2.97$ p= 0.23
Completed education	5.8 (1.8)	5.3 (2.2)	4.7 (1.8)	F= 5.49 p= 0.00
PANSS Scores				
Mean positive scale (SD)	7.4 (1.3)	7.5 (1.1)	12.4 (5.1)	F= 10.00 p= 0.00
Mean negative scale (SD)	8.0 (0.2)	8.2 (1.0)	10.8 (3.3)	F= 9.15 p=0.00
Mean disorganization scale (SD)	10.2 (0.4)	10.2 (0.5)	31.4 (4.1)	F= 8.65 p= 0.00
Mean excitement scale (SD)	8.3 (0.6)	8.4 (1.1)	9.9 (2.1)	F= 5.38 P= 0.00
Mean emotional distress scale (SD)	9.5 (2.5)	9.9 (2.3)	14.2 (5.0)	F= 7.56 P= 0.00
Mean alcohol use present state (SD)	6.1 (8.7)	7.6 (9.2)	4.9 (6.6)	F= 1.64 P= 0.19
Mean cigarettes use present state (SD)	1.1 (3.9)	1.8 (4.6)	12.1 (11.9)	F= 7.86 P= 0.00
Cannabis use				
Mean cannabis use last 12 months (SD)	1.6 (8.8)	3.4 (12.6)	34.3 (99.8)	F= 1.68 p= 0.18
Mean cannabis use lifetime (SD)	16.8 (34.4)	19.6 (35.7)	54.2 (48.0)	F= 4.49 P= 0.00
Hard drug use				
Mean hard drug use last 12 months (SD)	1.6 (9.2)	0.0 (0.0)	5.0 (18.2)	F= 0.78 p= 0.51
Mean hard drug use life time(SD)	3.4 (13.0)	5.1 (15.4)	40.5 (73.05)	F= 3.48 p= 0.02
Antipsychotics				
Type AP (typical:atypical)			2:16	
Mean haloperidolequivalent present state(SD)			2.4 (1.9)	
Mean lifetime AP use in haloperidolequivalent (SD)			48.2 (47.1)	
Mean lifetime estrogen exposure ¹ (SD)	17589 (31700)	18474 (29410)	672 (2013)	F= 9.23 p= 0.00
Scan type (MDEFT:ADNI)	23:09	26:11	16:04	$\chi^2= 0.7$ P= 0.72

Abbreviations: PANSS, Positive and Negative Syndrome Scale; AP, antipsychotic

¹months use of oral contraceptives multiplied with micrograms estrogen (of the corresponding oral contraceptive) per month

F/Chi² and P-values refer to between-group differences

Figure 1. Emotional stress-reactivity stratified by hippocampal volume, in control, sibling and patient groups: Multilevel estimates of the effects of daily events on negative affect. The models control for age, gender, intracranial volume and scan type. Effects are unstandardized regression coefficients.

Figure 2. Biological stress-reactivity stratified by hippocampal volume, in sibling and control groups: Multilevel estimates of the effects of daily events on cortisol. The dependent variable is log-transformed cortisol (Incort). The models control for time, age, gender, oral estrogen exposure, recent food intake, recent smoking and intracranial volume, and scantype. Effects are unstandardized regression coefficients.

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6

General discussion

Psychotic disorder is a highly heterogeneous disorder of which the aetiology remains elusive. Childhood trauma, cannabis use and stress are known environmental risk factors that increase the risk for developing a psychotic disorder. However, the impact of these environmental risk factors is less than the risk related to having a first-degree relative (1). Using twin and adoption studies, researchers have found that 80% of the liability for psychotic disorder has a genetic origin (2, 3) although consistent evidence for genes involved in psychotic disorder is still lacking. With regard to genetic effects on the brain in individuals with psychotic disorder, there is evidence for both indirect and direct genetic effects. In this respect, environmental factors are assumed to contribute to brain alterations in psychotic disorder, and genetic effects could be misinterpreted when not taking environmental factors into account (4). For example, the pathway to psychotic disorder may involve gene-environment interactions influencing pathological processes of early brain development, such as neuronal loss and synaptic pruning, which continue to unfold as the brain matures during adolescence. These neurodevelopmental abnormalities may lead to the activation of pathological neural circuits and consequently to the emergence of symptoms in response to cumulative genetic and environmental interplay (5). In other words, the brain may be differentially sensitive to certain environments due to genetic vulnerability associated with the risk for psychotic disorder (i.e., gene-environment interaction). Alternatively, it may be that genes contribute to the tendency of individuals to expose themselves to adverse environments, which, in turn, may lead to an indirect genetic, causal effect of the environment on brain alterations associated with the illness phenotype (i.e., gene-environment correlation).

In summary, it is likely that the interplay between genes and environment is related to brain phenotypes in psychotic disorder. However, this association is customarily ignored in neuroimaging studies. The studies described in this thesis aimed at identifying genetic and environmental factors that are associated with brain alterations in psychotic disorder using a genetically sensitive study sample.

STRUCTURAL CEREBRAL INTERMEDIATE PHENOTYPE

Cortical thickness

Investigating genetic effects without measuring genes directly can be done using the intermediate phenotype approach. The rationale behind this is to find phenotypes

