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ISBN 978-90-393-6412-3

Printed by: Proefschrift-AIO
Cover photo: Dim Balsem
Cover design: Proefschrift-AIO

Family Matters: imaging the vulnerability for schizophrenia

Familiebanden: beeldvorming van de kwetsbaarheid voor schizofrenie

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 1 oktober 2015 des middags te 4.15 uur

door

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geboren op 4 maart 1983 te Amsterdam

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Chapter 1

Introduction

The research presented in this thesis centers on family matters to investigate if family matters. That is, how are family members of schizophrenia patients affected.

Background

Schizophrenia is a devastating psychiatric disorder characterized by positive symptoms such as delusions and hallucinations, negative symptoms including affective flattening and social withdrawal, as well as cognitive impairments (American Psychiatric Association, 2000). Underlying these symptoms may be dysfunctions in the frontal lobe and the striatum: dopamine hyperactivity in the striatum and dopamine hypoactivity in the frontal cortex (Davis et al., 1991). Striatal hyperactivation is linked to psychosis. Indeed, treatment of schizophrenia consists of the administration of antipsychotics, which work by blocking increased dopamine activity in the striatum (Seeman and Lee, 1975). In addition, frontal hypoactivation is associated with negative symptoms (Davis et al., 1991). The frontal cortex and the striatum together form the so-called fronto-striatal network (*Figure 1*). Besides the clinical symptoms, impairments in this network are also thought to underlie the cognitive deficits characteristic of schizophrenia (Pantelis et al., 1997).

Schizophrenia is a highly heritable psychiatric disorder (Gottesman and Shields, 1973). More than 40% of monozygotic twins of those with schizophrenia are also affected (Picchioni and Murray, 2007). About 13% of the children is affected if one parent has schizophrenia whereas this risk increases to about 50% if both parents are affected. Finally, siblings of patients have a tenfold risk to develop schizophrenia (Gejman et al., 2011). Although these relatives are not (yet) ill, they do show cognitive impairments intermediate between patients and controls (Barrantes-Vidal et al., 2007; Brahmbhatt et al., 2006; Chen et al., 2009; Delawalla et al., 2008; Snitz et al., 2006). This may very well indicate fronto-striatal network dysfunction in these relatives in the absence of the illness.

Therefore, the aim of this thesis is to present data on fronto-striatal network function in first-degree relatives. Specifically, unaffected siblings as well as offspring of schizophrenia patients will be investigated. Siblings share on average 50% of their genes with their ill relative (Gottesman and Gould, 2003; Meyer-Lindenberg and Weinberger, 2006), allowing the investigating of genetic vulnerability for schizophrenia in adult state (Part I). Furthermore, the association between fronto-striatal function and genetic predisposition for schizophrenia will be investigated in siblings (Part II). Finally, adolescent

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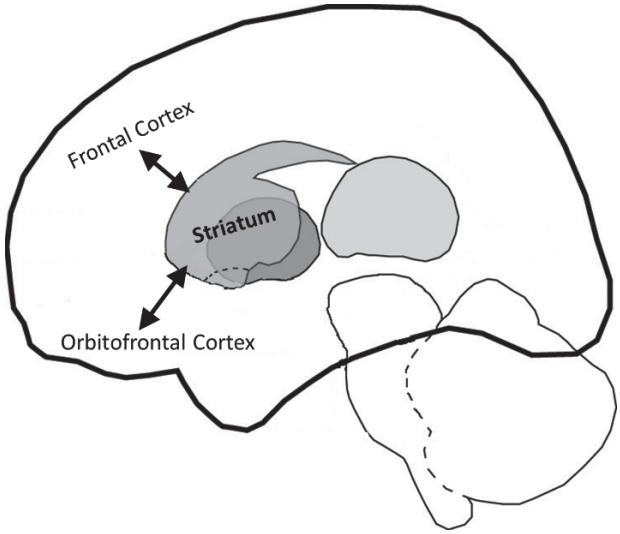

Schematic representation of the fronto-striatal circuits.

offspring of patients will be tested, thereby unravelling the impact of genetic vulnerability on fronto-striatal network development (Part III). Before discussing studies and their background, a brief overview of functional Magnetic Resonance Imaging as well as Diffusion Tensor Imaging is provided.

Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) allows the visualization of task related brain activation. It was first developed in the early 1990's (Belliveau et al., 1991; Ogawa et al., 1992) and has since become one of the main techniques to map activity of the brain. The most commonly used fMRI technique is called BOLD-fMRI. It uses the so called 'Blood Oxygen Level-Dependent' (BOLD)-contrast (Ogawa and Lee, 1990). It does not directly measure neuronal activation, but maps the hemodynamic response (change in relative oxygenation in blood flow) related to neural activity in the brain. Increased neural activity causes an increased demand for oxygen, which is provided via an increase in cerebral blood flow. The vascular system actually overcompensates for the decrease in oxygen, delivering an oversupply for oxygenated blood (Fox and Raichle, 1986), increasing the amount of oxygenated hemoglobin relative to deoxygenated hemoglobin. Deoxy- and oxyhemoglobin have different magnetic properties; deoxyhemoglobin is paramagnetic and introduces an inhomogeneity into the nearby magnetic field

(and lowers the measured fMRI signal), whereas oxyhemoglobin is diamagneticly weaker and has little effect. The changing concentrations of oxyhemoglobin vs. deoxyhemoglobin constitute the basis of the BOLD effect (Kwong et al., 1992; Ogawa et al., 1992).

Diffusion tensor imaging

Diffusion tensor imaging (DTI) is a magnetic resonance imaging technique that probes the diffusion profile of water molecules in brain tissue to obtain information about its microstructure. It was developed in 1990 to map out the orientation of the white matter tracks in the brain (Douek et al.). In white matter, water has a clear preferred diffusion direction as water diffuses much more easily in the direction along the axons then in the directions perpendicular to the axons. The fractional anisotropy (FA) is a scalar measure that reflects the level of preferred direction of a diffusion profile. It ranges between zero and one where a value of zero means that diffusion is isotropic, i.e. no preferred direction and a value of one means that diffusion occurs only in one direction and is fully restricted along all other directions. In white matter, differences in FA may reflect (amongst others) differences in axonal directionality, axonal density, axonal diameter, and level of myelination.

Fronto-striatal network dysfunction in schizophrenia

Functional MRI studies in schizophrenia patients have demonstrated abnormal fronto-striatal activity in the context of various cognitive tasks (Ehrlich et al., 2012; Koch et al., 2008; Quidé et al., 2013; Tu et al., 2006; van Veelen et al., 2011, 2010; Vink et al., 2006; Wolf et al., 2011; Zandbelt et al., 2011). First, fronto-striatal hypoactivation in schizophrenia patients is described during working memory (Karch et al., 2009; Koch et al., 2008; van Veelen et al., 2011, 2010). Specifically, patients displayed most pronounced hypoactivation during correct information retrieval (Koch et al., 2008), and increasing working memory load (Karch et al., 2009). Second, schizophrenia patients show fronto-striatal deficits during the processing of reward (Esslinger et al., 2012; Gradin et al., 2013; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; Nielsen et al., 2012; Schlagenhauf et al., 2009). Specifically, studies reported on blunted ventral striatum activation during reward anticipation (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; Nielsen et al., 2012; Schlagenhauf et al., 2009), as well as decreased activation in the orbitofrontal cortex during successful receipt of reward and increased activation during (unsuccessful) reward outcome (Schlagenhauf et al., 2009). Moreover, these impairments, in particular decreased ventral striatal activity, have been associated with symptom severity

in schizophrenia patients (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Nielsen et al., 2012; Simon et al., 2010). Third, schizophrenia patients fail to activate the fronto-striatal network during response inhibition (Vink et al., 2006; Zandbelt et al., 2011). Specifically, reduced proactive inhibition was associated with a failure to activate the right striatum and the right inferior frontal cortex.

In addition to functional measurements, structural MRI studies have revealed reductions in brain volume of the frontal cortex (Buchanan et al., 1998; Harms et al., 2010; Oertel-Knöchel et al., 2012) and striatum (Keshavan et al., 1998; Oertel-Knöchel et al., 2012) in schizophrenia patients.

Finally, DTI studies have reported on disrupted anatomical pathways connecting frontal and striatal regions in schizophrenia. Quan et al. (2013) reported reduced FA in the tract connecting the left inferior frontal gyrus with the striatum in schizophrenia patients compared to matched controls (Quan et al., 2013). Bracht et al. (2014) reported on increased probability indices forming part of a bundle of interest (PIBI) for the tract connecting the nucleus accumbens with the dorsolateral prefrontal cortex, suggesting reduced white-matter tract integrity (Bracht et al., 2014).

Unfortunately, although these results provide compelling evidence in support of the idea that schizophrenia is characterized by fronto-striatal dysfunctions, it is the antipsychotic medication aimed to alleviate this dysfunction that may confound neuroimaging results. Furthermore, and also directly related to the illness, another potential confounder is the fact that schizophrenia patients typically tend to perform poor on cognitive task used to engage the fronto-striatal network.

Part I studies in siblings: neuro-imaging

Fronto-striatal network dysfunction in schizophrenia siblings

Since schizophrenia has a genetic penetrance (Gottesman and Shields, 1973), fronto-striatal activations may also be affected in first-degree relatives. In this thesis, non-affected siblings are recruited for the investigation of fronto-striatal activation. These siblings do not have confounds that affect study results such as medication use (Davis et al., 2005; Ettinger et al., 2011). If siblings show abnormal fronto-striatal processing, this would provide evidence in support for a genetic vulnerability underlying this phenotypic abnormality.

Several studies investigated the fronto-striatal network in siblings, but found inconsistent results. For example during working memory (WM), fMRI studies have reported fronto-striatal hypoactivation (Meda et al., 2008), hyperactivation

(Brahmbhatt et al., 2006; Callicott et al., 2003; Delawalla et al., 2008; Whitfield-Gabrieli et al., 2009) and equal activation (Karch et al., 2009) compared to healthy controls. The main reasons for these inconsistencies are task design and poor WM performance (Brahmbhatt et al., 2006; Delawalla et al., 2008; Karch et al., 2009). Although these studies have corrected for the medication confound, the performance confound was not addressed. Indeed, siblings show behavioral WM deficits, intermediate between patients and controls (Barrantes-Vidal et al., 2007; Brahmbhatt et al., 2006; Chen et al., 2009; Delawalla et al., 2008; Snitz et al., 2006). In this thesis, WM performance will be matched across group in order to eliminate this potential confound.

In addition to WM, one recent study in first-degree relatives of schizophrenia patients reported on attenuated ventral striatal activity during reward anticipation (Grimm et al., 2014). However, relatives of several generations were used, including siblings, parents, and adolescent offspring, ranging in age between 18 and 50. Moreover, they only tested activation in the ventral striatum during reward anticipation. Finally, siblings showed a failure to activate the striatum during inhibitory control (Vink et al., 2006; Zandbelt et al., 2011). However, in contrast to schizophrenia patients, their frontal activation was relatively unaffected.

In addition to functional deficits, structural fronto-striatal abnormalities have been demonstrated in unaffected siblings of schizophrenia patients (Harms et al., 2010; Mamah et al., 2008; Oertel-Knöchel et al., 2012). However, in contrast to schizophrenia patients, tracts connecting the striatum and frontal cortex have never been investigated in siblings. Therefore, it remains unclear whether these fronto-striatal white matter tract dysfunctions are related to the illness itself or to a genetic vulnerability for the disorder.

Part II studies in siblings: imaging genetics

The genetic underpinnings of fronto-striatal network dysfunction in schizophrenia

The fact that siblings show deficits similar to those seen in patients suggest that disturbances in the fronto-striatal network are linked to a genetic predisposition. Since various lines of research have consistently implicated dopamine as the major neurotransmitter underlying these abnormalities, it is likely that genes involved in dopamine function may contribute to fronto-striatal activation abnormalities in schizophrenia. Indeed, a recent Genome-Wide Association Study (GWAS) based on the Psychiatric GWAS Consortium (PGC) data showed that the rs2514218 genetic polymorphism in the gene encoding dopamine D2 receptor (DRD2) is

strongly associated with schizophrenia (Ripke et al., 2014). Furthermore, DRD2 is particularly distributed in the striatum, the main subcortical input region for cortical afferents (Lerner and Kreitzer, 2011). Here, the impact of carrying this risk allele on fronto-striatal functioning will be investigated. As discussed above, schizophrenia patients show confounds on imaging results in terms of medication use. Therefore, this thesis will report on the impact of rs2514218 polymorphism in siblings who are medication-free.

Part III studies in offspring: neuro-imaging

Normal and dysfunctional fronto-striatal development

During adolescence, the brain goes through tremendous changes to prepare for the challenges of adulthood. Importantly, these changes occur at different rates (Eveline A Crone and Dahl, 2012): data from rodent studies show that subcortical structures such as the striatum develop earlier than the frontal cortex (Spear, 2011, 2000) resulting in an imbalance in the network during adolescence (Casey et al., 2008, 1997). Given the involvement of the network in important cognitive functions, this imbalance may cause typical adolescent behavior such as impulsivity and risk taking (Paus et al., 2008). As adolescence progresses, fronto-striatal (anatomical) connectivity is strengthened and the activity patterns of frontal cortex and striatum become balanced and integrated (Eveline A. Crone and Dahl, 2012; Somerville and Casey, 2010; Vink et al., 2014). This integration facilitates higher-order cognitive functioning and behavior characteristic of adulthood. Given that this network does not function properly in adult schizophrenia, suggest that development of the fronto-striatal network may be abnormal. In fact, the genetic vulnerability, probably in interaction with environmental factors, may be at the basis of an abnormal fronto-striatal brain development in offspring of schizophrenia patients (Paus et al., 2008) that precedes the overt manifestation of schizophrenia. Evidence in support of such a developmental hypothesis of schizophrenia comes primarily from neuropsychological studies showing deficits in cognition (Agnew-Blais and Seidman, 2013; De Herdt et al., 2013) and behavior (Keshavan et al., 2008) in at-risk adolescents prior to the clinical diagnosis. Moreover, adolescence is the time of onset of many psychiatric illnesses (Paus et al., 2008), such as schizophrenia (Rössler et al., 2005). Indeed, the first signs of the illness, such as prodromal symptoms and cognitive impairments, begin to surface around the age of 12 (Kessler et al., 2012). Since environmental factors also play an important role, offspring of schizophrenia patients are considered to be at increased familial risk.

Fronto-striatal network dysfunction in schizophrenia offspring

There have been three cross-sectional fMRI studies in schizophrenia offspring: two reporting frontal hypoactivation during working memory (Bakshi et al., 2011; Diwadkar et al., 2011a) and sustained attention (Diwadkar et al., 2011b) compared to controls (range 10-19 years), and one study reported on hyperactivation in both in the frontal cortex and the striatum during working memory (range 8-19 years) (Diwadkar et al., 2012).

Furthermore, three structural MRI studies in adolescent offspring of schizophrenia patients were performed, reporting on increased volumes of subcortical regions across age (Dougherty et al., 2012), reduced frontal surface volume (Prasad et al., 2010) and no volumetric differences in the frontal lobe (Sişmanlar et al., 2010). Finally, no data on structural connectivity between the frontal cortex and striatum is available since no DTI studies have been performed.

Aims and outline

The objective of the research presented in this thesis was threefold. First (Part I, Chapter 2, 3 and 4), it is investigated whether fronto-striatal functional and structural abnormalities, as shown in schizophrenia patients, are related to the genetic vulnerability for schizophrenia by examining non-affected siblings. Second (Part II, Chapter 5), the genetic underpinnings of abnormal fronto-striatal activation in siblings is examined. Third (Part III, Chapter 6 and 7), the impact of the genetic vulnerability on fronto-striatal network development in schizophrenia offspring is explored.