with a simpler genetic basis than the genetically complex and etiologically heterogeneous psychotic disorder genotype. Before a biological marker can be defined as an intermediate phenotype, several definitions have to be fulfilled: the intermediate phenotype (1) should be associated with psychotic disorder, (2) should be heritable, (3) should be present even when the illness is not active, (4) should co-segregate with psychotic disorder within families, (5) should be present at higher rates in unaffected family members when compared to the general population (6). In **chapter 2**, a family study design was used to examine the influence of different genetic risk levels for psychotic disorder on surface-based cortical thickness measures. Analyses showed some decreases in overlapping regions in patients with psychotic disorder and their healthy siblings. Shared cortical decrements were found in frontal, temporal, parietal and occipital regions in the two groups at higher than average genetic risk for psychosis. These regions, which may be potential intermediate phenotypes, were part of two different brain networks: i) the heteromodal association cortical regions and ii) the network involved in sensory gating. The heteromodal association cortex is involved in the integration of sensory, motor and behavioural activities with drive and mood, resulting in an integrated network critically important for attention, language, working memory and executive function (7, 8). Whereas the heteromodal association cortex is involved in integration, sensory gating reflects the ability to filter out redundant and/or distracting sensory information, thus allowing the brain to focus on a (first) stimulus preventing overstimulation or distraction by other stimuli. Delusions and hallucinations may, consequently, arise from an individual's response to an erroneous attribution of salience to external and internal stimuli. In short, the widespread cortical alterations in patients and siblings provides further evidence for the suggestion that psychotic disorder is not a disorder of a single brain area but rather a disorder of several brain regions being part of various networks (9, 10). In the highest genetic risk group, alterations were much more pronounced and widespread than in the sibling group, possibly reflecting a more dysfunctional brain network. Siblings shared some of the cortical thickness decrements with their ill family member and, additionally, showed some specific alterations though they have not expressed the clinical phenotype. It could be hypothesised that brain networks are flexible and able to compensate for certain failures up to a certain threshold. When the threshold is exceeded, failures in one or more networks may result in altered information processing and the expression of overt psychotic symptoms. Accordingly, smaller failures in the network

may contribute to the development of symptoms on a subclinical level.

The finding that siblings shared some brain alterations with their ill family member suggests a genetic influence, which is in accordance with twin studies reporting grey matter volume reductions in twins discordant for schizophrenia and showing that the greatest similarity in affected brain areas was present in monozygotic twins as compared to dizygotic twins (11, 12). It can be hypothesised that with lower genetic loading, brain alterations become more subtle. The brain alterations in siblings were indeed subtle and some of the variation in cortical thickness could be explained by cannabis use (as was also the case in patients), which was demonstrated by the association between cannabis use and cortical thickness and by the fact that the group-specific thinning in siblings disappeared after correction for cannabis use. Upon correction, the shared regions between patients and siblings remained significant, suggesting that these alterations are not fully conditional on this environmental influence and may be caused by shared genetic factors in the patients and siblings.

In summary, the genetic vulnerability for psychotic disorder may be associated with cortical thinning in regions involved in brain networks associated with several cognitive functions and with psychotic symptomatology. In addition to the shared genetic vulnerability that may influence cortical thickness in certain brain areas, environmental factors, specific (epi)genetic effects or gene-environment interactions may be determinants of patient- or sibling-specific cortical thinning in other areas.

DIFFERENTIAL SENSITIVITY TO THE ENVIRONMENT

Childhood trauma and cortical thickness

There is accumulated evidence for an association between psychotic disorder and childhood trauma (13-15), but studies investigating this relationship are prone to bias. For example, patients with psychotic disorder may report more childhood trauma because of their illness. However, if indeed a reporting bias would be the basis of the association between psychotic disorder and childhood trauma, this could not explain why a difference in cortical thickness between exposed and non-exposed patients (and particularly siblings) is greater than between exposed and non-exposed controls. Therefore, in an attempt to circumvent bias, the study presented in **chapter 3** used an objective biological measure (i.e., cortical thickness) as an outcome measure instead of the usual illness phenotype. It was examined

whether the effect of trauma on cortical thickness was conditional on genetic risk for psychotic disorder. The analyses revealed that patients exposed to higher levels of trauma in childhood had significantly lower cortical thickness values. This was not found in the controls. Siblings (although not significantly different from controls) displayed increased cortical thickness with increasing childhood trauma levels. This directionally opposite finding in siblings may represent chance or noise. Alternatively, siblings and controls may differ from patients in that the effect of exposure to traumatic experiences on cortical thickness may be neutral (controls) or even trophic (siblings). The existence of differential sensitivity to trauma is likely not the only moderator of the outcome. Differences in psychological adaptation, resilience and coping styles may be important mitigators of the risk for later psychotic outcomes.

Moreover, as siblings in this study experienced less childhood trauma compared to their ill relatives, mediation (i.e., genetically influenced differential exposure to the environment) may constitute part of the underlying mechanisms leading to psychosis. Research has shown that harsh parental behaviours directed at one person in the family may have protective effects for the other children in the family, a phenomenon called the sibling barricade (16), suggesting that, given shared genetic liability, differential exposure to trauma may result in illness in only one sib of the sibling pair (17). As mentioned before, patients reported more childhood trauma than controls and siblings with no difference in childhood trauma exposure between the latter two groups. Therefore, no definite conclusions can be drawn regarding the exact mechanism, but as mediation does not exclude moderation and vice versa, the difference in clinical expression between patients and siblings may well be due to a combination of both mechanisms (differential *exposure* and *sensitivity* to the environment).

Lastly, the findings may reflect genetic plasticity (64), indicating that the same gene may confer positive sensitivity to an exposure in some environments but negative sensitivity in others. For example, research has shown that a polymorphism in FKBP5, a modulator of glucocorticoid receptor (GR) sensitivity, influences the association between childhood trauma and later development of posttraumatic stress. FKBP5 showed reduced expression in PTSD, consistent with enhanced GR responsiveness, but only in interaction with developmental trauma (18). FKBP5 is located on chromosome 6p21, a chromosomal region associated with psychotic disorder (19) and, therefore, a candidate gene to use in the investigation of the relation between

developmental trauma and psychotic disorder. It could be argued that differences in FKBP5 aggravate or diminish the effects of childhood trauma on the stress hormone system, thereby altering HPA axis sensitivity and risk for psychotic disorder.

Cannabis use and cortical thickness

Cannabis use has been linked to the development of psychosis (20), but the role of cannabis use in brain alterations evidenced in psychotic disorder is not clear. In **chapter 3**, it was examined whether there was an effect of cannabis use on cortical thickness and, in addition, whether this was conditional on a genetic risk for psychotic disorder. The results showed that exposure to cannabis was associated with cerebral cortical thinning in individuals at high (patients) and intermediate (siblings) genetic risk for psychotic disorder whereas this was not the case for those at low genetic risk (controls). Furthermore, there was a suggestion of a similar pattern of regional thinning in the frontal and parahippocampal regions in patient and sibling cannabis users. The finding that cannabis use had a differential impact on cortical thickness not only in patients but also in siblings extended earlier findings, indicating that gene-environment interactions may underlie the association between cannabis use and psychosis (21-24). For example, evidence for a gene-environment interaction between psychosis and cannabis use has been found for the catechol-O-methyltransferase (COMT) VAL¹⁵⁸Met gene. Val allele carriers of the COMT gene are more prone to develop psychotic symptoms or later psychotic disorder with cannabis use than carriers of the MET allele (21, 23). Furthermore, a recent study provided evidence for the involvement of AKT1 in the association between psychosis and cannabis use (25). Genetic variation in AKT1 moderated the relationship between recent cannabis use and the level of schizotypy in siblings of patients with psychotic disorder. Also, increased risk allele loading in patients was associated with increased sensitivity to lifetime cannabis use. In healthy individuals, the AKT1 A allele was found to be associated with lower grey matter volume in the prefrontal lobe. In addition, evidence was found for gene-gene (epistatic) interaction in that subjects with both the COMT Val allele and the AKT1 A allele had lower prefrontal grey matter volumes than subjects with only the AKT1 A allele, relating the AKT1 polymorphism to mechanisms that may influence altered dopamine signalling (26).]

Stress-sensitivity and volumetric proxy measures for HPA axis functioning

Previous research suggests that patients with psychotic disorder and their siblings demonstrate greater increases in negative affect as a response to daily life stressors than healthy control subjects, suggesting that increased reactivity to stress is associated with a genetic risk for psychotic disorder (27, 28). Altered volumes of the pituitary gland have also been associated with stress and with increased vulnerability for psychotic disorder; for example, increased pituitary volumes have been found in patients with psychotic disorder (29), siblings of patients with psychotic disorder (30) and in subjects at ultra-high risk (31). The pituitary gland is a crucial organ involved in the HPA axis stress response and regulates cortisol secretion in response to stressful events (Figure 1A). Persistently elevated cortisol levels can decrease the activity of the negative feedback mechanism on the HPA axis, controlled by the hippocampus (32), resulting in a sensitised state of HPA axis hyperactivity (Figure 1B). Pituitary volume can, therefore, be viewed as a proxy measure for HPA axis functioning. As a result of HPA axis hyperactivity, patients become more sensitive to stressors, a phenomenon called stress-sensitisation (33). Stress-sensitisation occurs when early and/or frequent exposures to stressors alter the stress response system, sensitising individuals to later stress and leading to increased biological and behavioural responses to that stressor even when less severe than the original stressor (33). Given the fact that many environmental risk factors associated with psychotic disorder may reflect certain types of stressors (trauma, life events and daily life stressors, discrimination, migration, urbanicity), stress-sensitisation possibly represents a common underlying mechanism (33). Nevertheless, stress-reactivity has never been directly examined in relation to HPA axis functioning in psychotic disorder. In **chapter 4**, the relationship between biological (cortisol) and emotional stress-reactivity and pituitary volume was examined as a function of genetic risk for psychotic disorder. Cortisol measures were only available for siblings and controls. Although pituitary volume was not significantly different between the groups, higher pituitary volume was significantly associated with increased emotional stress-reactivity in all three groups (patients with psychotic disorder, siblings and controls). This association was significantly stronger in the patient group than in the siblings and controls, suggesting a process of progressive sensitisation mediating the clinical outcome. The siblings did not differ from controls with regard to cortisol reactivity nor was there an interaction between group status and pituitary volume in the model of

biological stress-reactivity. Emotional and biological stress-reactivity in siblings appeared to be associated and showed a similar pattern as in controls although baseline cortisol measures were higher in siblings than in controls. It should be noted that power issues may have led to type II errors in the sibling analyses.

Higher pituitary volume may be the result of differential functional states, such as increased stress-sensitivity/hypercortisolism or, *vice versa*, pituitary volume may contribute causally to increased stress-sensitivity. If the latter were true, it can be hypothesised that pre-existing individual differences in regional brain morphology partly increase vulnerability to (or decrease resiliency against) life stressors. These individual differences may emerge early in life, resulting from a combination of genetic and developmental influences (34).

Another proxy for HPA axis functioning may be the hippocampal volume as the hippocampus plays an essential role in regulating emotional responses to stressful stimuli and has an important role in the negative feedback mechanisms on the HPA axis (35-37). However, repeated heightened cortisol levels can decrease the activity of the negative feedback mechanism from the hippocampus on the hypothalamus (38), which may result in a sensitised state of HPA axis hyperactivity (Figure 1B). In humans, hippocampal atrophy is cross-sectionally accompanied by hyperactivity of the HPA axis and elevated levels of cortisol (39). Furthermore, reduced hippocampal size has been well established in patients with a psychotic disorder (40-43), and recent studies have shown that similar brain abnormalities in hippocampal volume reduction are present in first-degree relatives of patients with psychotic disorder (44, 45), suggesting that hippocampal volume changes may constitute part of the liability for psychosis. However, the association of hippocampal volume with biological and emotional real life stress-reactivity has never been examined. In **chapter 5**, it was hypothesised that increased stress-reactivity could be associated with lower hippocampal volume and that this association would be moderated by the genetic risk for psychotic disorder. Results indicated that patients with smaller hippocampal volume had a greater increase in negative affect in reaction to stress, which was not found in siblings. With regard to biological stress-reactivity, siblings with reduced hippocampal volume exhibited increased cortisol reactivity. This discrepancy between the emotional and biological stress-reactivity pattern in siblings might suggest that, even with an increased biological reactivity, siblings manage to cope better with stress and/or have higher resilience and, consequently, display a

relatively healthy emotional stress-reactivity. Although no definite conclusion can be drawn on the direction of this association using a cross-sectional study, studies in mice have shown that repeated exposure to stress and associated glucocorticoid excess can alter hippocampal neurons or induce changes in the gross morphology of the hippocampus (46). It could be hypothesised that repeated stress can alter hippocampal morphology, which, in turn, may lead to a malfunctioning negative feedback mechanism resulting in HPA axis hyperfunctioning