Part I Studies in siblings: neuro-imaging

Chapter 2 investigates working memory (WM) and default-mode network (DMN) brain activity using fMRI in 23 unaffected siblings of schizophrenia patients and 24 matched controls. Participants perform a modified version of the Sternberg working memory task (Sternberg, 1966). Working memory load is adjusted individually, so that despite WM deficits in siblings (Barrantes-Vidal et al., 2007; Brahmbhatt et al., 2006; Chen et al., 2009; Delawalla et al., 2008; Snitz et al., 2006) all participants perform at 90% accuracy. In addition to preventing performance confounds, this high level of performance will result in maximum recruitment of both the WM and DMN networks (Altamura et al., 2007; Anticevic et al., 2010). Furthermore, the Sternberg task allows the separate investigation of the three phases of WM, being temporary storage (encoding), manipulation (maintaining) and retrieval of information (Baddeley, 1992). This is important

because, for example, (DMN) abnormalities in schizophrenia patients have been found mainly during the encoding phase of WM (Anticevic et al., 2010). In **Chapter 3** brain activity is investigated using fMRI during reward processing in 27 unaffected siblings of schizophrenia patients and 29 matched healthy controls. All subjects performed a modified Monetary Incentive Delay task (Figee et al., 2011; Hoogendam et al., 2013; van Hell et al., 2010). Task performance is manipulated online so that all subjects won the same amount of money. Reward processing can be divided into at least two sub-processes: anticipation of reward and receipt of reward (Knutson et al., 2001). Activation during reward anticipation and receipt of reward is investigated in regions involved in processing of reward: the bilateral ventral striatum, dorsal striatum, insula, supplementary motor area and orbitofrontal cortex (Diekhof et al., 2012; Dillon et al., 2008; Hoogendam et al., 2013; Knutson et al., 2001, 2000; Martin-Soelch et al., 2001; Schmack et al., 2008). Moreover, since patients show associations between decreased ventral striatum activity and symptom severity (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Nielsen et al., 2012; Simon et al., 2010), activation of the ventral striatum in siblings is correlated with sub-clinical symptoms as measured with the Community Assessment of Psychic Experiences (Stefanis et al., 2002). In **Chapter 4** fractional anisotropy is examined in fronto-striatal pathways using DTI in 24 schizophrenia patients, 30 unaffected siblings and 58 healthy controls. FreeSurfer software (Fischl et al., 2004) is used to parcellate the gray matter regions used to trace the fiber bundles of interest. The frontal cortex is subdivided into three regions: dorsolateral prefrontal cortex (DLPFC), medial orbital frontal cortex (mOFC) and inferior frontal gyrus (IFG), all of which are consistently reported to be abnormal in schizophrenia patients and their siblings in functional (Nielsen et al., 2012; van Buuren et al., 2011; Zandbelt et al., 2011) as well as structural MRI studies (Byun et al., 2012; Harms et al., 2010). Neurons from these frontal regions project to the caudate nucleus, putamen, and nucleus accumbens separately, together forming the fronto-striatal network (Postuma and Dagher, 2006). Mean FA is then computed along averaged tracts starting in each of these striatal subregions directing to the frontal cortex regions.

Part II Studies in siblings: imaging genetics

The study in **Chapter 5** measures the impact of the DRD2 rs2514218 polymorphism on striatal activity using fMRI during response inhibition in 45 unaffected siblings of schizophrenia patients. Twenty-four siblings carry the schizophrenia-risk allele of the rs2514218 polymorphism encoding DRD2 and 21 matched unaffected siblings do not. All subjects perform a stop-signal anticipation task (Zandbelt & Vink, 2010). This task engages the striatum during both reactive

and proactive inhibitory control. Specifically, cues are presented to indicate the probability of having to inhibit a response.

Part III Studies in offspring: neuro-imaging

In **Chapter 6** the impact of familial risk on fronto-striatal functioning is investigated. Functional MRI data is obtained from 25 adolescent offspring of schizophrenia patients and 36 age-matched healthy controls (age 10-19 years). Subjects perform a modified version of the Monetary Incentive Delay task (Chapter 3; Hoogendam et al., 2013) which is optimized to analyze changes in brain activation related to the anticipation and receiving of reward separately. Activation in the ventral striatum and the orbitofrontal cortex are analyzed. In **Chapter 7** fronto-striatal tracts are explored by examining FA using DTI in 21 adolescent offspring of schizophrenia patients and 30 matched typically developing adolescents (age 10-18 years). First, the tract connecting the left nucleus accumbens and left DLPFC is examined since decreased mean FA is reported in both schizophrenia and siblings in this tract specifically (Chapter 4). Mean FA is compared between adolescent offspring of schizophrenia patients and healthy control adolescents, and investigated across age for both groups. Second, as in siblings (Chapter 4), mean FA is computed in the remaining fronto-striatal tracts: striatal subregions (caudate nucleus, putamen, and nucleus accumbens) directing to the frontal cortex regions (DLPFC, mOFC and IFG).

Chapter 8 provides a general discussion of the findings from Chapters 2 to 7.

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PART I

Studies in siblings: neuro-imaging

Chapter 2

Working Memory and Default Mode Network abnormalities in unaffected siblings of schizophrenia patients

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Based on
Schizophrenia Research, 2013 November; 150(2-3): 555-62

Abstract

Background: Impaired working memory (WM) is a hallmark of schizophrenia. In addition to classical WM regions such as the dorsolateral prefrontal cortex (DLPFC) and the striatum, dysfunctions in the default-mode network (DMN) contribute to these WM deficits. Unaffected siblings of patients also show WM impairments. However, the nature of the functional deficits underlying these impairments is unclear, mainly because of impaired performance confounding neuroimaging results.

Methods: Here, we investigated WM and DMN activity in 23 unaffected siblings of schizophrenia patients and 24 healthy volunteers using fMRI and a Sternberg WM task. WM load was determined prior to scanning to ensure 90% accuracy for all subjects.

Results: Siblings showed hyperactivation during the encoding phase of WM in the right medial prefrontal cortex (MPFC) which is the anterior part of the DMN. No differences were found during the maintenance phase. During the retrieval phase, siblings showed hyperactivation in WM regions: DLPFC, inferior parietal cortex and the striatum. Siblings who showed hyperactivity in the MPFC during encoding showed DLPFC and striatum hyperactivation during retrieval.

Conclusions: Our finding of hyperactivation in WM and DMN areas indicates that siblings fail to adequately inhibit DMN activity during demanding cognitive tasks and subsequently hyperactivate WM areas. This failure may reflect dopamine hyperactivity in the striatum which prevents adequate DMN suppression needed for effective WM. This study provides support for the notion that aberrant WM and DMN activation patterns may represent candidate endophenotypes for schizophrenia.

Introduction

Working memory (WM) deficits are at the core of cognitive impairments of schizophrenia and remain relatively stable despite fluctuations in symptoms (Weinberger and Gallhofer, 1997). There have been many studies in schizophrenia reporting robust WM-deficits in terms of poor performance (Hahn et al., 2012; van Veelen et al., 2011b, 2010; Weinberger and Gallhofer, 1997). Although the unaffected siblings of schizophrenia patients do not have symptoms to the same extent as their affected relative, they do show behavioral WM deficits, intermediate between patients and controls (Barrantes-Vidal et al., 2007; Brahmbhatt et al., 2006; Chen et al., 2009; Delawalla et al., 2008; Snitz et al., 2006). Since siblings share on average 50% of their genes with their ill brother or sister, these WM deficits, or so-called endophenotypes, may reflect the expression of shared susceptibility genes for schizophrenia (Tsuang, 1993). However, the underlying neural substrates of WM deficits, as measured with functional MRI, may be an even more sensitive endophenotype than WM performance alone (Gottesman, 2003). Moreover, studying endophenotypes in unaffected siblings of schizophrenia patients is advantageous since they do not use medication which may affect study results (Davis et al., 2005; Ettinger et al., 2011).

Functional MRI studies consistently report a cortico-striatal network subserving WM processes that includes dorsolateral prefrontal cortex (DLPFC), parietal cortex and striatum (Murty et al., 2011; Tan et al., 2007). In unaffected siblings of schizophrenia patients, fMRI studies of these WM areas are inconsistent as they include hypoactivation (Meda et al., 2008), hyperactivation (Brahmbhatt et al., 2006; Callicott et al., 2003; Delawalla et al., 2008; Whitfield-Gabrieli et al., 2009) and equal activation (Karch et al., 2009) compared to healthy controls. The main reasons for these inconsistencies are task design and poor WM performance (Brahmbhatt et al., 2006; Delawalla et al., 2008; Karch et al., 2009). Another factor that may contribute to these inconsistent findings is that WM network activation can be influenced by other networks such as the default-mode network (DMN) (Mayer et al., 2010). The DMN encompasses regions that are active during rest and are suppressed during cognitive demands (Raichle et al., 2001).

Here, we investigate working memory and default-mode network brain activity in 23 unaffected siblings of schizophrenia patients and 24 matched controls. Participants perform a modified version of the Sternberg working memory task (Sternberg, 1966) while being scanned with functional MRI. Importantly, working memory load is adjusted individually, so that despite intermediate WM deficits in siblings all participants perform at 90% accuracy. In addition to preventing

performance confounds, this high level of performance will result in maximum recruitment of both the WM and DMN networks (Altamura et al., 2007; Anticevic et al., 2010). Furthermore, the Sternberg task allows the separate investigation of the three phases of WM, being temporary storage (encoding), manipulation (maintaining) and retrieval of information (Baddeley, 1992). This is important because, for example, DMN abnormalities in schizophrenia patients have been found mainly during the encoding phase of WM (Anticevic et al., 2010).

Since siblings typically display poorer WM performance than to healthy controls, we hypothesize that they will show aberrant activation in WM and DMN circuits when they perform at an equally high level (90% accuracy) as controls. Specifically, we hypothesize that if siblings show aberrant DMN activity, (a) this will be most pronounced during the encoding phase of working memory, and (b) may predict (aberrant) activation levels in WM regions during retrieval.

Methods and materials

Participants

Twenty-three unaffected siblings of patients with schizophrenia and 24 unrelated healthy control subjects participated in this study (*Table 1*). All subjects were right-handed. None of the participants received psychotropic medication, had any contraindications for MRI, suffered from alcohol or drug dependence, or had a history of a neurological or psychiatric diagnosis as verified by either the Mini International Neuropsychiatric Interview (8 subjects) (Sheehan et al., 1998) or the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1, 41 subjects) (Wing et al., 1990). All participants were unrelated to each other and healthy control subjects who had a first-degree relative suffering from a psychotic disorder were excluded. Siblings had (at least) one affected brother or sister, with none of the other first degree relatives being affected. Participants were recruited from the database of an ongoing multicenter longitudinal study, the Genetic Risk and Outcome of Psychosis (GROUP) study, and received monetary compensation for participation. All gave written informed consent. The ethics committee of the University Medical Center of Utrecht approved this study.

Sternberg Working Memory task

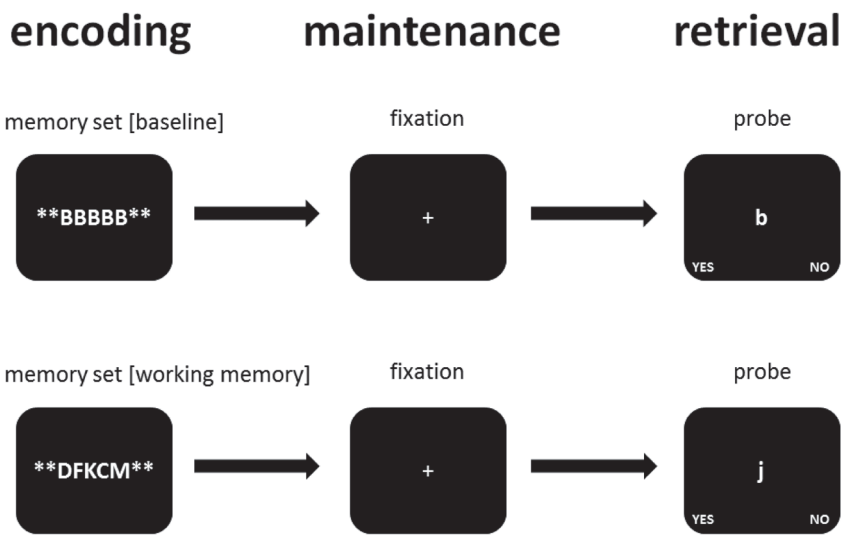
A schematic representation of the task is presented in *Figure 1*. At the beginning of each trial (encoding phase), a set of uppercase letters (memory set) was presented for 4 seconds and consisted of identical uppercase letters (baseline condition) or different letters (WM condition). The size of the memory set was determined

t. 1

	Siblings ($n = 23$)	Healthy controls ($n = 24$)	p
Age (years)	30.1 ± 4.2	28.3 ± 5.4	0.82
Sex (M/F)	14/9	12/12	0.46
Participant's Education Level (0-8)	7.6 ± 2.1	7.3 ± 1.1	0.50
Father's Education Level (0-8)	6.6 ± 2.8	5.8 ± 2.0	0.31
Mother's Education Level (0-8)	6.3 ± 2.4	5.3 ± 2.2	0.23

Demographic characteristics of the diagnostic groups. Age and education data represent mean ± SD. Level of education was measured on a 9-point scale ranging from no education (0) to university degree (8). Two-sample t-tests were performed to test for differences between the groups.

f. 1



Schematic representation of the task. For details see methods section.

individually prior to scanning so that accuracy was 90%. Next, the maintenance phase during which a fixation-cross was presented for 0.5 to 3.5 seconds. Finally, the retrieval phase in which a probe item (lowercase consonant) was presented for 2 sec (retrieval phase) and participants had to indicate whether or not it had appeared in the memory set, by pressing one of two buttons on a response device. There was a 50% chance that a probe was a part of the memory set. No feedback was given during the task. The task consisted of 30 trials per condition and had a total duration of 11 minutes.

Prior to scanning, WM load (size of memory set) was determined using a slightly different version of the task. In this version, the memory set was followed by 25 probes, spaced 1 second apart. Across the experiment, the memory set size varied from three to nine letters in a pseudorandom fashion. Each set size was presented twice, so that participants classified a total of 50 probes per memory set size. The total duration of the task was about 14 minutes. All participants performed this pre-scan session to determine for each individual the WM load (i.e. the amount of letters in a memory set) at which 90% accuracy is achieved. This number of letters in the memory set (90% accuracy) for each participant individually was then used for the task during scanning.

Functional magnetic resonance imaging

Measurements

All imaging was performed on a Philips 3.0-T Achieva whole-body MRI scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a two dimensional echo planar imaging-sensitivity encoding (EPI-SENSE) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1600 msec; echo time (TE) = 23 msec; flip angle = 72.5 ; 30-slice volume; SENSE-factor R = 2.4 (anterior-posterior). Three hundred twenty functional images were acquired during the task. Next, a whole-brain three-dimensional fast field echo T1-weighted structural image was acquired for within-subject registration purpose, scan parameters: voxel size 1 mm isotropic; TR = 25 msec; TE=2.4 msec; flip angle = 30°; 150 slices.

Image preprocessing

Image preprocessing and analyses were carried out with SPM5 (http://www.fil.ion.ucl.ac.uk/spm5). After realignment, the structural scan was coregistered to the mean functional scan. Next, using unified segmentation, the structural scan was segmented, and normalization parameters were estimated. Subsequently, all scans were registered to a Montreal Neurological Institute T1-standard brain using these normalization parameters and a three-dimensional Gaussian

smoothing kernel (8-mm full width at half maximum) was applied to all functional images.

First-level analyses

The preprocessed functional images were submitted to a General Linear Model regression analysis to estimate the event-related effects of the WM condition on brain activation in three different WM phases (encoding, maintenance, retrieval) against the baseline condition in each individual separately. To correct for head motion, the six realignment parameters were included in the design matrix as regressors of no interest. A high-pass filter was applied to the data with a cutoff frequency of 0.0058 Hz to correct for drifts in the signal. Subsequently, for each subject, first level contrast images were created for the WM condition compared with baseline during encoding, maintenance and retrieval. Head motion parameters were investigated to ensure that there were no differences in motion between the groups and that the maximum motion did not exceed predefined thresholds (Van Dijk et al., 2012).

Second-level whole-brain analyses

Whole-brain group-wise analyses were performed to investigate activity for correct responses during the three phase of WM (encoding, maintenance and retrieval). To test whether activity differed between siblings and matched healthy control subjects during these stages, whole-brain two-sample t-tests were performed. All group activation maps were tested for significance at a family-wise error (FWE) corrected cluster level of $p = 0.05$ (cluster-defining threshold of $p = 0.001$, critical cluster size of 25 voxels).

Second-level region of interest analyses

In addition to the whole-brain analyses, we performed region of interest (ROI) analyses to test for group differences in brain activity in WM related networks. To this end, we used ROIs determined from an independent sample to avoid double dipping (Kriegeskorte et al., 2009). This sample consisted of 26 healthy controls (all right handed; mean age = 20.7, SD ± 1.2, level of education = 5.8 SD ± 2.6, mean WM load = 6.5, SD ± 0.99, WM accuracy = 92% SD ± 0.70). ROIs were defined as regions showing activation in a group t-map calculated for the contrast WM condition versus baseline (threshold of $p \pm 0.05$ family-wise-error-corrected). This resulted in ROIs that are specific for the Sternberg WM task per phase and included supplementary motor cortex, anterior cingulate, parietal cortex, dorsolateral prefrontal cortex, insula, ventro-lateral prefrontal cortex, and inferior temporal cortex. For each

ROI, the average level of brain activation (i.e. b-value or regression-coefficient) was obtained for each subject (siblings and healthy controls). These subject-wise values were then submitted to two-sample t-test to investigate group differences (control, siblings) in activation within ROIs of WM during encoding, maintenance and retrieval.