In short, lower hippocampal volume was associated with increased emotional reactivity in patients and increased cortisol reactivity in siblings, suggestive of a genetic influence. The lack of emotional reactivity in siblings may be a true effect, thus reflecting better coping styles or higher resilience, or it may be a false negative finding.

The studies described in **chapters 4 and 5** provide further evidence for a malfunctioning of the HPA axis in patients with psychotic disorder assuming hippocampal volume, pituitary volume and elevated cortisol levels can be considered proxy measures for HPA axis functioning. Integrating the results from both chapters, that is higher pituitary volume and lower hippocampal volume associated with increased stress-reactivity at the behavioural level in patients with psychotic disorder, it may be concluded that the neural diathesis-stress model of stress-induced HPA axis hyperactivity (32, 38)(Figure 1B) can also be applied to daily life stressors. A comparable observation in depression is that HPA axis hyperactivity is thought to reflect a lack of negative inhibitory feedback by circulating cortisol on the HPA axis, especially at the level where pituitary cells produce ACTH, leading to an increase in the size and number of these cells (47). In siblings, increased stress-reactivity (at the endocrinological level) was associated with lower hippocampal volumes. This finding was consistent with the results on the behavioural level (emotional stress-reactivity) in patients. However, with regard to pituitary volume, there was no interaction between group and pituitary volume on cortisol reactivity. The only evidence for HPA axis hyperactivity was higher baseline cortisol levels in siblings than in controls. It cannot, therefore, be concluded at this stage whether emotional stress-reactivity patterns in siblings and patients are associated and qualitatively different from controls or dissociated from each other. In both studies, however, there was evidence for certain biological differences in siblings that may fit the altered (emotional) stress-reactivity pattern in patients, indicating genetically

influenced HPA axis hyperactivity in psychotic disorder.

One could speculate in two ways about the direction of the effect: do smaller hippocampal volume and, consequently, a disruption of the negative feedback lead to increased pituitary volume or does larger pituitary volume lead to an excess of cortisol, causing a toxic effect on the hippocampus, reflected by smaller volumes? Although the start of the sensitisation process might not be clear, both brain structures are part of a functional brain circuitry, likely affecting each other. Evidence suggests that alterations in both structures are present before the onset of the disease (31, 48), but detailed information about the timing of these structural alterations during development is lacking. There are studies, however, suggesting that the morphology of the two structures may change over time. For example, FEP patients have shown increased pituitary volumes whereas patients with a chronic psychotic disorder have shown decreased pituitary volume (29). Similarly, hippocampal volumes may change over time, (49) and hippocampal deficits may be reversed after antidepressant therapy (50), suggesting that various processes influence the plasticity of brain structures over time.

Taken together, the studies described in **chapters 4 and 5** indicate that volumetric alterations in brain structures involved in HPA axis functioning are associated with increased stress-reactivity in patients with psychotic disorder and, to a certain extent, in siblings of these patients. The association between altered brain structures involved in the HPA axis and increased reactivity to stress extends the neural diathesis-stress model of psychotic disorder to small daily life stressors.

Figure 1. Exposure to stress activates the HPA axis through the release of corticotropin releasing hormone (CRH) in the hypothalamus, resulting in secretion of adrenocorticotrophic hormone (ACTH) in the pituitary, stimulating the release of cortisol in the adrenal gland. Cortisol, through a negative feedback mechanism (via the hippocampus, hypothalamus and pituitary) regulates HPA axis activity (A). One theory could be that persistently elevated cortisol levels associated with increased pituitary volume, may lead to smaller hippocampi and consequently to a diminished negative feedback, resulting in a sensitised state of HPA axis hyperactivity (B).

The studies in this thesis provide evidence for associations between brain alterations and environmental risk factors such as childhood trauma, cannabis use, and daily life stress. This relationship could inherently result in positive psychotic symptoms through a common mechanism, that is, dopamine sensitisation (1, 51-53). Bearing in mind that an oversimplification is made of very complex mechanisms, such as psychosis, in which several neurotransmitter systems are involved, dopamine dysregulation may be a final step in a complex developmental cascade that starts early in life and ends with the onset of full-blown psychotic disorder at the adolescent or adult age (Figure 2). Taking cannabis use as an example, this exposure may ultimately impact dopamine sensitisation. In this thesis, cannabis-associated regional cortical thinning was found in individuals at high compared to low genetic risk for psychotic disorder with preliminary evidence for alterations in the frontal pole, entorhinal cortex and parahippocampal gyrus. It could be hypothesised that genetically determined frontal sensitivity to cannabis may affect the stress-buffering system, implying less top-down control, with subsequently increased subcortical dopamine levels. This, in combination with a direct effect of delta-9-tetrahydrocannabinol (δ -9-THC), the active ingredient of cannabis, on dopamine transmission in the ventral tegmental area (VTA) and striatum (54), may eventually induce dopamine sensitisation from which psychotic symptoms arise. In addition, hippocampal alterations may be at the origin of striatal dopamine alterations (55, 56). Recent animal studies have suggested that altered activity of the ventral hippocampus may induce an increase in the number of spontaneously firing dopamine neurons in the VTA, which, in turn, may lead to pathological high levels of dopamine release in the limbic striatum (57). To date, it remains unclear whether the cannabis-associated alterations in cortical thickness reflect neuronal or glial loss, reduction in synaptic density or a change in cell size, all of which have been reported in rodent studies investigating the hippocampus (58, 59). In addition, glutamatergic effects of δ -9-THC may affect grey matter volume and, possibly, cortical thickness through a mechanism of neurotoxicity (60, 61).