Regression Analyses

Finally, to determine whether the abnormal activation during encoding was predictive for aberrant activation patterns during the retrieval phase of WM, we performed a regression analysis. First, we identified brain regions showing abnormal activation in siblings compared to controls during encoding and during retrieval. Next, the average b-value (or beta) was obtained for these regions for each individual subject. These values were entered in the regression analysis as independent (activation during encoding) and dependent (activation during retrieval) variable, respectively. The regression analysis was performed across all subjects. However, as this may be confounded by group effects, we also performed regression analyses for siblings and for controls separately.

Results

Behavioral results

The mean WM load prior to scanning in siblings performing at 90% accuracy was 6.09 letters for siblings (SD ± 1.04) and 6.25 letters for controls (SD ± 0.85), and this did not differ between the groups ($t(45) = 0.59$, $p = 0.56$).

During scanning, WM accuracy for siblings (91%, SD ± 5.9) and controls (91%, SD ± 5.3) did not differ ($t(45) = -0.20$, $p = 0.84$). There was no correlation between load and accuracy in either group (for siblings $R^2 = 0.013$, $p = 0.60$, for controls $R^2 = 0.004$, $p = 0.76$). Accuracy for both groups was equal for the baseline condition (controls: 98%, SD ± 3.44, siblings: 97%, SD ± 3.76; $t(45) = 0.79$, $p = 0.43$). Siblings and control subjects did not differ in reaction time during the WM condition (controls: 1122 sec, SD ± 121, siblings: 1072 sec, SD ± 111; $t(45) = 1.45$, $p = 0.14$) or baseline condition (controls: 889 sec, SD ± 112, siblings = 847 sec, SD ± 92; $t(45) = 1.41$, $p = 0.17$).

Imaging results

Encoding phase

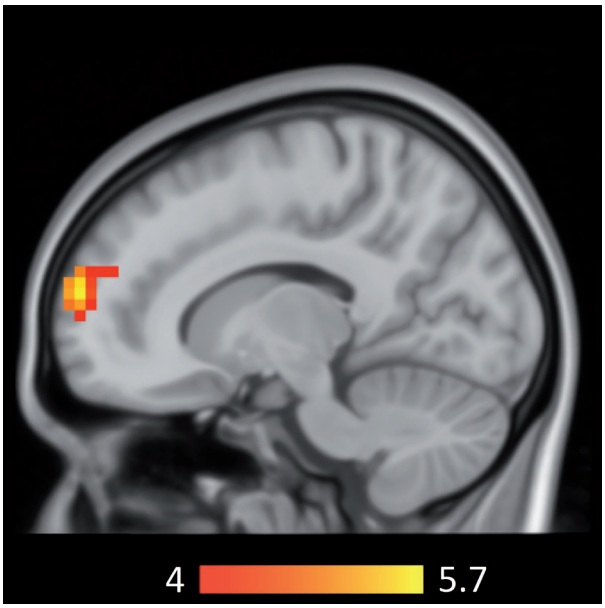
As expected, healthy controls showed activation within regions commonly associated with working memory (*Table 2*), including the left DLPFC and bilateral

t2

	Control group ($n = 24$) MNI Coordinates					Siblings group ($n = 23$) MNI Coordinates					Siblings > Controls MNI Coordinates				
Brain region	**x**	**y**	**z**	**max t**	**voxels**	**x**	**y**	**z**	**max t**	**voxels**	**x**	**y**	**z**	**max t**	**voxels**
L premotor cortex	-48	0	28	6.44	228	-52	0	24	5.99	181					
R premotor cortex	36	-8	44	6.18	145	48	-4	36	6.45	260					
SMA	-4	0	64	6.11	100	-4	0	64	7.85	97					
R parietal cortex	28	76	24	8.94	223	24	-68	36	6.53	256					
L parietal cortex	-24	-68	40	7.01	186	-28	-76	16	8.42	225					
R posterior cingulate	4	-40	48	4.38	55										
L posterior cingulate						-4	-44	24	5.16	71					
R anterior cingulate	8	16	44	4.71	45	12	12	44	5.33	34					
L anterior cingulate	-12	16	36	5.60	39	-4	40	16	4.97	48					
R ventral striatum	12	24	0	5.60	68	16	16	8	4.56	92					
L ventral striatum	-12	28	0	5.16	47	-20	16	4	6.00	124					
L hippocampus	-20	-20	-8	6.45	37	-24	-28	-8	8.37	24					
R hippocampus						16	-28	-4	6.08	49					
R DLPFC	40	8	28	8.31	35										
L postcentral	-40	-28	56	-7.98	108	-40	-28	56	-10.19	259					
MPFC						**-8**	**52**	**8**	**5.71**	**163**	**16**	**64**	**20**	**5.40**	**28**
R parahippocampus						28	-20	-16	5.92	31					
L parahippocampus						-32	-16	-20	5.18	35					

Whole-brain group comparison during the encoding phase of WM, performing at 90% accuracy. In bold are the regions showing significant group differences in activation. Montreal Neurological Institute (MNI) Coordinates represent the location of the peak voxels; max t is the maximum t-value. Cluster-defining threshold of $p < 0.001$ and a $p = 0.05$ FWE-corrected critical cluster extend of 25 voxels. Note that there were no significant clusters for the contrast Controls > Siblings.

f·2



Results of the whole-brain-analyses revealed that the right MPFC showed significantly increased activity in siblings compared to the controls during the encoding phase of WM. No areas showed more activity in the control subjects relative to the siblings. Activation is overlaid on a mean brain-extracted anatomical image. Colorbar represent t-values. Cluster-defining threshold of $p < 0.001$ and a p = 0.05 FWE-corrected critical cluster size of 25 voxels.

parietal cortices. Siblings activated a similar network. When compared directly (whole-brain two-sample t-test) we found significant hyperactivation in the right medial prefrontal cortex (MPFC) in siblings (*Figure 2*). This region is considered to the anterior part of the default mode network (DMN). Similar to the whole-brain analysis, the ROI analysis did not reveal differences in WM related regions during encoding phase.

Maintenance phase

Healthy controls and siblings activated WM circuits (*Table 3*). Furthermore, regions considered to be part of the DMN such as the MPFC, posterior cingulate cortices and medial temporal lobes were deactivated in healthy controls and siblings during maintaining of information. A whole brain two-sample t-test did not reveal any differences in activation patterns between siblings and healthy controls. Similarly, the ROI analysis did not reveal any significant differences between the groups.

t3

	Control group (n = 24) MNI Coordinates					Siblings group (n = 23) MNI Coordinates				
Brain region	**x**	**y**	**z**	**max t**	**voxels**	**x**	**y**	**z**	**max t**	**voxels**
L premotor cortex	-44	0	40	5.42	135	0	12	52	7.24	171
R premotor cortex						44	0	48	5.12	61
SMA	-4	4	64	7.31	77					
L parietal cortex	-28	-56	40	6.05	112	-32	-56	40	11.98	148
R parietal cortex						24	-68	44	4.80	35
R anterior cingulate	4	12	52	4.82	53					
L ventral striatum	-20	8	4	5.33	58	-20	12	0	6.26	59
L insula	-44	-16	8	-6.04	180	-44	-12	4	-5.21	99
R insula	52	-12	12	-5.72	169					
L postcentral	-36	-32	60	-4.27	48	-44	-24	56	-6.94	59
R calcarinus	16	-56	12	-5.11	30					

Whole-brain group comparison during the maintenance phase of WM, performing at 90% accuracy. Montreal Neurological Institute (MNI) coordinates represent the location of the peak voxels; max t is the maximum t-value. Cluster-defining threshold of $p < 0.001$ and a $p = 0.05$ FWE-corrected critical cluster extend of 25 voxels. Note that there were no significant clusters for the contrast Siblings > Controls and Controls > Siblings.

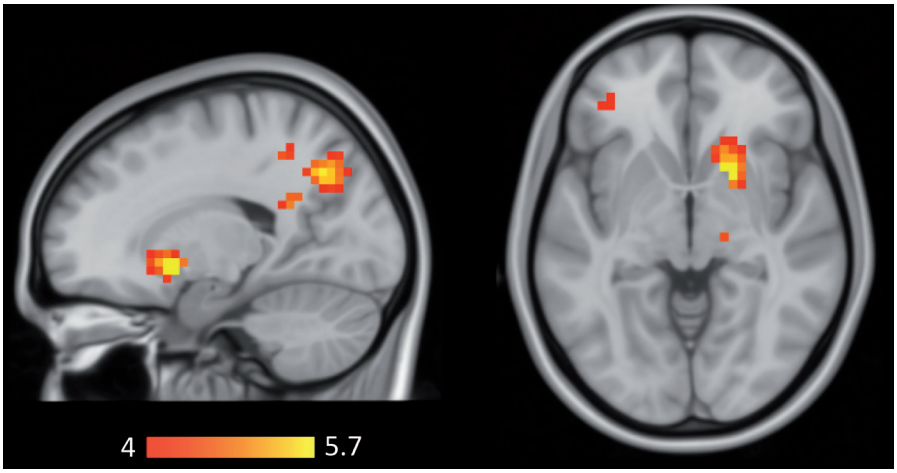
Retrieval phase

Healthy controls and siblings activated WM circuits during the retrieval of information, similar to the other first two phases of WM (*Table 4*). A direct comparison between controls and siblings (whole-brain two-sample t-test) revealed hyperactivation in the right striatum and right inferior parietal cortex in siblings (*Figure 3*). The ROI analysis also revealed hyperactivation in the left DLPFC in siblings compared to controls ($t(45) = 2.82$; $p = 0.003$).

Regression analyses

We investigated the relationship between DMN activation during encoding (in the MPFC) and WM activation during retrieval (left DLPFC, right parietal cortex, and striatum) using regression analysis. This analysis revealed that MPFC activation during encoding predicted activation in the striatum during retrieval ($t(45) = 1.61$, $p = 0.01$). Furthermore, MPFC activation during encoding predicted activation in the DLPFC during retrieval for siblings ($t(22) = 2.12$, $p = 0.046$): those siblings showing the least amount of MPFC suppression also showed the highest level of DLPFC activation. This effect was not modulated by WM load. There was no such a relation for controls ($t(23) = 1.28$, $p = 0.18$). We did not find such a relation between MPFC and the other WM regions for either siblings or controls.

f·3



Results of the whole-brain-analyss revealed that the right caudate and the right inferior parietal lobe showed significantly increased activity in siblings compared to the controls during the retrieval phase of WM. No areas showed more activity in the control subjects relative to the siblings. Activation is overlaid on a mean brain-extracted anatomical image. Colorbar represent t-values, left = left (for transversal slide). Cluster-defining threshold of $p < 0.001$ and a $p = 0.05$ FWE-corrected critical cluster size of 25 voxels.

Discussion

This study investigated working memory (WM) and default-mode network (DMN) brain activity with functional MRI in 23 non-medicated unaffected siblings of schizophrenia patients and 24 matched healthy controls using a Sternberg WM task. Activation was examined during the three phases of WM: encoding, maintenance, and retrieval of information. WM load was determined individually so that each participant (siblings, controls) performed at 90% accuracy.

During the encoding phase of WM, siblings showed hyperactivity in the medial prefrontal cortex (MPFC) (whole-brain analysis, *Figure 2*). The MPFC is the anterior part of the default mode network (DMN) and is normally suppressed during WM processing (Mayer et al., 2010; Raichle et al., 2001). During the maintenance phase, there were no differences between siblings and controls. Finally, during the retrieval phase, siblings showed hyperactivation in regions of the WM network such as the striatum, the inferior parietal cortex (whole-

t-4

	Control group ($n = 24$) MNI Coordinates					Siblings group ($n = 23$) MNI Coordinates					Siblings > Controls MNI Coordinates				
Brain region	**x**	**y**	**z**	**max t**	**voxels**	**x**	**y**	**z**	**max t**	**voxels**	**x**	**y**	**z**	**max t**	**voxels**
L premotor cortex	-4	0	56	12.32	118	-28	-16	56	7.76	247					
R premotor cortex	24	-4	56	8.62	235	32	-8	56	7.46	209					
SMA	-4	8	52	9.81	432	4	4	56	6.88	349					
R parietal cortex	**32**	**-56**	**40**	**6.34**	**291**	**24**	**-56**	**44**	**11.47**	**630**	**20**	**-64**	**40**	**4.84**	**70**
L parietal cortex	-28	-56	48	11.35	265	-24	-64	44	10.41	424					
R posterior cingulate	4	-56	16	-5.28	68										
L posterior cingulate	-4	-48	24	-7.78	263	-4	-52	24	-5.14	77					
anterior cingulate						-4	12	44	10.66	351					
R ventral striatum						**8**	**4**	**8**	**7.14**	**276**	**20**	**12**	**-4**	**5.77**	**34**
L ventral striatum						-20	-4	4	7.65	201					
R insula	32	24	0	7.28	113	32	24	4	11.74	192					
L insula						-32	24	4	8.43	67					
L temporal cortex	-48	-64	28	-7.71	223	-52	-64	24	-7.50	116					
R temporal cortex	48	-60	24	-7.72	205	52	-56	20	-4.91	24					
L postcentral						-44	-28	48	11.99	107					

Whole-brain group comparison during the retrieval phase of WM, performing at 90% accuracy. In bold are the regions showing significant group differences in activation. Montreal Neurological Institute (MNI) Coordinates represent the location of the peak voxels; max t is the maximum t-value. Cluster-defining threshold of $p < 0.001$ and a $p = 0.05$ FWE-corrected critical cluster extend of 25 voxels. Note that there were no significant clusters for the contrast Controls > Siblings.

brain analysis, *Figure 3*) and the dorsolateral prefrontal cortex (DLPFC) (region of interest (ROI) analysis). Using regression analysis, we showed that the amount of activation in the striatum and the DLPFC during retrieval was related to the amount of activation in the MPFC during the encoding phase. Specifically, siblings who failed to suppress the MPFC during encoding showed hyperactivity of DLPFC and the striatum during retrieval.

These results suggest that siblings are impaired in encoding and retrieving of information, but not in maintaining that information. Furthermore, the deficits shown here in unaffected siblings mimic those found in schizophrenia patients (Anticevic et al., 2013; Whitfield-Gabrieli et al., 2009) and provide support for the notion that cortico-striatal network dysfunction potentially represents an endophenotype and reflect the expression of shared susceptibility genes for schizophrenia (Vink et al., 2006; Zandbelt et al., 2011). Moreover, we found that these cortico-striatal deficits are linked to a failure to adequately suppress DMN activation.

Our finding of hyperactivity in regions of the WM network in unaffected siblings is consistent with previous fMRI studies in unaffected siblings during the N-back task (Brahmbhatt et al., 2006; Callicott et al., 2003) and the AX-CPT task (Delawalla et al., 2008; Thermenos et al., 2004). We extend these studies by showing that this hyperactivation occurred specifically during the retrieval phase of WM. To date, two studies have used the Sternberg task to investigate WM processing in siblings (Karlsgodt et al., 2007; Meda et al., 2008). Consistent with our data, Karlsgodt et al. (2007) report hyperactivation in the DLPFC as well as the parietal cortex in patients (n=8) and unaffected co-twins (n=10) compared to controls (n=13) at accuracy levels above 80 percent. Meda et al. (2008) reported hypoactivation for a WM load of four items during the retrieval phase of WM in the DLPFC and parietal cortex in unaffected siblings compared to controls. For higher WM loads (6 items), which is more similar to the WM load we used, this hypoactivation disappeared, suggesting that siblings progressively increased their activation with increasing load more so than healthy controls. This is in line with our findings of increased activation in siblings.

The current results of hyperactivation in the frontal cortex (DLPFC) and the striatum may reflect abnormalities in dopamine transmission in siblings. For example, it has been shown that the fMRI signal in the striatum reflects dopamine release in the striatum (Knutson and Gibbs, 2007; Schott et al., 2008). Moreover, effective WM processing depends upon increased striatal activity (Dahlin et al., 2008) which is related to enhanced dopamine release (Bäckman et al., 2011). Taken together, our finding of striatal hyperactivation in siblings suggests enhanced dopamine release in the striatum in siblings. This is consistent with

a PET study in unaffected siblings showing increased dopamine sensitivity to stress (Brunelin et al., 2010). Normally, (dopamine) activation in the striatum is regulated by the frontal cortex via glutamate afferents (Lovinger and Tyler, 1996). In schizophrenia, this frontal regulation fails, leading to striatal hyperactivation (Davis et al., 1991). Indeed, using PET imaging, Meyer-Lindenberg and co-workers found that frontal activation predicted exaggerated striatal dopamine activity in schizophrenia patients (Fusar-Poli and Meyer-Lindenberg, 2013a, 2013b). The data of the current study provide further evidence that in siblings this frontal control over the striatum fails. This is consistent with previous studies showing cortico-striatal deficits in schizophrenia patients and in unaffected siblings during higher cognitive functions such as inhibition (Vink et al., 2006; Zandbelt et al., 2011) and eye-movements (Raemaekers et al., 2006). As such, this cortico-striatal dysfunction may be considered a candidate endophenotype for schizophrenia.

Our results also show hyperactivation in the MPFC in unaffected siblings compared to controls, which is the anterior part of the Default Mode Network (DMN). Since DMN activity must be suppressed during WM tasks (Mayer et al., 2010; Raichle et al., 2001), these results suggest that siblings fail to deactivate DMN. Whereas there are a number of studies reporting on DMN dysfunction in siblings (Jang et al., 2011; Meda et al., 2012; Repov and Barch, 2012; Repovs et al., 2010; van Buuren et al., 2011), to date only one has reported DMN dysfunction during WM processing (n-back task) (Whitfield-Gabrieli et al., 2009). Consistent with our results, they found that patients (n=13) and siblings (n=13) showed MPFC hyperactivation compared to controls (n=13). However, in their study overall performance (RT and accuracy) was lower in siblings compared to healthy controls. We replicated these findings of hyperactivation in a larger group of siblings and show that even with equal performance siblings still hyperactive the MPFC.

Moreover, we found that this failure to suppress DMN activity was related to the hyperactivity of the cortico-striatal network in siblings. This is consistent with studies in healthy controls showing that the amount of DMN deactivation is related to dopamine activation in the striatum (Braskie et al., 2011). Moreover, Tomasi et al. (2009) suggested that striatal dopamine activation may modulate (task-related) attention by facilitation of brain deactivation in the DMN (Tomasi et al., 2009). Our finding of striatal hyperactivation in siblings may therefore be causally related to their inability to suppress the DMN. Furthermore, our findings are in line with connectivity data of Whitfield et al. (2009) showing reduced anti-correlations (negative connectivity during rest and n-back task) between MPFC and DLPFC in siblings. Together, our findings indicate that siblings who show the most pronounced striatal hyperactivation fail in suppressing the DMN

during encoding and subsequently hyperactivate the DLPFC during retrieval. In healthy controls, we did not find such a relation between DMN suppression and DLPFC activation, suggesting that if DMN activity is adequately suppressed during encoding, DLPFC activity does not need to be enhanced during the retrieval phase of WM.

A potential limitation of this study is that we did not investigate brain activation at varying levels of accuracy. To get more insight in the dynamics of WM in siblings, further experiments should focus on activation patterns between siblings and healthy controls on lower accuracy levels.

Another potential limitation is that some siblings in our sample already passed the age of onset for schizophrenia (Häfner et al., 1993). Thus, even if these siblings have WM and DMN deficits, they are unlikely to develop the illness. In addition, it is unclear as of yet whether those who are still at risk will actually go on to develop schizophrenia. Therefore, it can be argued that our findings do not necessarily represent a risk factor for the illness. Since we have not tested for heritability or segregation of these deficits with illness within families, we also cannot determine whether these deficits could be seen as endophenotypes for schizophrenia. Nevertheless, our findings do meet one of the criteria for endophenotype, since it is associated with the illness (Gottesman, 2003; Meyer-Lindenberg and Weinberger, 2006; Rasetti and Weinberger, 2011): abnormal WM and DMN activations in siblings are similar to those seen in patients in previous studies (Anticevic et al., 2013; Whitfield-Gabrieli et al., 2009). Moreover, combined with the observation of WM deficits shown in unaffected co-twins using fMRI (Karlsgodt et al., 2007), our findings add to the suggestion that such deficits are, at least to some extent, heritable and may be candidates for a schizophrenia endophenotype. Future studies should focus on longitudinal tracking of at-risk subjects with WM and DMN deficits, to clarify whether they elevate one's risk for developing schizophrenia. Studying multiple ill and well members within families will also determine whether they could be endophenotypes for schizophrenia.

Alternatively, our finding of increased right inferior parietal cortex activation may be related to impaired lateralization. Indeed, schizophrenia is characterized by impaired lateralization (Angrilli et al., 2009), and we have shown that lateralization is abnormal even in first-degree medication-naïve schizophrenia patients (van Veelen et al., 2011a). To date, there is only one study investigating language lateralization in siblings (Li et al., 2007). This study by Li et al. showed that in a group of 30 siblings and young high-risk individuals, right inferior parietal was hyperactivated compared to controls during a visual word discrimination task. Their finding of parietal hyperactivity along with other hyperactivated regions was most prominent during word trials together with

controls symbols. However, to what extent our finding of inferior parietal cortex hyperactivity reflects reduced language lateralization during cognitive tasks needs to be investigated in future experiments.

In sum, compared with matched controls, unaffected siblings of schizophrenia demonstrated exaggerated activity in WM regions such as the DLPFC, inferior parietal cortex and in the striatum during information retrieval, and hyperactivation of the anterior DMN during information encoding. Moreover, these findings are related: dopamine hyperactivity in the striatum may obstruct adequate DMN suppression needed for effective WM encoding. The fact that these WM and DMN disturbances occur in unaffected siblings provides support for the notion that these deficiencies potentially represent candidate endophenotypes for schizophrenia.

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Chapter 3

Fronto-striatal dysfunction during reward processing in unaffected siblings of schizophrenia patients

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Based on
Schizophrenia Bulletin, 2015 January; 41(1): 94-103

Abstract

Schizophrenia is a psychiatric disorder that is associated with impaired functioning of the fronto-striatal network, in particular during reward processing. However, it is unclear whether this dysfunction is related to the illness itself or whether it reflects a genetic vulnerability to develop schizophrenia. Here, we examined reward processing in unaffected siblings of schizophrenia patients using functional MRI. Brain activity was measured during reward anticipation and reward outcome in 27 unaffected siblings of schizophrenia patients and 29 healthy volunteers using a modified Monetary Incentive Delay task. Task performance was manipulated online so that all subjects won the same amount of money. Despite equal performance, siblings showed reduced activation in the ventral striatum, insula and supplementary motor area during reward anticipation compared to controls. Decreased ventral striatal activation in siblings was correlated with sub-clinical negative symptoms. During the outcome of reward, siblings showed increased activation in the ventral striatum and orbitofrontal cortex compared to controls. Our finding of decreased activity in the ventral striatum during reward anticipation and increased activity in this region during receiving reward may indicate impaired cue processing in siblings. This is consistent with the notion of dopamine dysfunction typically associated with schizophrenia. Since unaffected siblings share on average 50% of their genes with their ill relatives, these deficits may be related to the genetic vulnerability for schizophrenia.

Introduction

Schizophrenia is a highly heritable psychiatric disorder characterized by positive symptoms such as delusions and hallucinations, negative symptoms including affective flattening, as well as cognitive impairments (van Os and Kapur, 2009). Underlying these symptoms may be dysfunctions in the frontal lobe and the striatum (Hahn et al., 2012; McGuire et al., 2008; van Veelen et al., 2011, 2010; Waltz and Gold, 2007; Weinberger and Gallhofer, 1997; Zandbelt et al., 2011). Indeed, functional MRI (fMRI) studies have demonstrated abnormal fronto-striatal activity in the context of various cognitive tasks (Ehrlich et al., 2012; Koch et al., 2008; Murty et al., 2011; Quidé et al., 2013; Tu et al., 2006; van Veelen et al., 2011, 2010; Vink et al., 2006; Wolf et al., 2011; Zandbelt et al., 2011), in particular those that require the processing of rewards (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; M. Ø. Nielsen et al., 2012; Schlagenhauf et al., 2009).

Reward processing can be divided into at least two sub-processes: anticipation of reward and receipt of reward (Brian Knutson et al., 2001), both of which are consistently reported to be abnormal in schizophrenia (Esslinger et al., 2012; Gradin et al., 2013; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; M. Ø. Nielsen et al., 2012; Schlagenhauf et al., 2009). Functional MRI studies have revealed that in the healthy human brain the striatum is most active during reward anticipation, while during receipt of reward the frontal cortex, particularly the orbitofrontal cortex, becomes most activated (Diekhof et al., 2012; Dillon et al., 2008; B Knutson et al., 2001; Brian Knutson et al., 2001). However, in schizophrenia patients, several studies reported blunted ventral striatum activation during reward anticipation (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; M. Ø. Nielsen et al., 2012; Schlagenhauf et al., 2009), whereas one study showed increased activation in the orbitofrontal cortex during outcome of reward (Schlagenhauf et al., 2009). Moreover, these impairments, in particular decreased ventral striatal activity, have been associated with symptom severity in schizophrenia patients (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; M. Ø. Nielsen et al., 2012; Simon et al., 2010). However, as of yet it remains unclear whether these abnormal activations are related to the illness itself or to a genetic vulnerability for the disorder.

Therefore, we investigated reward processing in non-affected siblings. These siblings do not have the illness, but share on average 50% of their genes with their ill relative (Gottesman and Gould, 2003; Meyer-Lindenberg and Weinberger, 2006) and have a 10-fold increased risk to develop schizophrenia

(Gejman et al., 2011). Consequently, if siblings show impaired reward processing similar to that observed in patients, this would provide evidence in support for a genetic vulnerability underlying this phenotypic abnormality. Indeed, one recent study in first-degree relatives of schizophrenia patients reported on attenuated ventral striatal activity during reward anticipation (Grimm et al., 2014). However, relatives of several generations were used, including siblings, parents, and adolescent offspring, ranging in age between 18 and 50. Moreover, they only tested activation in the ventral striatum during reward anticipation. Other regions as well as receipt of reward were not investigated. Impaired reward processing in siblings is anticipated given reports on fronto-striatal dysfunction during other cognitive functions such as inhibitory control (Vink et al., 2006; Zandbelt et al., 2011), emotion processing (van Buuren et al., 2011a) and working memory (de Leeuw et al., 2013; Meda et al., 2008).

Here, we measured brain activity using fMRI during reward processing in 27 unaffected siblings of schizophrenia patients and 29 matched healthy controls. All subjects performed a modified Monetary Incentive Delay task (Figee et al., 2011; Hoogendam et al., 2013; van Hell et al., 2010). Activation during reward anticipation and receipt of reward was investigated in regions involved in processing of reward: the bilateral ventral striatum, dorsal striatum, insula, supplementary motor area and orbitofrontal cortex (Hoogendam et al., 2013; Brian Knutson et al., 2001; Knutson et al., 2000; Martin-Soelch et al., 2001; Schmack et al., 2008).

As outlined above, we hypothesized that siblings show fronto-striatal deficits during reward processing comparable to that of schizophrenia patients. Specifically, we hypothesized that, similar to patients, activation in the ventral striatum is decreased during reward anticipation. Furthermore, since schizophrenia patients show hyperactivation of the orbitofrontal cortex during receipt of reward (Schlagenhauf et al., 2009), we hypothesized that activity in this region is likely increased in siblings. Finally, because schizophrenia patients show associations between decreased ventral striatum activity and symptom severity, we hypothesize that reduced activation of the ventral striatum in siblings may be associated with sub-clinical symptoms as measured with the Community Assessment of Psychic Experiences (Stefanis et al., 2002).

t.1

	Healthy controls ($n = 29$)	Siblings ($n = 27$)	p
Age (years)	30.3 ± 1.7	31.7 ± 1.2	0.51
Gender (M/F)	12/17	14/13	0.37
Edinburgh Handedness	0.89 ± 0.03	0.93 ± 0.02	0.22
IQ (WAIS)	117 ± 3	119 ± 2	0.71
Participant's Education Level [a]	7.0 ± 0.22	7.0 ± 0.23	0.89
Father's Education Level	5.4 ± 0.40	5.2 ± 0.45	0.78
Mother's Education Level	4.8 ± 0.41	5.4 ± 0.52	0.18
Depression in medical history	0	7	
Cigarette smokers	1	8	0.01
Cigarettes per day	2 ± 0	10 ± 1	0.07
Cannabis users for the last 12 months	3	8	0.07
CAPE positive dimension (range)	0.14 ± 0.03 (0 - 0.4)	0.21 ± 0.05 (0 - 1.1)	0.43
CAPE negative dimension (range)	0.69 ± 0.05 (0.4 - 1.0)	0.60 ± 0.06 (0 - 1.5)	0.44
CAPE depressive dimension (range)	0.81 ± 0.06 (0.4 - 1.4)	0.86 ± 0.08 (0 - 1.9)	0.70
Amount won (euro) [b]	14.41 ± 0.21	14.76 ± 0.19	0.23
Reaction time potentially rewarding trials (ms)	303 ± 7	311 ± 7	0.41
Reaction time non-rewarding trials (ms)	323 ± 8	329 ± 9	0.60

Demographic, clinical and behavioral characteristics of the diagnostic groups.
Note: Values represent mean ± SEM. IQ, Intelligence Quotient; WAIS, Wechsler Adult Intelligence Scale; CAPE, Community Assessment of Psychic Experiences.

[a] Level of education was measured on a 9-point scale ranging from no education (0) to university degree (8).

[b] Note that the maximum amount participants could win was 15 euro.

Methods

Participants

Twenty-seven unaffected siblings of patients with schizophrenia and 29 healthy control subjects participated in this study (*Table 1*). All individuals were participating in an ongoing longitudinal study at the Department of Psychiatry at the University Medical Center Utrecht (Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011). All subjects were right-handed. None of the participants received psychotropic medication, had any contraindications for MRI, suffered from alcohol or drug dependence, or had a neurological diagnosis. Sub-clinical symptoms in all participants were measured using the Community Assessment of Psychic Experiences (CAPE) (see *supplementary material* for details) (Stefanis et al., 2002). Seven siblings had a history of at least one depressive episode as verified by either the Mini International Neuropsychiatric Interview (Sheehan et al., 1998) or the Schedules for Clinical Assessment in Neuropsychiatry (SCAN

2.1) (Wing et al., 1990). None of the healthy controls had a history of psychiatric diagnosis. Healthy control subjects who had a first-degree relative suffering from a psychotic disorder were excluded. Participants received monetary compensation for participation. All gave written informed consent. The ethics committee of the University Medical Center of Utrecht approved this study.

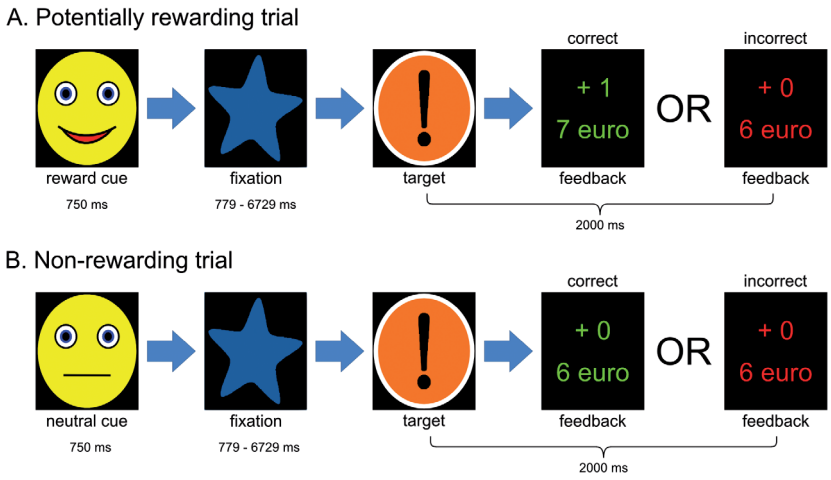
Monetary Incentive Delay task

Participants performed a reward task (Figee et al., 2011; Hoogendam et al., 2013; van Hell et al., 2010) (*Figure 1*) based on the Monetary Incentive Delay task (Brian Knutson et al., 2001). This task allows the investigation of anticipation and receipt of reward, separately. At the beginning of each trial, a cue is presented for 750 milliseconds signaling whether the subject can win money (potentially rewarding trial) or not (non-rewarding trial). For the potentially rewarding trials, this cue is a smiling face and for the non-rewarding trials a neutral face. Following this cue, subjects have to respond as fast as possible, by pressing a button, when a target stimulus (exclamation mark) appears on the screen. Subsequent feedback notifies participants of their performance, indicating if they have earned money on the trial, as well as their cumulative total at that moment. Subjects can win €1 during a potentially rewarding trial.

For both potentially rewarding and non-rewarding trials, subjects have to respond to the target stimulus within a certain time limit, i.e. target duration. Responses are considered correct (correct feedback) if subjects responds within this time limit. Responses given after the time limit are considered incorrect (incorrect feedback).

The time limit is individually adjusted to ensure that each participant succeeds in 50% of the trials. This adjustment is based on twenty practice trials which are presented prior to the start of the experiment (when subjects were already in the scanner). From these practice data, the shortest reaction time to the target is used to determine the individual time limit for responses to the target. In 50% of the trials, 200 ms is added to the duration of the individual time limit, enabling participants to be successful in these trials. In the remaining trials, 150 ms is subtracted from the time limit, to make sure that participants cannot respond in time. This procedure resulted in about 50% correct feedback for both rewarding and non-rewarding trials, separately. The total amount of money won is presented at the end of the task. Participants are told that they receive the cumulative total amount of reward of the actual experiment in addition to the standard compensation for participation. The task consisted of 60 trials with a mean duration of 9571 ms (range 4946 - 16107 ms, inter-trial-interval range 1029 - 6979 ms), resulting in a total task duration of 9 minute 35 seconds.

f.1



Schematic representation of the reward task, based on the Monetary Incentive Delay Task. There were two types of trials: a potentially rewarding (A) and a non-rewarding trial (B). The inter-trial-interval ranged from 1029 to 6979 ms.