The pathway from stress to psychotic symptoms may also involve dopamine sensitisation. As mentioned before, altered frontal cortical alterations may affect the stress-buffering system, resulting in increased dopamine release in the limbic striatum. (62-64). In line with this hypothesis, it has been reported that patients with

psychotic disorder show higher dopamine metabolite increases during metabolic stress than controls, and this dopamine metabolite increase was associated with lower (prefrontal) grey matter volume (65-67). In a recent PET study, subjects at increased psychometric risk for psychotic disorder had increased dopamine release compared to controls following a social stress task (68). Not only has current psychological stress been found to be associated with increased striatal dopamine release, but adverse early life experiences may moderate this association (69). Developmental trauma or other types of (chronic) adversity may alter HPA axis functioning (70) through hypersensitive glucocorticoid release or abnormalities in glucocorticoid receptors (47). In addition, studies in this thesis suggested that proxy measures for HPA axis hyperfunctioning (higher pituitary and lower hippocampal volume) are associated with increased reactivity to daily life stress in patients with psychotic disorder. As there is evidence for a synergistic relation between the activation of the HPA axis and the activation of dopaminergic circuits, increased glucocorticoid secretion may lead to increased dopamine activity in certain brain regions such as the ventral striatum and amygdala (64). In addition, some brain regions, such as the hippocampus or the prefrontal lobe, may be particularly sensitive to stressors (46, 64, 71), possibly aggravating the effects by inducing dopamine sensitisation. In summary, although the exact mechanisms need to be elucidated, dopamine release in the limbic system can be increased by stress and cannabis exposure (72). Repeated exposure to these environmental factors may lead to prolonged changes in the functioning of the dopamine system and, eventually, to dopamine sensitisation (73). As subcortical dopamine systems are involved in incentive or motivational salience and reward prediction (74-76), dopamine sensitisation may lead to an aberrant assignment of salience to innocent stimuli (75, 77, 78). Delusions and hallucinations could consequently arise from an individual's response to erroneous attribution of salience to external and internal stimuli (79).

Figure 2 Pathways towards psychotic disorder

THE PROBLEM OF HETEROGENEITY

A significant amount of structural neuroimaging studies investigating brain alterations in psychotic disorder have become available over the last decade. This literature suggests that brain alterations related to psychotic disorder are smaller and more subtle than researchers originally believed would be the case. Furthermore, no specific brain alteration has been found to be pathognomonic for psychotic disorder. For instance, ventricle enlargement, which is reported in approximately 80% of the studies, is also observed in Alzheimer's disease (80) and bipolar disorder (81). Many, if not all, brain structures have been described in relation to schizophrenia. Reviews and meta-analyses provided evidence for decreased volume in the hippocampus

in psychotic disorder, together with moderate evidence for frontal and temporal reductions (9, 41, 42, 82-85). The lack of consistency in 'group differences' in cortical and subcortical phenotypes among individual studies is likely the result of great variety in structural imaging processing and analysing techniques, available in a wide range of different software packages, applied to a highly heterogeneous phenotype. Thus, there not only is much heterogeneity in imaging methods but also there exists great variance in the clinical expression of psychotic disorder. Psychotic disorder is, according to the DSM-IV classification system, a categorical defined set of symptoms, suggesting qualitative differences between an ill and healthy individual. However, an extensive amount of epidemiological data have shown that (subclinical) psychotic symptoms are also present in the general population and that there is not only phenomenological continuity but also etiologic continuity (86). In other words, the factors associated with these minor psychotic symptoms are the same as those associated with the risk for psychotic disorder, that is, male, poor education, ethnic minority, and cannabis use (87). Therefore, suggestions have been made to drop the syndrome-oriented categorical representation and replace it with a symptom-oriented continuum representation of psychotic disorder (86, 88). Measuring differences in cortical thickness or other brain measurements between illness groups based on a dimensional classification rather than the classical categorical approach may increase consistency between results and may facilitate detection of etiologic overlap between disorders. To date, an interesting outcome of GWAS data analyses is that there appears to be a considerable genetic overlap between schizophrenia and bipolar disorder, consistent with the idea that the two disorders exist on a clinical continuum with overlapping symptom dimensions (89). Similarly, genes such as DISC1(90), NRG1(91) and ANK3(92) are associated with both psychotic disorder and bipolar disorder. A comparable genetic overlap may also exist with autism spectrum disorders, for which deletions in the neurexin 1 gene have been associated with both autism and psychotic disorder (93). The demonstration of shared structural deficits in psychotic disorder and bipolar disorder has provided further support to this etiologic overlap between two, as yet categorically, defined illnesses (81, 94, 95).

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Using structural MRI measurements as a diagnostic tool or clinical guidance is still a future perspective. Structural brain images lack specificity although considerable predictive power has been reported for subgroups (96). Despite the absence of (as yet) clinical applications of MRI scanning, investigating gene-environment relationships associated with brain phenotypes may help to further elucidate the aetiology of psychotic disorder and lead to the development of more targeted treatments to avert relapse. For example, as daily life stress has been associated with HPA axis dysfunction, the potential for drugs acting upon glucocorticoid systems (e.g., CRH antagonists) and thereby intervening at a more upstream level in the brain than at the traditional postsynaptic dopamine receptor level could become a target of further investigation.