Behavioral data analyses

Repeated-measures ANOVAs were performed to test for effects of condition (potentially rewarding trials, non-rewarding trials) and group (controls, siblings) on reaction time. The amount of money that participants won was compared between controls and siblings using a two-sample t-test.

Functional magnetic resonance imaging

Measurements

All imaging was performed on a Philips 3.0-T Achieva whole-body MRI scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a two dimensional echo planar imaging-sensitivity encoding (EPI-SENSE) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1600 ms; echo time (TE) = 23 ms; flip angle = 72.5°; 30-slice volume; SENSE-factor R = 2.4 (anterior-posterior). Three hundred twenty functional images were acquired during the task. Next, a whole-brain three-dimensional fast field echo T1-weighted structural image was acquired for within-subject registration purpose, scan parameters: voxel size 1 mm isotropic; TR = 25 ms; TE=2.4 ms; flip angle = 30°; 150 slices.

Image preprocessing

Image preprocessing and analyses were carried out with SPM5 software (http://www.fil.ion.ucl.ac.uk/spm). After slice timing and realignment, the structural scan was coregistered to the mean functional scan. Next, using unified segmentation, the structural scan was segmented, and normalization parameters were estimated. Subsequently, all scans were registered to a Montreal Neurological Institute T1-standard brain using these normalization parameters and a three-dimensional Gaussian smoothing kernel (8-mm full width at half maximum) was applied to all functional images.

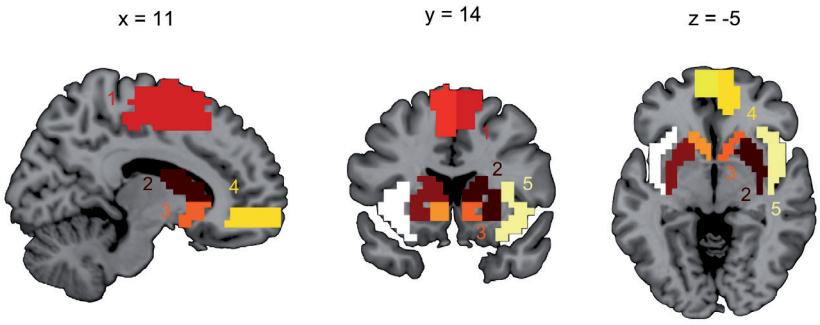
Individual analyses

Individual datasets were analyzed using multiple-regression, to estimate brain activation time-locked to anticipation of reward, anticipation of non-reward, correct reward outcome, incorrect reward outcome, correct non-reward outcome, and incorrect non-reward outcome. To correct for head motion, the six realignment parameters were included in the design matrix as regressors of no interest. A high-pass filter was applied to the data with a cutoff frequency of 0.0058 Hz to correct for drifts in the signal. For each subject, we calculated brain activation related to reward anticipation (anticipation of reward versus anticipation of non-reward) and receipt of reward (correct reward outcome versus correct non-reward outcome). Head motion parameters were investigated to ensure there were no differences in motion between the groups and that for none of the subjects the maximum motion exceeded predefined thresholds (scan-to-scan: > 3 mm).

Region of Interest analyses

In the manner of previous functional MRI studies investigating cognitive functioning (Hoogendam et al., 2013; M. Ø. Nielsen et al., 2012; Nieto-Castanon et al., 2003; van Buuren et al., 2011a), region of interest (ROI) analyses were performed to test for group differences in five predefined regions that are known for their involvement in reward processing, including the bilateral ventral striatum, dorsal striatum, insula, supplementary motor area and orbitofrontal cortices (also referred to as ventromedial prefrontal cortex, vmPFC) (*Figure 2*) (Hoogendam et al., 2013; Brian Knutson et al., 2001; Knutson et al., 2000). Regions were created using the automated anatomical labeling-atlas (Tzourio-Mazoyer et al., 2002). For each ROI, the average level of brain activation (i.e. percent signal change) was obtained for each subject. These subject-wise values were then submitted to independent samples t-test to investigate group differences (controls, siblings) in activation

f-2



Region of interests (ROIs) in five predefined regions that are known on their involvement in reward processing (Brian Knutson et al., 2001). ROIs were the 1) supplementary motor area, 2) dorsal striatum, 3) ventral striatum, 4) orbitofrontal cortex, and 5) insula.

within ROIs of reward processing during anticipation and receiving. All results were corrected for multiple testing of ten regions (five regions left and right).

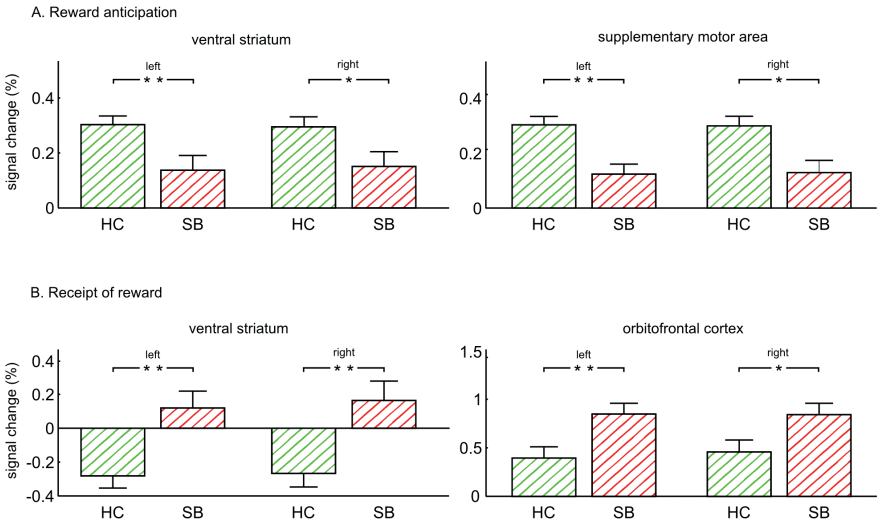
Whole-brain analyses

In addition to the region of interest analyses, whole-brain group-wise analyses were performed to investigate brain activity outside the predefined ROIs during reward anticipation and receipt of reward (Friston et al., 1995). To test group differences in activity between siblings and matched controls, whole-brain two-sample t-tests were performed. All group activation maps were tested for significance at a family-wise-error corrected cluster level of $p = 0.05$ (cluster-defining threshold of $p = 0.001$, critical cluster size of 34 voxels).

Correlations

Pearson correlations were used to test for associations between brain activation in all regions during reward processing, intelligence quotient (IQ) and sub-clinical symptoms as measured with the CAPE. In addition, we investigated the influence of past depressive episodes on brain activity in siblings. For this reason, we performed two-sample t-tests to compare brain activity between siblings with and without a history of depression. Finally, we and others have shown that cigarette smoking and cannabis use impact activity during reward processing in the ventral striatum (Peters et al., 2011; van Hell et al., 2010). We therefore compared ventral striatum activation, using two-sample t-tests, between cigarette smokers and non-smokers as well as between cannabis users and non-users. Importantly, none of the participants used other drugs for the last 12 months.

f-3



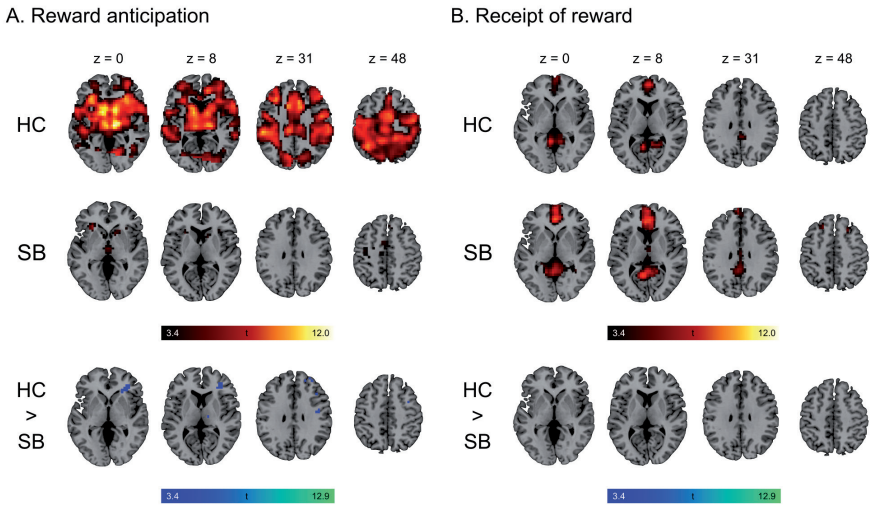
Region of interest analyses revealed decreased activity in the left ventral striatum and the left SMA in siblings compared with controls during reward anticipation (anticipation of reward versus anticipation of non-reward) (A). During receiving reward (correct reward outcome versus correct non-reward outcome), siblings showed increased activity in the ventral striatum bilaterally and the left orbitofrontal cortex (B). Error bars indicate standard error of means. * = significant ($p < 0.05$) group differences. * * survived Bonferroni correction for multiple testing. HC = controls, SB = siblings.

Results

Behavioral results

All behavioral data of controls and siblings are presented in *Table 1*. A main effect of reward on reaction time was found, with all subjects responding significantly faster to the target on potentially rewarding trials compared to non-rewarding trials ($F(1,53) = 37$, $p < 0.01$). This effect of reward did not differ between the groups (group by reward effect interaction: $F(1,53) = 0.14$, $p = 0.71$). There was no main effect of group ($F(1,53) = 0.48$, $p = 0.49$), indicating that there was no overall difference in response speed. Siblings and control subjects won the same amount of money ($t(54) = 1.21$, $p = 0.23$). This was expected, since task difficulty was individually determined based on a training session prior to the experiment to ensure that all subjects won the same amount of money. In this way, potential performance confounds which may affect imaging results were excluded.

f-4



Whole-brain activation during reward anticipation (anticipation of reward versus anticipation of non-reward) (A) and receipt of reward (correct reward outcome versus correct non-reward outcome) (B) in controls (HC) and siblings (SB). During reward anticipation, siblings showed decreased activity in the right anterior insula compared to the controls (center of mass in Talairach coordinates [30 32 0]). Significant activation clusters ($p < 0.05$, family-wise error-corrected) are displayed on the normalized and skull-stripped group-average brain (neurological orientation). Note that the colors for controls and siblings are scaled to the same colormap. Right = right.

Imaging results

Reward anticipation

During reward anticipation (anticipation of reward versus anticipation of non-reward), ROI analyses revealed that the ventral striatum, dorsal striatum, insula and supplementary motor area were less active in siblings than in healthy controls (*Figure 3*, *Table S1 in supplementary material*). After correcting for multiple comparisons for the number of ROIs, only the difference between groups in the left ventral striatum and the left supplementary motor area remained significant (*Figure 3*). In the orbitofrontal cortex no significant activation differences between siblings and controls were found.

To investigate whether there were activation differences between the groups outside these ROIs, we performed whole-brain analyses. As expected, healthy controls and siblings activated regions within the neural circuitry of reward anticipation including ventral and dorsal striatum, insula, parietal cortices,

thalamus and the supplementary motor area, corroborating the findings from the ROI analysis (*Figure 4*). When compared directly by the two-sample t-test, siblings showed significant less activation in the right anterior insula as compared to controls (*Figure 4*).

Receipt of reward

During the receipt of reward, ROIs analyses revealed increased activity in siblings as compared to controls in the ventral striatum and orbitofrontal cortex (*Figure 3, Table S1 in supplementary material*). It is important to note that we contrasted receipt of reward (i.e. correct reward outcome) to correct non-reward outcome. For a complete overview of all outcome conditions, see *Figure S1 in supplementary material*. After correcting for multiple comparisons for the number of ROIs, the effect in bilateral ventral striatum and left orbitofrontal cortex remained significant (*Figure 3*).

Whole-brain analyses showed that healthy controls and siblings activated circuits that are typical for receipt of reward including the orbitofrontal cortex (*Figure 4*). However, a direct comparison of activation levels in the voxel-wise whole-brain analysis did not reveal significant group differences (*Figure 4*).

Correlations

Correlation analyses in siblings revealed that reduced levels of ventral striatum activation during reward anticipation were related to the increased levels of ventral striatum activation during the receipt of reward (striatum left: $r = -0.6$, $p = 0.001$, striatum right: $r = -0.6$, $p = 0.0004$) (*Figure S2 in supplementary material*). Next, left ventral striatal activity in siblings was negatively correlated with the effect of reward on reaction time (mean reaction time during non-rewarding trials minus mean reaction time during potentially rewarding trials) ($r = -0.6$, $p = 0.0005$). This indicates that siblings who showed the most pronounced decrease in left ventral striatum activity did not respond faster on potentially rewarding trials compared to non-rewarding trials.

For siblings, we found a negative correlation between the CAPE negative dimension and activation of the left ventral striatum during reward anticipation ($r = -0.5$, $p = 0.01$) (*Figure S3 in supplementary material*). We did not find such a correlation in healthy controls ($r = -0.01$, $p = 0.98$). No correlations were found between brain activity during reward processing and IQ. There were no differences in brain activation for any of the regions during reward processing between siblings with and without a history of depression. Smoking status as well as cannabis use did not affect activation in the ventral striatum in siblings.

Discussion

This study investigated brain activity during reward processing in 27 unaffected siblings of schizophrenia patients and 29 matched controls using functional MRI. During reward anticipation, siblings showed reduced activation in the left ventral striatum, left supplementary motor area and the right anterior insula as compared to healthy controls. Moreover, decreased ventral striatal activity was correlated with the degree of sub-clinical negative symptoms only in siblings. In contrast, during the receipt of reward siblings showed increased activation in the bilateral ventral striatum and the left orbitofrontal cortex as compared to healthy controls. Since siblings share on average 50% of their genes with their ill relative, these results suggest that impaired reward processing may be related to the genetic vulnerability for schizophrenia (Gottesman and Gould, 2003).

Our finding of decreased ventral striatal activation during reward anticipation in siblings of schizophrenia patients as compared to healthy controls is consistent with several studies in medicated schizophrenia patients (Morris et al., 2012; Murray et al., 2008; Waltz et al., 2009), but not with others (Dowd and Barch, 2012; Juckel et al., 2012; Simon et al., 2010). The latter inconsistency may be partly due to the varying effects of antipsychotics on activation in the fronto-striatal network (Abler et al., 2008; Juckel et al., 2006; Kirsch et al., 2007; M. O. Nielsen et al., 2012; Schlagenhauf et al., 2008). Indeed, findings in medication-free (Juckel et al., 2006; Schlagenhauf et al., 2009) and medication-naïve patients (Esslinger et al., 2012; M. Ø. Nielsen et al., 2012) consistently show diminished ventral striatal activity during reward anticipation. In addition to what has been found in schizophrenia patients and consistent with our results in siblings, a recent study shows decreased ventral striatal activity during reward anticipation in first-degree relatives of schizophrenia, including parents, siblings and adolescent offspring of schizophrenia patients (Grimm et al., 2014). Here, we extend this finding by showing decreased striatal activity in unaffected siblings of schizophrenia patients as compared to controls. Consequently, relative to controls and schizophrenia patients, siblings show intermediate activation levels at best. The attenuated striatal activation during reward anticipation has been suggested to represent a ceiling effect due to an overall increased dopaminergic tone as shown in schizophrenia patients (Davis et al., 1991; Heinz and Schlagenhauf, 2010; Howes and Kapur, 2009). Whether decreased striatal activity in siblings reflects increased dopamine tone should be further explored by PET studies. For example, using PET, Brunelin et al. (Brunelin et al., 2010) reported increased ventral striatal dopamine release in siblings compared to healthy controls in response to metabolic stress.

Siblings with lower activation of the ventral striatum during reward anticipation showed higher scores of sub-clinical measures in the negative domain as measured with the CAPE (Stefanis et al., 2002). High scores in the negative dimension of the CAPE represent sub-clinical symptoms including apathy and lack of motivation. Our finding is consistent with results from Simon et al. who reported similar correlations between decreased ventral striatal activity during reward anticipation and apathy in schizophrenia patients (Simon et al., 2010). Moreover, apathy is associated with dysfunctional dopamine activation in the reward network (Bressan and Crippa, 2005).

In contrast to reduced activation in the ventral striatum during reward anticipation, we found increased activity in the ventral striatum during the receipt of reward in siblings compared to controls. This finding is consistent with one study showing increased ventral striatal activity in schizophrenia patients during successful reward feedback (Schlagenhauf et al., 2009). On the other hand, increased ventral striatal activation is not consistent with the study of Simon et al. (Simon et al., 2010), who found no such an increase in fifteen medicated schizophrenia patients during receiving reward. This discrepancy is likely caused by the fact that all patients received antipsychotic medication which has a direct effect on activation of the fronto-striatal network. Indeed, several studies reported normalization of striatal activation as a result of antipsychotic treatment (Abler et al., 2008; Juckel et al., 2006; Kirsch et al., 2007; M. O. Nielsen et al., 2012; Schlagenhauf et al., 2008).

We found that for siblings the increase in ventral striatal activation during the receipt of reward was related to the reduced activation during reward anticipation. This argues against a dopaminergic ceiling effect as explanation for reduced ventral striatum during anticipation in siblings of patients. Normally, ventral striatal activation occurs in response to an unpredicted reward (Schultz, 2007). When a cue is coupled with a particular outcome (reward), activation shifts from the outcome period to cue presentation, i.e. anticipation (Knutson and Cooper, 2005; Schultz, 2007). This activation shift is known to rely on adequate dopaminergic transmission (Shohamy et al., 2008) and involves the ventral striatum (Schott et al., 2008; Vink et al., 2013). Reduced activity of the ventral striatum during reward anticipation in siblings as compared to controls suggest that this shift from receipt to anticipation of reward does not occur, possibly due to impairments in dopamine transmission (Schultz, 2007). In other words, the hypothesized dopamine dysfunction in siblings may underlie the failure of cues signaling potential reward to trigger activation in the ventral striatum in siblings. As a consequence, striatal activity is relatively increased during receipt of reward. This hypothesis was confirmed by our behavior data indicating that

those siblings who display the most pronounced decrease in ventral striatal activity during reward anticipation together with the most increased ventral striatal activity during receipt of reward, showed the least effect of reward on cues in response times. The failure in siblings to shift activation from outcome to cue is probably not specific for reward, but may reflect impaired cue processing in general. Indeed, we also observed similar striatal hypoactivation in siblings and patients during inhibition (Vink et al., 2006; Zandbelt et al., 2011). Although it is clear that dopamine is pivotal for reward processing (Schott et al., 2008; Schultz et al., 1997), abnormal ventral striatum functioning in siblings may, at least in part, also be attributed to abnormalities involving other neurotransmitters such as glutamate (E M P Poels et al., 2014; Eline M P Poels et al., 2014).

Our finding of decreased activity in the right anterior insula in siblings as compared to controls during reward anticipation is consistent with studies in schizophrenia patients showing reduced anterior insula activity during reward processing (Dowd and Barch, 2012; Morris et al., 2012). Given the fact that insular function is highly dependent on dopamine transmission (Wang et al., 1995; Williams and Goldman-Rakic, 1998), aberrant insular functioning as shown here in siblings suggests impaired dopamine function in siblings similar to that in schizophrenia patients (Gradin et al., 2013; Heinz and Schlagenhauf, 2010).

In addition to reduced activation in the anterior insula, we found decreased activity in the supplementary motor area (SMA) during reward anticipation in siblings compared to controls. This is not surprising given the role of the SMA in motor preparation and the fact that this region is highly interconnected with the ventral striatum (Tanji, 1994; Vink et al., 2013, 2005). Indeed, SMA activity has been consistently found during reward anticipation (Bijleveld et al., 2014; FitzGerald et al., 2009; Roesch and Olson, 2003; Wunderlich et al., 2009). Our finding therefore suggests decreased reward-based motor preparation in siblings.

Finally, we found increased activity in the orbitofrontal cortex during the receipt of reward in siblings compared to controls. This finding is consistent with one study showing exaggerated orbitofrontal cortex activation during reward feedback in schizophrenia patients (Schlagenhauf et al., 2009). However, they contrasted reward outcome with no-reward outcome (i.e. incorrect reward trial), while we contrasted reward outcome with correct non-reward outcome. Post-hoc analyses of our data showed that this particular contrast yielded similar results (*see Figure S1 in supplementary material*).

Our findings of impaired fronto-striatal functioning in non-medicated, disorder-free siblings may represent a genetic vulnerability for schizophrenia. However, common environmental factors cannot be ruled out. In order to fully investigate the genetic determinants of impaired reward processing, future

studies should include monozygotic and dizygotic twins who are discordant for schizophrenia. In another cognitive domain, i.e. spatial working memory, it has been shown that unaffected monozygotic co-twins show the greatest impairments compared to unaffected dizygotic co-twins and healthy control twin pairs, indicating a strong effect of genetic loading (Bachman et al., 2009; Cannon et al., 2000; Glahn et al., 2003).

A potential limitation of this study is that we did not observe behavioral differences between siblings and controls: both groups responded faster on potentially rewarding trials compared to neutral trials. However, this lack of a direct association between brain deficits and performance has been reported on before (Bernacer et al., 2013; Murray et al., 2010; Zandbelt et al., 2011). In contrast, studies in schizophrenia patients using a similar task, have shown that patients respond significantly slower on potential rewarding trials compared to healthy controls (Murray et al., 2008; M. Ø. Nielsen et al., 2012; Schlagenhauf et al., 2009). However, reward experiments in siblings have never been performed, so it is unclear if behavioral deficits are to be expected (de Leeuw et al., 2013; van Buuren et al., 2011b; Zandbelt et al., 2011). Moreover, there have been various studies with other cognitive tasks showing brain activity abnormalities in siblings despite performance being equal to that of healthy participants. The task we used was relatively simple; more complex tasks may provoke behavioral deficits. Finally, using smiling and neutral faces may have had an effect apart from anticipatory processes we aimed to investigate. One could argue that a smiling face by itself is potentially rewarding. We opted to use these intuitive cues rather than the abstract cues developed by Knutson et al. (Brian Knutson et al., 2001), to minimize cognitive load, specifically for the siblings.

Here, we show fronto-striatal impairments during reward processing in unaffected siblings of schizophrenia patients. Specifically, ventral striatum activation was decreased during reward anticipation and increased during receipt of reward in siblings as compared to matched healthy controls. Moreover, decreased ventral striatal activation in siblings was correlated with sub-clinical negative symptoms. These data suggest that impaired cue processing, possibly as a result of dopamine dysfunction, may be related to the genetic vulnerability to develop schizophrenia.

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Supplementary material

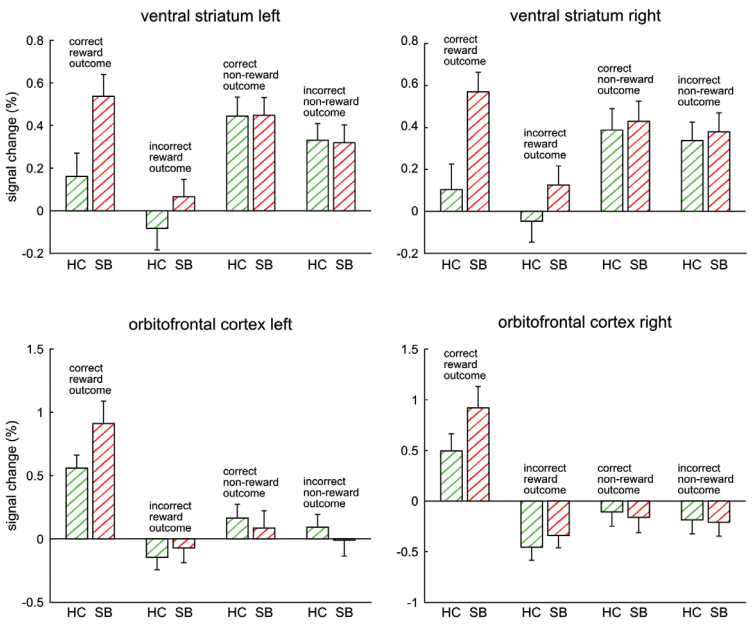
St.1

Region		Reward anticipation		p	Receipt of reward		p
		HC ($n = 29$)	SB ($n = 27$)		HC ($n = 29$)	SB ($n = 27$)	
Ventral striatum	L	0.31 (± 0.03)	0.14 (± 0.05)	0.004*	-0.28 (± 0.07)	0.12 (± 0.09)	0.001*
	R	0.28 (± 0.03)	0.14 (± 0.05)	0.019	-0.28 (± 0.08)	0.18 (± 0.12)	0.002*
Dorsal striatum	L	0.19 (± 0.02)	0.07 (± 0.03)	0.006	-0.20 (± 0.07)	0.00 (± 0.07)	0.041
	R	0.18 (± 0.02)	0.07 (± 0.04)	0.008	-0.22 (± 0.06)	0.01 (± 0.07)	0.028
Insula	L	0.20 (± 0.03)	0.07 (± 0.04)	0.012	0.01 (± 0.09)	0.12 (± 0.10)	0.420
	R	0.18 (± 0.02)	0.07 (± 0.04)	0.018	0.04 (± 0.07)	0.06 (± 0.10)	0.845
SMA	L	0.30 (± 0.03)	0.11 (± 0.05)	0.002*	-0.05 (± 0.08)	0.06 (± 0.11)	0.376
	R	0.28 (± 0.04)	0.11 (± 0.05)	0.006	-0.05 (± 0.10)	0.05 (± 0.11)	0.524
Orbitofrontal cortex	L	0.05 (± 0.05)	-0.07 (± 0.06)	0.113	0.39 (± 0.12)	0.87 (± 0.11)	0.005*
	R	-0.04 (± 0.07)	-0.11 (± 0.07)	0.472	0.60 (± 0.17)	1.14 (±0.15)	0.018

Brain activation levels during reward anticipation and the receipt of reward.

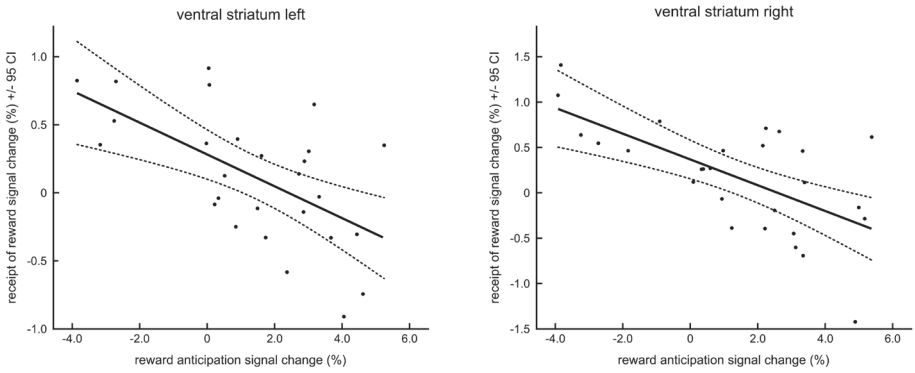
Note: Values represent percent signal change ± SEM. P-values with * survived Bonferroni correction for multiple testing. HC = healthy controls, SB = siblings, SMA = Supplementary Motor Area, L = left, R = right.

Sf.1



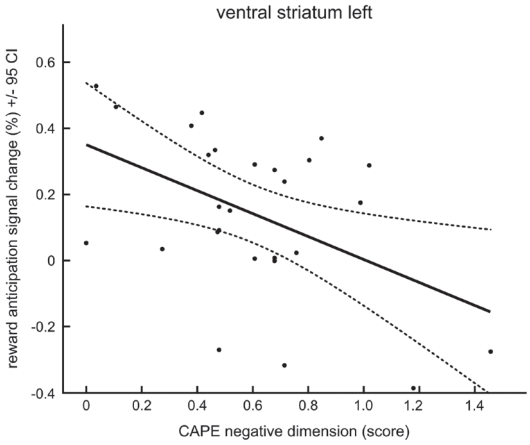
Region of interest analyses in the ventral striatum and orbitofrontal cortex during the outcome phase. Error bars indicate standard error of means. HC = controls, SB = siblings.

Sf. 2



Correlation analyses in siblings revealed that reduced levels of ventral striatum activation during reward anticipation were related to the increased levels of ventral striatum activation during the receipt of reward (striatum left: r = - 0.6, p = 0.001, striatum right: r = - 0.6, p = 0.0004). CI = confidence interval.

Sf. 3



For siblings, a negative correlation between the CAPE negative dimension and activation of the left ventral striatum was found during reward anticipation (r = - 0.5, p = 0.01). CAPE = Community Assessment of Psychic Experiences, CI = confidence interval.

Sub-clinical psychotic measures

The Community Assessment of Psychic Experiences (CAPE; www.cape42.homestead.com, 42 self-reported items) was used to rate self-reports of psychotic experiences in the preceding three years. The CAPE measures frequency as well as distress associated with subclinical positive, negative and depressive symptoms. The original 1 - 4 scale was recoded into a scale of 0 - 3 (zero indicating that psychotic experiences were absent). In order to account for partial non-response, scores were weighted for the number of valid answers per dimension. Studies using the CAPE in general population samples have shown good psychometric properties in terms of reliability and validity (Hanssen et al., 2006; Konings et al., 2006; Stefanis et al., 2002).

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Chapter 4

Reduced fronto-striatal white matter integrity in schizophrenia patients and unaffected siblings: a DTI study

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Based on
npj Schizophrenia, 2015 April; 1: 15001

Abstract

Background/Objectives: Schizophrenia is characterized by impairments in the fronto-striatal network. Underlying these impairments may be disruptions in anatomical pathways connecting frontal and striatal regions. However, the specifics of these disruptions remain unclear and whether these impairments may be related to the genetic vulnerability of schizophrenia is not known. *Methods:* Here, we investigated fronto-striatal tract connections in 24 schizophrenia patients, 30 unaffected siblings and 58 healthy controls using diffusion tensor imaging (DTI). Mean fractional anisotropy (FA) was calculated for tracts connecting the striatum with frontal cortex regions including the dorsolateral prefrontal cortex (DLPFC), medial orbital frontal cortex (mOFC) and inferior frontal gyrus (IFG). Specifically, the striatum was divided into three subregions (caudate nucleus, putamen and nucleus accumbens) and mean FA was computed for tracts originating from these striatal subregions. *Results:* We found no differences between patients, siblings and controls in mean FA when taking the whole striatum as a seed region. However, subregion analyses showed reduced FA in the tract connecting the left nucleus accumbens and left DLPFC in both patients ($p = 0.0003$) and siblings ($p = 0.0008$) compared to controls. *Conclusions:* The result of reduced FA in the tract connecting the left nucleus accumbens and left DLPFC indicates a possible reduction of white matter integrity, commonly associated with schizophrenia. As both patients and unaffected siblings show reduced FA, this may represent a vulnerability factor for schizophrenia.

Introduction

Underlying the clinical and cognitive symptoms in schizophrenia may be dysfunctions of the frontal lobe and the striatum (Davis et al., 1991; Hoptman et al., 2014; van Veelen et al., 2011, 2010; Zandbelt et al., 2011). Indeed, functional MRI studies have demonstrated abnormal fronto-striatal activity (Morris et al., 2012; Nielsen et al., 2012; Quidé et al., 2013; van Veelen et al., 2011, 2010; Vink et al., 2006; Zandbelt et al., 2011). Moreover, structural MRI studies have revealed reductions in brain volume of the frontal cortex (Buchanan et al., 1998; Harms et al., 2010; Oertel-Knöchel et al., 2012) and striatum (Keshavan et al., 1998; Oertel-Knöchel et al., 2012) in schizophrenia patients.

In addition to functional and structural brain measurements, anatomical pathways connecting frontal and striatal regions may also be disrupted in schizophrenia. Two studies have investigated fronto-striatal white-matter tracts using diffusion tensor imaging (DTI). Quan et al. (2013) reported reduced fractional anisotropy (FA) in the tract connecting the left inferior frontal gyrus (IFG) with the striatum in 16 schizophrenia patients compared to 18 matched controls (Quan et al., 2013). However, they only compared tracts connecting frontal regions with the whole striatum, using the striatum as a single seed region, rather than subdividing it into specific subregions. This subdivision is important since different striatal components are involved in specific functional networks (Shohamy, 2011). Indeed, Bracht et al. (2014) investigated white matter tracts connecting the nucleus accumbens with frontal and subcortical regions in schizophrenia patients (n = 24) and controls (n = 22) (Bracht et al., 2014). They reported on increased probability indices forming part of a bundle of interest (PIBI) for the tract connecting the nucleus accumbens with the dorsolateral prefrontal cortex (DLPFC), suggesting reduced white-matter tract integrity. However, they did not find a difference in FA in this tract. This inconsistency makes it unclear if and how this white-matter tract is impaired by schizophrenia. Furthermore, in both studies it remains unclear whether these fronto-striatal white matter tract dysfunctions are related to the illness itself or to a genetic vulnerability for the disorder.