Siblings of patients with psychotic disorder showed evidence of higher cortical thickness values when exposed to trauma during childhood than siblings not exposed to childhood trauma. Furthermore, it was determined that siblings of patients do show increased biological response to stressors whereas this increased stress response was not apparent at the behavioural level. These differences between patients and siblings may reflect the importance of protective factors resulting from different coping and/or attributional styles, increasing their resilience. Therefore, delivering psychoeducation for patients and individuals at a higher than average genetic risk for psychotic disorder and focusing on different coping strategies in dealing with stress and stress-related symptoms are essential strategies in preventing relapse or transition to psychosis.

Furthermore, replications on brain alterations deriving from differential sensitivity to certain environmental factors, such as cannabis use, are urgently needed as this knowledge may further guide early intervention and prevention programs. This may include the development of awareness campaigns and psycho-education for young people at risk for psychotic disorder who are or who are not (yet) in contact with mental health services. Moreover, as a result of better insight into the pathways to psychotic disorder, stigma and self-stigma can be potentially reduced.

With the above mentioned potential implications in mind, future research on brain alterations and mechanisms in psychosis should make use of symptom dimensions besides categorical illness phenotypes and further elucidate differential sensitivities to environmental factors as these areas, to date, are far from being explored. New

multivariate methods can be used to investigate the association between complex brain phenotypes and a large number of single nucleotide polymorphisms (SNPs)(97). Investigating SNPs of COMT, AKT1 and FKBP5 are interesting because of their relationship with environmental risk factors such as cannabis use and stress (18, 25).

In summary, although it will be a difficult task, integrating genetic and environmental data with brain imaging data, and thereby using relevant dimensional symptom profiles instead of traditional diagnostic constructs, is the challenge and could be the future for personalised medicine in terms of tailor-made treatments and prevention strategies.

CONCLUDING REMARKS

This thesis examined potential genetic and environmental determinants of brain alterations in psychotic disorder. It was found that regional cortical thickness alterations may be an indicator of genetic risk for psychotic disorder. Furthermore, cannabis use was associated with cortical thinning in patients with psychotic disorder and their siblings, suggestive of underlying gene-environment interactions associated with psychotic disorder. With regard to trauma, increased levels of childhood trauma were associated with decreased cortical thinning in patients, but not in siblings and controls, possibly reflecting specific disease-related processes. In addition, volumetric alterations of the pituitary and hippocampus, structures that are involved in the HPA axis stress response were associated with stress-reactivity in patients with psychotic disorder and, to a certain extent, in their siblings.

The findings have deepened the relationship between neural substrates and various environmental factors in individuals at high genetic risk for psychotic disorder. It was shown that combining research fields such as neuroimaging and genetic epidemiology is feasible and that neuroimaging phenotypes play an important role in identifying gene-environment relationships in psychotic disorders.

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Summary

The field of genetic epidemiology and neuroimaging has mainly consisted of disparate research fields. In other words, the integration of findings from epidemiologic and neuroimaging studies on psychotic disorder has not received much attention to date. As both genetic and environmental factors are assumed to play a role in the development of certain brain alterations that may contribute to the risk of developing a psychotic disorder, this thesis *“The role of neuroimaging phenotypes in ecogenetic studies of psychotic disorder”*, attempted to combine both fields. Genetic risk levels, environmental factors and their interactions were analysed in relation to different brain phenotypes. The study samples used in this thesis consisted of patients with psychotic disorder (highest genetic risk group), siblings of patients with psychotic disorder (higher than average genetic risk group), and controls (average genetic risk group).

In **chapter 2**, cortical thickness was investigated in relation to genetic risk for psychotic disorder. The goal of this study was to identify biological markers of genetic risk such as intermediate phenotypes. Previous studies have provided some evidence for a cortical thickness intermediate phenotype whereas more extensive and more recent studies have failed to confirm this. The study described in **chapter 2** indicated that patients and their siblings had cortical thickness reductions in overlapping areas, in the frontal, temporal, parietal and occipital regions combined with patient-specific alterations and some sibling-specific alterations. After controlling for cannabis use, the number of significant clusters was reduced in siblings and patients, but most shared cortical thickness alterations between siblings and patients remained significant. In summary, the results suggest that a cortical thickness intermediate phenotype may exist in psychotic disorder. In addition, we concluded that some of the cortical changes may be influenced by environmental factors such as cannabis use.

In **chapter 3**, the relationship between cortical thickness and two environmental risk factors, cannabis use and childhood trauma, was investigated using the same genetically sensitive sample. The aetiology of schizophrenia is thought to involve differential – likely genetically mediated – sensitivity to environmental exposures. However, examination of differential sensitivity in models of psychopathological constructs is subject to bias as psychopathology itself may distort exposure assessment. The use of neuroimaging phenotypes may provide unbiased evidence

for differential sensitivity to environmental exposures. In **chapter 3**, an association was found between reduced cortical thickness and higher levels of cannabis use in patients with psychotic disorder and their siblings, but not in controls, which extends earlier findings indicating that gene-environment interactions may underlie the association between cannabis use and psychotic disorder. Patients who reported higher levels of childhood trauma also showed cortical thinning, which was not found in controls or siblings with a history of childhood trauma. Siblings and controls may differ from patients in that the effect of exposure to traumatic experiences may be neutral (controls) or even trophic (siblings). To the degree that the effects of trauma on the brain are mediated by long-term psychological adaptation, resilience and coping may moderate the outcome of traumatic experiences over time, possibly mitigating the risk for later psychotic outcomes.