Therefore, we investigated fronto-striatal tracts in a large cohort of schizophrenia patients, unaffected siblings and matched healthy controls using DTI. Siblings do not have the illness, but share on average 50% of their genes with their ill relative (Meyer-Lindenberg and Weinberger, 2006). Furthermore, they have a 10-fold increased risk to develop schizophrenia (Gejman et al., 2011). Consequently, if siblings show impairments in fronto-striatal tract connections similar to those observed in patients this would provide evidence in support for

a genetic vulnerability underlying this phenotypic abnormality. Fronto-striatal tract abnormalities in siblings are anticipated given reports on functional (de Leeuw et al., 2014, 2013; van Buuren et al., 2011; Vink et al., 2014, 2006; Zandbelt et al., 2011) as well as structural abnormalities (Harms et al., 2010; Mamah et al., 2008; Oertel-Knöchel et al., 2012) in this network. Moreover, abnormalities in white matter integrity have already been shown in siblings in other brain regions including the fasiculus arcuatus (Boos et al., 2013; Kubicki et al., 2013; Muñoz Maniega et al., 2008), medial frontal cortex (Camchong et al., 2009), prefrontal cortex (Hao et al., 2009), cingulate and angular gyri (Hoptman et al., 2008), inferior occipitofrontal fasiculus (Kubicki et al., 2013), anterior limb of the internal capsules (Muñoz Maniega et al., 2008), corpus callosum genu (Wang et al., 2011) , cuneus (Moran et al., 2014), and temporal lobe (Wang et al., 2011).

Here, we examined FA in fronto-striatal pathways using DTI in 24 schizophrenia patients, 30 unaffected siblings and 58 healthy controls. FreeSurfer software (Fischl et al., 2004) was used to parcellate the gray matter regions used to trace the fiber bundles of interest. We subdivided the frontal cortex into three regions: DLPFC, medial orbital frontal cortex (mOFC) and IFG, all of which are consistently reported to be abnormal in schizophrenia patients and their siblings in functional (Nielsen et al., 2012; van Buuren et al., 2011; Zandbelt et al., 2011) as well as structural MRI studies (Byun et al., 2012; Harms et al., 2010). Neurons from these frontal regions project to the caudate nucleus, putamen, and nucleus accumbens separately, together forming the fronto-striatal network (Postuma and Dagher, 2006). Mean FA was then computed along averaged tracts starting in each of these striatal subregions directing to the frontal cortex regions.

Given reports on reduced FA in various tracts (for recent review, see Fitzsimmons et al. (Fitzsimmons et al., 2013)), we hypothesize that FA in fronto-striatal white-matter tracts will be reduced in schizophrenia patients. Furthermore, we hypothesize that if these deficits signify a genetic vulnerability, then similar deficits are also present in unaffected siblings of schizophrenia patients.

Methods

Participants

Twenty-four schizophrenia patients, 30 unaffected siblings and 58 healthy control subjects participated in this study. All subjects were right-handed and there were no differences between groups for age and gender (*Table 1*). None of the participants had any contraindications for MRI or suffered from alcohol or drug dependence,

	HC ($n = 58$)	SB ($n = 30$)	SZ ($n = 24$)	Test Statistic	p
Age (years)	28.8 ± 1.0	31.4 ± 1.2	31.1 ± 0.7	F = 2.04	0.14
Gender (M/F)	35/23	17/13	19/5	$\chi^2 = 3.39$	0.18
Participant's education level	7.2 ± 0.2	6.3 ± 0.3	4.8 ± 0.4	F = 15.10	<0.001
Father's education level	5.2 ± 0.5	5.6 ± 0.5	5.4 ± 0.5	F = 0.23	0.80
Mother's education level	4.9 ± 0.5	5.4 ± 0.4	4.6 ± 0.5	F = 0.85	0.43
Cigarette smokers	2	13	12	F = 1.68	0.20
Cigarettes per day	3.5 ± 1.5	10.4 ± 2.1	14.3 ± 3.7	F = 1.16	0.33
Duration of illness (years)			6.2 ± 0.9		
Paranoid/disorganized/undifferentiated type			23/0/0		
Schizoaffective disorder			1		
Chlorpromazine equivalent doses (mg)			321.5 ± 53.4		
PANSS positive symptoms score			14.5 ± 0.8		
PANSS negative symptoms score			13.3 ± 0.7		
PANSS general symptoms score			27.0 ± 1.1		
History of depressive episode		4			

Demographic characteristics of the diagnostic groups.
Abbreviations: HC, healthy controls; SZ, schizophrenia patients; SB, unaffected siblings of schizophrenia patients; M, male; F, female; PANNS, positive and negative syndrome scale. Values represent mean ± SEM. Level of education was measured on a 9-point scale ranging from no education (0) to university degree (8).

which was assessed with the Composite International Diagnostic Interview. Patients were outpatients recruited from the Department of Psychiatry at the University Medical Center Utrecht and participating in an ongoing longitudinal study (Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011). The diagnosis of schizophrenia, schizophreniform or schizoaffective disorder in patients was assessed with the Structured Clinical Interview for DSM IV or the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992). Symptom severity in terms of positive, negative and general symptoms were assessed with the positive and negative syndrome scale (PANSS) (Kay et al., 1987). All schizophrenia patients received antipsychotic medication (medication use is listed in Supplementary information). Four siblings had a history of at least one depressive episode, as verified by the CASH. None of the healthy control subjects had a history of a neurological or psychiatric diagnosis as verified by either the Mini International Neuropsychiatric Interview (Sheehan et al., 1998) or the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) (Wing et al., 1990). Healthy control subjects who had a first-degree relative suffering from a

psychotic disorder were excluded. Participants received monetary compensation for participation. All gave written informed consent. The ethics committee of the University Medical Center of Utrecht approved this study.

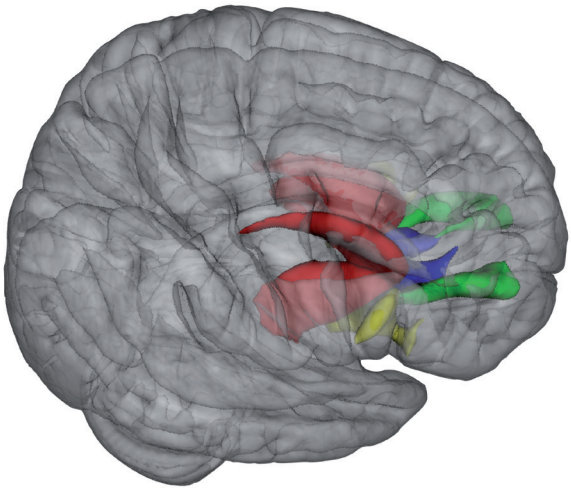
Diffusion tensor imaging

Image acquisition and preprocessing

A T1-weighted structural MRI scan and a set of two diffusion-weighted scans were obtained from each subject using a 3.0 T Achieva scanner (Philips, Best, the Netherlands). One three-dimensional T1-weighted scan (185 slices; repetition time = 8.4 ms; echo time = 3.8 ms; flip angle = 8°; field of view, 252 x 185 x288 mm; voxel size: 1 mm isotropic) of the whole head was made for anatomical reference. The T1-weighted scans were used to extract anatomically delineated region of interest (ROIs) of the caudate nucleus, putamen, nucleus accumbens, DLPFC (consisting of the rostral middle frontal gyrus (Kikinis et al., 2010)), mOFC and IFG (consisting of the Pars Opercularis, Pars Orbitalis and Pars Triangularis) (*Figure 1, Figure 2*) in each hemisphere using the FreeSurfer 5.1.0 structural imaging pipeline (Fischl et al., 2004).

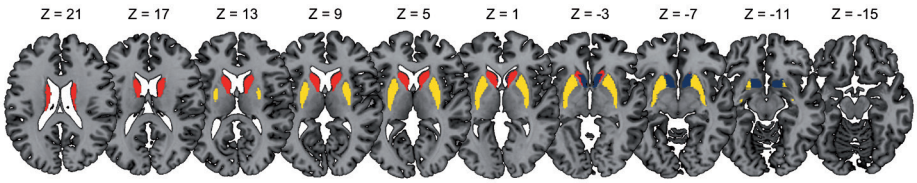
A set of two transverse diffusion-weighted (DWI) scans were acquired (30 diffusion-weighted volumes with different non-collinear diffusion directions with b- factor = 1000 s/mm^2 and 8 diffusion-unweighted volumes with b- factor = 0 s/mm^2; parallel imaging SENSE factor = 2.5; flip angle = 90°; 60 slices of 2.5 mm; no slice gap; 96 x 96 acquisition matrix; reconstruction matrix 128 x 128; FOV = 240 mm; TE = 88 ms; TR = 9822 ms; no cardiac gating; and total scan duration = 296 s). The second DWI scan is identical to the first except that the k-space readout is reversed which allows for correction of susceptibility artifacts during preprocessing. Preprocessing of the DWI scans was performed with the diffusion toolbox of Andersson et al. (Andersson and Skare, 2002; Andersson et al., 2003) and in-house developed software (Mandl et al., 2010). First, susceptibility artifacts were corrected by calculating a distortion map based on the two b = 0 images acquired with reversed k-space readout. Subsequently it was applied to all DWI volumes. This resulted in one corrected DWI set consisting of a single b = 0 volume (averaged over 8 b = 0 volumes) and 30 corrected weighted volumes (Andersson et al., 2003). Finally the DWI set was corrected for eddy-current distortions and small head movements (Andersson and Skare, 2002).

f.1



Mean fractional anisotropy was compared along averaged fibers connecting the striatum (red) with the with frontal cortex regions: striatum - DLPFC (green), striatum - mOFC (blue), and striatum - IFG (yellow). Right = right.

f.2



The striatum was divided into three subregions: nucleus accumbens (blue), caudate nucleus (yellow), and putamen (red) and mean fractional anisotropy was computed for tracts originating from these striatal subregions directing to frontal cortex regions including DLPFC, mOFC and IFG. Right = right.

Fronto-striatal fiber tractography and diffusion parameter reconstruction

Diffusion modeling and probabilistic tractography were carried out using the FMRIB Diffusion Toolbox (FDT, version 2.0, http://fsl.fmrib.ox.ac.uk/fsl/fsl-4.1.9/fdt/fdt—probtrackx.html). This process involves generating connectivity distributions from user-specified seed voxels to target voxels. First, the whole striatum (nucleus accumbens, putamen and caudate nucleus) was used as a seed mask and the three ROI's of the frontal cortex (DLPFC, mOFC and IFG) were defined as target ROI's, such that for each subject three fiber distributions from striatum to frontal cortex were obtained (*Figure 1*). Each frontal ROI was specified as a waypoint and as a termination mask to ensure that only those streamlines running between the seed mask and target ROI were captured in the fiber distribution. The default parameters (5000 streamline samples, step length of 0.5 mm, and curvature threshold of 0.2) were used during the probabilistic fiber tracking procedure.

Subsequently, tracts originating from the three anatomical subregions of the striatum were analyzed separately by using these predefined ROI's as separate seed masks directing to the frontal cortex regions as described above (*Figure 2*). In this way, a total of twelve tracts were traced for and within each hemisphere between the frontal cortex and the striatum, leaving 24 fiber distributions for each subject in total.

Because the seed points could be volumetrically dependent on individual or group differences, a group average fiber was reconstructed for each of the 24 fiber distributions. First, the Tract Based Spatial Statistics (TBSS) toolbox (version 1.2) (Smith et al., 2006) was applied to subjects' fractional anisotropy (FA) maps for warping into FMRIB58—FA standard space. This non-linear registration was also applied to each of the 24 individually obtained fiber distributions. By only selecting the top 1% of streamlines in each fiber distrubution that overlapped in all participating subjects, a total of 24 group average tracts were reconstructed. The group average tracts were made binary and subsequently they were projected onto the warped FA-maps, allowing for the estimation of a mean FA measure per individual per tract.

Statistical analysis

Demographic data between schizophrenia patients, siblings and healthy controls were compared using independent sample t-tests. General linear model (GLM) analyses were performed to test for effects of group (schizophrenia patients, siblings and controls) on FA of the tracts connecting the striatum with the frontal regions (DLPFC, mOFC and IFG). Subsequently, similar GLMs were performed

to test for effects of group (schizophrenia patients, siblings and controls) on fractional anisotropy of the tracts connecting the three subregions of the striatum (caudatus, putamen and nucleus accumbens) with the frontal regions (DLPFC, mOFC and IFG). All results were Bonferroni corrected for multiple testing (three seeds * three targets * two hemispheres = 18), resulting a critical p value of 0.0028. In schizophrenia patients, Pearson's correlations were used to calculate associations between mean FA and symptom severity, as measured with the PANSS. Finally, it has been shown that nicotine use may impact FA measures in fronto-striatal tracts (Savjani et al., 2014), we compared mean FA, using two-sample t-tests, between cigarette smokers and nonsmokers in the whole sample as well as in healthy controls, schizophrenia patients and siblings.

Results

Group differences in fractional anisotropy

There were no significant differences among patients, siblings and controls in mean FA along tracts averaged for the whole striatum with the frontal cortex regions (*Table 2*). However, when investigating tracts projecting from subregions of the striatum, schizophrenia patients as well as their unaffected siblings showed reduced mean FA in the tract between the left nucleus accumbens and left DLPFC (patients versus controls: $t(80) = 3.80$, $p = 0.0003$; siblings versus controls: $t(86) = 3.49$, $p = 0.0008$) (*Table 3*). Patients and siblings did not differ ($t(52) = 0.56$, $p = 0.58$) indicating a similar reduction in FA. Symptoms severity in schizophrenia patients as measured with the PANNS scores did not correlate with mean FA in this tract (PANSS positive symptoms score: $r = 0.1$, $p = 0.65$, PANSS negative symptoms score: $r = -0.6$, $p = 0.77$, PANSS general symptoms score: $r = -0.2$, $p = 0.38$). Finally, smoking status did not affect mean FA for this tract in the whole sample ($t(60) = 0.68$, $p = 0.50$), nor in healthy controls ($t(8) = -1.86$, $p = 0.10$), schizophrenia patients ($t(20) = 0.45$, $p = 0.66$) or siblings ($t(28) = 0.68$, $p = 0.50$). Mean FA in the other tracts did not differ between the groups.

t-2

		HC ($n = 58$)	SB ($n = 30$)	SZ ($n = 24$)	p (HC vs. SB)	p (HC vs. SZ)	p (SB vs. SZ)
Striatum - DLPFC	R	0.36 ± 0.02	0.36 ± 0.02	0.36 ± 0.02	0.80	0.53	0.74
	L	0.35 ± 0.02	0.34 ± 0.02	0.35 ± 0.02	0.67	0.25	0.17
Striatum - mOFC	R	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.71	0.97	0.73
	L	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.61	0.87	0.53
Striatum - IFG	R	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.96	0.30	0.33
	L	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.47	0.93	0.49

Fractional anisotropy for tracts connecting the whole striatum with the frontal cortex regions.

t-3

		HC ($n = 58$)	SB ($n = 30$)	SZ ($n = 24$)	p (HC vs. SB)	p (HC vs. SZ)	p (SB vs. SZ)
Nucleus Accumbens - DLPFC	R	0.38 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.16	0.22	0.92
	L	0.35 ± 0.01	0.34 ± 0.02	0.33 ± 0.02	0.0008*	0.0003*	0.58
Nucleus Accumbens - mOFC	R	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.24	0.63	0.55
	L	0.21 ± 0.01	0.21 ± 0.02	0.21 ± 0.01	0.82	0.80	0.72
Nucleus Accumbens - IFG	R	0.27 ± 0.01	0.27 ± 0.02	0.27 ± 0.01	0.42	0.48	0.94
	L	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.92	0.10	0.16
Caudate - DLPFC	R	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.86	0.23	0.38
	L	0.36 ± 0.02	0.35 ± 0.02	0.36 ± 0.03	0.57	0.17	0.09
Caudate - mOFC	R	0.29 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.70	0.92	0.80
	L	0.28 ± 0.02	0.28 ± 0.01	0.29 ± 0.02	0.92	0.48	0.55
Caudate - IFG	R	0.35 ± 0.01	0.35 ± 0.02	0.35 ± 0.01	0.78	0.19	0.20
	L	0.33 ± 0.01	0.33 ± 0.02	0.33 ± 0.01	0.72	0.95	0.72
Putamen - DLPFC	R	0.39 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	0.89	0.75	0.86
	L	0.38 ± 0.02	0.38 ± 0.02	0.39 ± 0.02	0.55	0.27	0.11
Putamen - mOFC	R	0.32 ± 0.02	0.32 ± 0.02	0.31 ± 0.01	0.67	0.74	0.50
	L	0.28 ± 0.02	0.28 ± 0.01	0.28 ± 0.01	0.41	0.91	0.51
Putamen - IFG	R	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.72	0.33	0.50
	L	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.45	0.84	0.37

Fractional anisotropy for tracts connecting subregions of the striatum with the frontal cortex regions.

Abbreviations: HC, healthy controls; SZ, schizophrenia patients; SB, unaffected siblings of schizophrenia patients; L, left; R, right. Values represent mean ± SD. P-values with * survived Bonferroni correction for multiple testing.

Discussion

This study investigated fronto-striatal pathways in 24 schizophrenia patients, 30 unaffected siblings and 58 healthy controls using DTI. Results show reduced functional anisotropy (FA) in the tract connecting the left nucleus accumbens and left DLPFC in schizophrenia patients as well as their unaffected siblings, indicating reduced white matter integrity compared to controls. Taken together with the fact that siblings share on average 50% of their genes with their ill relative, these results indicate that reduced white matter integrity in this tract may represent a vulnerability factor for schizophrenia.