In **chapters 4** and **5**, we examined whether volumes of specific brain structures related to HPA axis functioning and stress-reactivity were associated and whether this association was influenced by the genetic risk for psychotic disorder. The pituitary and the hippocampus are involved in regulating the stress response in the HPA axis. Exposure to stress activates the HPA axis through the release of CRH in the hypothalamus, resulting in the secretion of the ACTH in the pituitary, thus stimulating the release of cortisol in the adrenal gland. Cortisol, through a negative feedback mechanism, regulates HPA axis activity. Persistently elevated cortisol levels can decrease the activity of the negative feedback mechanism, resulting in a sensitised state of HPA axis hyperactivity. As a result of HPA axis hyperactivity, patients become more sensitive to stressors, a phenomenon called stress-sensitisation. Stress-sensitisation has been hypothesised to be the underlying process related to increased stress-reactivity in individuals with (increased risk for) psychotic disorder. However, whether stress-reactivity is related to structural brain volumes associated with HPA axis functioning (i.e., pituitary and hippocampal volume) has never been examined. In **chapter 4**, pituitary volume was used as an index for HPA axis functioning. It was shown that an enlarged pituitary volume was associated with increased negative emotions as a response to daily life stressors in patients with psychotic disorder, their siblings and controls. This association was significantly stronger in patients when compared to controls, indicating a process of progressive sensitisation resulting in a clinical phenotype. Although baseline cortisol measures were higher in siblings than controls, biological stress-reactivity in siblings did not

differ from controls nor was there an interaction between group status and pituitary volume in the model of biological stress-reactivity. However, power issues may have led to type II errors in the sibling analyses. The study described in **chapter 5** used hippocampal volume as a proxy for HPA axis functioning, and it was found that lower hippocampal volume was associated with increased emotional reactivity in patients but not in siblings and controls. Siblings did show that lower hippocampal volume was associated with increased cortisol reactivity. This discrepancy between the emotional and biological stress-reactivity pattern might suggest that, even with an increased biological reactivity, siblings manage to cope better with stress or have higher resilience and, consequently, demonstrate a relatively healthy emotional stress-reactivity.

In **chapter 6**, the results of the different chapters were discussed and integrated into a wider context. The studies in this thesis provided evidence for associations between brain alterations in individuals at high genetic risk for psychotic disorder and environmental risk factors such as cannabis use, childhood trauma and daily life stress. Although the exact mechanisms need to be elucidated, it is attractive to speculate that differential sensitivity to the environment may be expressed in structural brain alterations impacting dopamine pathways and resulting in the emergence of psychotic symptoms. We have concluded that the neuroimaging phenotype is an important variable in identifying gene-environment relationships in schizophrenia, and this new paradigm is likely to lead to future clinical valorisation of which examples have been provided.

Samenvatting

Genetische epidemiologie en neuroimaging zijn twee afzonderlijke onderzoeksgebieden en de integratie van de bevindingen van beide onderzoeksvelden op het gebied van psychotische stoornissen heeft tot op heden weinig aandacht gekregen. Omdat zowel genetische als omgevingsfactoren een rol spelen in de ontwikkeling van bepaalde veranderingen in de hersenen die geassocieerd zijn met een verhoogd risico voor het ontwikkelen van een psychotische stoornis zijn in dit proefschrift *"The role of neuroimaging phenotypes in ecogenetic studies of psychotic disorder"*, de twee bovengenoemde onderzoeksgebieden gecombineerd. Verschillen in genetisch risico, omgevingsfactoren en hun interacties werden onderzocht in relatie tot verschillende brein fenotypes. Er werden drie groepen onderzocht verschillend in mate van genetische kwetsbaarheid: een groep patiënten met een psychotische stoornis (hoogste genetische kwetsbaarheid), een groep broers en/of zussen van patiënten met een psychotische stoornis (hoger dan gemiddeld genetische kwetsbaarheid) en een groep gezonde controles (gemiddelde genetische kwetsbaarheid).

In **hoofdstuk 2** werden regionale verschillen in corticale dikte in relatie tot het genetisch risico voor psychotische stoornis onderzocht. Het doel van deze studie was om biologische markers voor genetisch risico, zoals intermediaire fenotypes, te identificeren. Eerdere studies hebben bewijs geleverd voor een corticaal intermediair fenotype, maar er zijn ook enkele grote, recente studies die het bestaan van een corticale dikte fenotype niet kunnen ondersteunen. De studie beschreven in hoofdstuk 2 vond dat patiënten en hun familieleden corticale veranderingen vertoonden in frontale, temporale, parietale en occipitale regio's van het brein. Daarnaast werden corticale dikte veranderingen gevonden die specifiek waren voor de patiënten alsmede enkele veranderingen die specifiek waren voor de familieleden. Na controle voor cannabisgebruik, verminderde het aantal significante clusters, maar de meeste gedeelte brein veranderingen bleven tussen patiënten en familieleden echter bestaan. Deze resultaten suggereren dat corticale dikte een potentiële intermediair fenotype is en dat een gedeelte van de corticale dikte veranderingen mogelijk beïnvloed wordt door omgevingsfactoren zoals cannabisgebruik.

In **hoofdstuk 3** werd de relatie tussen corticale dikte en twee omgevingsfactoren, cannabisgebruik en trauma in de kindertijd, onderzocht gebruik makende

van hetzelfde familiestudie design. De etiologie van psychotische stoornis is gerelateerd aan differentiële -waarschijnlijk genetisch gemedieerde- gevoeligheid voor omgevingsfactoren. Onderzoek naar deze differentiële gevoeligheid in psychopathologische populaties is echter aan bias onderworpen, omdat de psychopathologie zelf de uitkomstmaten kan verstoren. Door gebruik te maken van een MRI fenotype als uitkomstmaat, kan er mogelijk onvertekend bewijs voor differentiële gevoeligheid voor omgevingsfactoren gevonden worden. In hoofdstuk 3 werd een associatie gevonden tussen een dunnere cortex en een hogere mate van cannabisgebruik bij zowel patiënten met psychotische stoornis als hun familieleden. Dit geeft additioneel bewijs voor de al eerder gevonden relatie tussen cannabis en kwetsbaarheid voor psychotische stoornis. Patiënten die veel trauma in de kindertijd rapporteren vertoonden ook een lagere corticale dikte ten opzichte van hun gezonde broers/zussen en controlepersonen met vergelijkbare traumatische ervaringen. Het is mogelijk dat familieleden en controles verschillen van patiënten op zo een manier dat het effect van blootstelling aan traumatische ervaringen neutraal kan zijn (controles) of zelfs trofisch (familieleden). Er vanuit gaande dat de effecten van trauma op het brein gemedieerd worden door psychologische adaptatie op de lange termijn, zijn veerkracht en coping mogelijke mechanismen die de consequenties van traumatische ervaringen over de tijd kunnen beïnvloeden, door het risico op een psychotische stoornis te verminderen.

In **hoofdstuk 4 en 5** werd onderzocht of de volumes van specifieke hersenstructuren die gerelateerd zijn aan het functioneren van de HPA as geassocieerd waren met stressreactiviteit en of deze relatie beïnvloed werd door verschillen in genetische kwetsbaarheid voor psychotische stoornis. De hypofyse en hippocampus zijn betrokken bij de stressreactie van de HPA as. Blootstelling aan stress activeert de HPA as: de hypothalamus produceert CRH. Dit stimuleert de hypofyse tot de aanmaak van ACTH. ACTH stimuleert daarna de bijnier tot de aanmaak van cortisol. Cortisol remt vervolgens de hypothalamus en de hypofyse in de aanmaak van CRH en ACTH en zorgt zo voor een negatieve feedback op de HPA as. Continue verhoogde cortisolwaarden kan de activiteit van het negatieve feedback systeem verminderen, wat resulteert in een hyperactieve HPA as. Hierdoor kan het zijn dat patiënten gevoeliger zijn voor stressoren, ook wel stress-sensitisatie genoemd. Stress-sensitisatie wordt gezien als het onderliggende mechanisme gerelateerd aan de verhoogde stress-activiteit gezien in individuen met (verhoogd risico op) psychotische stoornis. Het is nog niet

onderzocht of stress-reactiviteit geassocieerd is met de hersenstructuren (hypofyse en hippocampus) van de HPA as. De studie beschreven in **hoofdstuk 4** gebruikte hypofyse volume als een proxy voor het functioneren van de HPA as. De studie toonde aan dat verhoogde hypofysevolumes geassocieerd zijn met verhoogde stress-reactiviteit in patiënten met psychotische stoornis, hun gezonde broers en zussen en controles. Deze associatie was significant sterker bij de patiënten, wat een indicatie is voor een proces van progressieve sensitatisatie resulterend in het klinisch fenotype. Ondanks het feit dat baseline cortisolwaarden hoger lagen voor de siblings dan voor de controles, was er geen verschil in biologische stress-reactiviteit tussen siblings en controles. Ook was er geen interactie tussen groep status en hypofyse volume in het model van biologische stress-reactiviteit. Het kan zijn dat power problemen geresulteerd hebben tot type II fouten in de analyse van de siblings. De studie beschreven in **hoofdstuk 5** gebruikte hippocampus volume als een proxy voor HPA functioneren en vond dat een lager hippocampusvolume geassocieerd was met verhoogde emotionele reactiviteit in patiënten maar niet in siblings en controles. Siblings hadden wel een hogere cortisol reactiviteit die geassocieerd was met lagere hippocampus volumes. Deze discrepantie tussen emotionele en biologische stress-reactiviteit patronen kunnen wijzen op het feit dat zelfs met een verhoogde biologische stress-reactiviteit, siblings beter omgaan met stress of een betere weerstand hebben tegen stress en dientengevolge een relatieve ‘gezonde’ emotionele stress-reactiviteit vertonen.

In **hoofdstuk 6** werden de resultaten van de verschillende hoofdstukken besproken en geïntegreerd in een bredere context. De studies in deze thesis hebben bewijs geleverd voor associaties tussen veranderingen in het brein in individuen met een hoog genetisch risico voor psychotische stoornis en omgevingsfactoren zoals cannabisgebruik, trauma in de kindertijd en dagelijkse stress. Ondanks het feit dat de exacte onderliggende mechanismen nog niet bekend zijn, is het aantrekkelijk om te speculeren dat verschillen in gevoeligheid voor de omgeving zich uiten in structurele veranderingen in het brein die invloed hebben op de transmissie van dopamine, met het ontwikkelen van psychotische symptomen tot gevolg. We hebben geconcludeerd dat het neuroimaging fenotype een belangrijke rol speelt bij het identificeren van gen-omgevings interacties in schizofrenie en dat dit nieuwe paradigma kansen biedt voor toekomstige klinische valorisatie, waarvan enkele voorbeelden werden gegeven.

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Curriculum Vitae

Petra An Marietje Habets werd op 10 mei 1981 geboren in Maaseik, België. Na voltooiën van het hoger secundair onderwijs aan het Koninklijk Atheneum in Maaseik in 2000, behaalde ze in 2005 haar diploma van Doctorandus in de psychologie, specialisatie neuropsychologie aan de Universiteit van Maastricht. Van 2004 tot 2005 was ze werkzaam als onderzoeksassistent bij de universiteit van Maastricht, eerst bij de afdeling Cognitive & Clinical Neuroscience daarna bij de afdeling Forensic Psychology. Van september 2005 tot maart 2011 heeft zij als assistent in opleiding het in dit proefschrift beschreven onderzoek uitgevoerd aan de School for Mental Health and Neuroscience afdeling Mental Health onder leiding van prof. Jim van Os en dr. Machteld Marcelis. Tijdens haar AiO-periode verbleef ze een tijdje in Cambridge, waar ze als onderzoeker verbonden was aan de University of Cambridge. Tevens werkte zij als case manager binnen een Assertive Community Treatment team van Psycope, een transmuraal zorgprogramma voor mensen met een psychotische aandoening. Sinds 1 juni 2011 is ze aangesteld als post-doctoraal onderzoeker bij de afdeling Mental Health van de School for Mental Health and Neuroscience.

Petra is getrouwd met Roberto Romano en samen hebben ze een dochter Nore.

List of publications

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