Our finding of reduced FA in the tract connecting the left nucleus accumbens and left DLPFC is consistent with findings from Bracht et al. (Bracht et al., 2014) who compared fronto-striatal tracts between 24 schizophrenia patients and 22 healthy controls. Although they did not find difference in FA, they computed a measure representing spatial extension of fiber tracts (PIBI: probability indices forming part of a bundle of interest). They found this measure to be increased in schizophrenia patients compared to controls in the tract between the left nucleus accumbens and left DLPFC, and suggest this to indicate volume reduction of this white matter pathway. We replicated and extended this finding by showing decreased FA in this particular tract in schizophrenia patients. Moreover, we found a similar FA reduction in this tract in siblings of schizophrenia patients, indicating that this deficit may represent a vulnerability factor for schizophrenia.

We did not find reduced FA in schizophrenia patients or siblings when averaging over all tracts connecting the entire striatum and DLPFC. This is in line with data from Quan et al. (2013), who also did not find differences between schizophrenia patients (n = 16) and controls (n = 18) in FA values between tracts connecting DLPFC and striatum (Quan et al., 2013). However, they did not investigate tracts originating from different subsections of the striatum.

Our finding of decreased mean FA in both schizophrenia patients and siblings may indicate decreased white matter integrity, since FA is used as an index for the microstructural integrity of white matter fiber bundles (Basser and Pierpaoli, 1996). White matter integrity abnormalities as measured with DTI have been reported in schizophrenia patients and their unaffected siblings in several regions of the brain including the frontal lobe, hippocampus and internal capsule (Boos et al., 2013; Camchong et al., 2009; Fitzsimmons et al., 2013; Hao et al., 2009; Hoptman et al., 2008; Kubicki et al., 2013; Muñoz Maniega et al., 2008; Wang et al., 2011). In the present study, we extend these results by showing white matter integrity reductions in schizophrenia patients and siblings specifically in fibers connecting the left nucleus accumbens and left DLPFC. As we did not

find deficits in other tracks, it is unlikely that our results is driven by a global impairment in white matter integrity. However, it should be noticed that the schizophrenia patients in our sample are relatively young, so that it cannot be ruled out that progression of the illness over time may result in abnormalities of other white matter tracks (Kochunov et al., 2013).

Both the nucleus accumbens and the DLPFC are part of reward pathways (Sesack and Grace, 2010) and are known to be involved in delayed reward processing (Maoz et al., 2013; McClure et al., 2004). Our finding of reduced FA in this particular tract is consistent with earlier reports on impaired frontostriatal reward processing in schizophrenia patients (Morris et al., 2012; Nielsen et al., 2012) and siblings (de Leeuw et al., 2014; Grimm et al., 2014). Our current finding of structural impairments in the reward pathway combined with previous reports of functional fronto-striatal impairments during reward processing adds to the evidence in support of fronto-striatal deficits representing a genetic vulnerability factor for schizophrenia.

The decrease in white matter integrity in schizophrenia patients was not related with symptom severity as measured with the PANSS. This was anticipated given that Bracht et al. also did not find such a relationship (Bracht et al., 2014). Furthermore, siblings showed similar white matter integrity reduction while being symptom-free. Taken together, this null-finding is consistent with the notion that our finding of reduced white matter integrity in the tract connecting left nucleus accumbens and left DLPFC is related to the genetic vulnerability for schizophrenia rather than to the clinical manifestations.

Our study has several limitations which need to be addressed. Schizophrenia patients used antipsychotic medication which may have influence on white matter integrity (Szeszko et al., 2014). However, this is not expected given that we have previously failed to show an effect of medication on white matter volume in schizophrenia patients (Haijma et al., 2013). Moreover, the fact that patients as well as unmedicated siblings show this deficit may indicate that this represents a genetic vulnerability for schizophrenia, rather than a medication effect. However, common environmental factors cannot be ruled out. In order to quantify the influence of genetic factors on the observed reduction in white matter tract integrity, a discordant twin-design would be most suitable. As it has already been shown that white matter integrity is substantially heritable (Bohlken et al., 2014), it is likely that genetic factors have a role in the effect observed in the present study. One other potential limitation is that the patients were not acutely ill as they show moderate PANSS score. However, since unaffected siblings also show decreased FA in the tract connecting the left nucleus accumbens and left DLPFC, it is unlikely that symptom severity impacts the structural impairment we report

on. In contrast, it may very well be that patients that are more severely affected by the illness display FA impairments in additional white matter tracts.

Here, we show impairments in fronto-striatal pathways in schizophrenia patients as well as in unaffected siblings. Specifically, we found mean FA to be decreased in the tract connecting the left nucleus accumbens and left DLPFC. This result is in line with the notion that schizophrenia is characterized by fronto-striatal deficits. Moreover, the present data add to the evidence suggesting that fronto-striatal deficits represent genetic vulnerability factors for schizophrenia. Future research should focus on how this network develops in adolescents at genetic risk for schizophrenia.

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Supplementary information

St.1

Medication	Frequency	Doses (range)
Clozapine	7	200 – 600 mg d.d.
Olanzapine	6	5 – 30 mg d.d.
Risperidon	4	2 – 4 mg d.d.
Aripiprazol	3	15 – 20 mg d.d.
Quetiapine	2	275 – 400 mg d.d.
Penfluridol	1	4 mg d.d.

Antipsychotic medication in the patient group.

St.2

Medication	Frequency	Doses (range)
Paroxetine	4	20 – 50 mg d.d.
Citalopram	4	20 – 40 mg d.d.
Clonazepam	3	0.5 – 3 mg d.d.
Oxazepam	1	10 mg d.d.
Temazepam	1	20 mg d.d.
Alprazolam	1	1 mg d.d.
Lithium	1	1000 mg d.d.
Biperiden	1	2 mg d.d.

Other psychotropic medication in the patient group.
Abbreviations: d.d., daily dose.

PART II

Studies in siblings: imaging genetics

Chapter 5

DRD2 schizophrenia-risk allele is associated with impaired striatal functioning in unaffected siblings of schizophrenia patients

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Abstract

A recent Genome-Wide Association Study (GWAS) showed that the rs2514218 genetic polymorphism in close proximity to DRD2 is strongly ($p = 2.75e\text{-}11$) associated with schizophrenia. To date however, the functional consequence of this polymorphism is unknown. Here, we used functional MRI to investigate the impact of this risk allele on striatal activation during proactive and reactive response inhibition in 45 unaffected siblings of schizophrenia patients. We included siblings to circumvent the illness specific confounds affecting striatal functioning independent from gene effects. Behavioral analyses revealed no differences between the carriers ($n = 21$) and non-carriers ($n = 24$). Risk allele carriers showed a diminished striatal response to increasing proactive inhibitory control demands, whereas overall level of striatal activation in carriers was elevated compared to non-carriers. Finally, risk allele carriers showed a blunted striatal response during successful reactive inhibition compared to the non-carriers. These data are consistent with earlier reports showing similar deficits in schizophrenia patients, and point to a failure to flexibly engage the striatum in response to contextual cues. This is the first study to demonstrate an association between impaired striatal functioning and the rs2514218 polymorphism. We take our findings to indicate that striatal functioning is impaired in carriers of the DRD2 risk allele, likely due to dopamine dysregulation at the DRD2 location.

Introduction

Evidence is accumulating in support of the idea that striatal dopamine dysfunction is at the core of schizophrenia (Grimm et al., 2014; Howes and Kapur, 2009). First, effective antipsychotics function by blocking the striatal dopamine receptor D2 (DRD2) (Davis et al., 1991). Second, functional imaging studies have consistently reported blunted striatal activation in schizophrenia patients across a variety of tasks such as reward processing (Juckel et al., 2006), anti-saccade eye-movements (Raemaekers et al., 2006) and proactive response inhibition (Vink et al., 2006; Zandbelt et al., 2011). Moreover, a recent Genome-Wide Association Study (GWAS) based on the Psychiatric GWAS Consortium (PGC) data showed that the rs2514218 genetic polymorphism in close proximity to DRD2 (i.e. the gene encoding DRD2) is strongly ($p = 2.75e-11$) associated with schizophrenia (Ripke et al., 2014). Since DRD2 is particularly distributed in the striatum, the main subcortical input region for cortical afferents (Alexander et al., 1986) it may very well be that the rs2514218 polymorphism contributes this striatal dysfunction in schizophrenia via a dysregulation of DRD2 availability and dopamine function. However, to date the functional consequences of the rs2514218 polymorphism have not yet been investigated. Other polymorphisms within the dopamine D2 receptor gene, such as rs1076560 (Sambataro et al., 2011), have been linked to striatal function, but show no association with schizophrenia.

Investigating these functional consequences in schizophrenia patients is complicated by the use of antipsychotics. One possible way to circumvent this confound is to test non-medicated siblings of schizophrenia patients who carry the risk allele. These siblings do not have the illness, but share on average 50% of their genes with their ill relative including schizophrenia-risk genes (Gottesman and Gould, 2003; Meyer-Lindenberg and Weinberger, 2006), and have a 10-fold increased risk to develop schizophrenia (Gejman et al., 2011). Although unaffected siblings of schizophrenia patients do not have symptoms, they do show striatal activation deficits during reward anticipation (de Leeuw et al., 2014), working memory (de Leeuw et al., 2013), antisaccade eye movements (Raemaekers et al., 2006), and proactive response inhibition (Vink et al., 2006; Zandbelt et al., 2011).

Here, we test for an association between the DRD2 rs2514218 polymorphism and striatal function using functional MRI in 45 unaffected siblings of schizophrenia patients. Twenty-four siblings carry the schizophrenia-risk allele with the rs2514218 polymorphism encoding DRD2 and 21 matched unaffected siblings do not. All subjects performed a stop-signal anticipation task (Zandbelt and Vink, 2010), which engages the striatum during both proactive and reactive inhibitory control. Specifically, cues are presented to indicate the probability

of having to inhibit a response. Importantly, striatal dopamine D2/D3 receptor availability has been found to be related with inhibition-related fMRI activation in the striatum (Ghahremani et al., 2012). We hypothesized that if disturbances in DRD2 availability underlie reduced striatal functioning in schizophrenia, siblings carrying the rs2514218 risk allele will show diminished striatal activation during response inhibition compared to siblings who do not carry the risk allele.

Methods

Participants

Forty-five unaffected siblings of patients with schizophrenia participated in this study (*Table 1*). All individuals were participating in an ongoing longitudinal study at the Department of Psychiatry at the University Medical Center Utrecht (Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011). All subjects were right-handed. None of the participants received psychotropic medication, had any contraindications for MRI, suffered from alcohol or drug dependence, or had a neurological diagnosis. Participants received monetary compensation for participation. All gave written informed consent. The ethics committee of the University Medical Center of Utrecht approved this study.

Genotypes

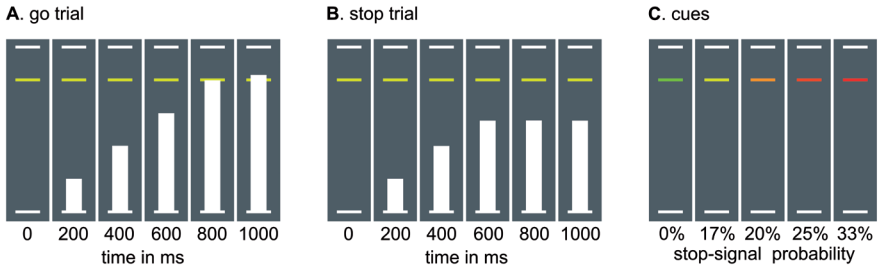
The rs2514218 genotype was retrieved by imputation, as described in the *Supplemental Material 1*. The imputation info score was 0.97. The Hardy-Weinberg equilibrium p-value was 0.11, and the minor allele frequency of rs2514218 in our sample was 0.37. We used SNAP (www.broadinstitute.org/mpg/snap) to output single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) with rs2514218 (r^2 cut-off of 0.5) and provide an overview of these SNPs in the *Supplemental Material 1*. We performed an in silico experiment to determine the relevance of rs2514218 to gene expression to the region of interest in the current study, the striatum, using GTExPortal (http://www.gtexportal.org). This tool allows users to specify regions, SNPs and genes of interest for expression quantitative trait locus (eQTL) analyses. We therefore entered rs2514218, DRD2 and basal ganglia (as this is the region listed that comes closest to the striatum). This demonstrates that rs2514218 has a cis eQTL in the basal ganglia ($p = 0.03$).

t.1

	Siblings carrying no-risk allele ($n = 21$)	Siblings carrying risk allele ($n = 24$)	Test Statistic	p
Age (years)	30.7 ± 1.6	31.8 ± 1.3	F = 0.41	0.60
Gender (M/F)	10/11	11/13	$\chi^2 = 0.14$	0.91
Participant's Education Level	5.6 ± 0.40	6.1 ± 0.36	F = 0.12	0.54
Father's Education Level	5.5 ± 0.53	5.4 ± 0.52	F = 0.71	0.91
Mother's Education Level	4.8 ± 0.51	5.3 ± 0.47	F = 0.04	0.52
SSRT (ms)	327 ± 3	328 ± 4	F = 2.76	0.83
Slope response time (ms)	147 ± 26	136 ± 21	F = 1.33	0.74

Demographic and behavioral characteristics of the diagnostic groups.
Abbreviations: SSRT, stop-signal response time. Values represent mean ± SEM. Level of education was measured on a 9-point scale ranging from no education (0) to university degree (8).

f.1



Stop-signal anticipation task. Three horizontal lines formed the background displayed continuously during the task. A: In each trial, a bar moved at constant speed from the bottom up, reaching the middle line in 800 ms. The main task was to stop the bar as close to the middle line as possible by pressing a button with the right thumb. In other words, the target response time was 800 ms. These trials are referred to as go trials. B: In a minority of trials, the bar stopped moving automatically before reaching the middle line (i.e., the stop-signal), indicating that a response had to be withheld. These trials are referred to as stop trials. C: The probability that a stop-signal would occur was manipulated across trials and was indicated by the color of the target response line. There were five stop-signal probability levels: 0% (green); 17% (yellow); 20% (amber); 25% (orange); and 33% (red).

Functional magnetic resonance imaging

Stop-Signal Anticipation Task

During the fMRI experiment, participants performed the stop-signal anticipation task (Zandbelt and Vink, 2010; Zandbelt et al., 2011), a stop-signal task designed to measure proactive and reactive inhibitory control. The task and experimental procedures were as described before (Zandbelt and Vink, 2010; Zandbelt et al., 2011) and are briefly explained in *Figure 1*. In short, participants are instructed to make timed responses in response to a moving bar (referred to as go trials). In some trials, the bar stops moving (referred to as the stop-signal) and subjects have to refrain from responding. A cue presented at the start of each trial indicates the probability that the bar will stop (stop-signal probability: 0, 17, 20, 25, and 33%). In total, 414 go trials (0%, n = 234; 17%, n = 30; 20%, n = 48; 25%, n = 54; 33%, n = 48) and 60 stop trials (17%, n = 6; 20%, n = 12; 25%, n = 18; 33%, n = 24) were presented in a single run in pseudorandom order. Stop-signal delay, the interval between trial onset and presentation of the stop-signal, was initially 550 ms and varied from one stop trial to the next according to a staircase procedure: if stopping was successful, then stopping was made more difficult on the next stop trial by increasing stop-signal delay by 25 ms. The process was reversed when stopping failed. Each trial lasted 1000 ms, and the intertrial interval was also 1000 ms. Prior to scanning, subjects were trained extensively on the task to ensure that they understood the task and the meaning of the cues.

Behavioral data analyses

Response latency and variability in response latency were calculated to assess baseline task performance. Reactive inhibitory control was indicated by the speed of inhibition, which was measured by the stop-signal reaction time (SSRT, (Zandbelt and Vink, 2010)). The SSRT, reflecting the latency of the inhibition process, was computed according to the integration method and calculated across the four stop-signal probability levels (17 – 33%). Proactive inhibitory control, the anticipation of a stop-signal based on contextual cues, was measured as the slope of response time to increasing stop-signal probability levels (0 – 33%). Hence, a steeper slope indicates better proactive inhibitory control.

Measurements

The experiment was performed on a 3.0 T magnetic resonance imaging scanner (Philips Medical System, Best, the Netherlands) at the University Medical Center Utrecht. We collected 622 whole-brain, T2*-weighted echo planar images with blood oxygen level-dependent contrast (repetition time = 1600 milliseconds, echo time = 23.5 milliseconds, flip angle = 72.5°) in a single run and a T1-weighted

image for within-subject registration purposes (for details, see (Zandbelt and Vink, 2010)).

Functional MRI data analysis

Image data were analyzed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). Preprocessing and first-level statistical analysis were performed as described before (Zandbelt and Vink, 2010). In brief, preprocessing involved correction for slice timing differences, realignment to correct for head motion, spatial normalization to the Montreal Neurological Institute template brain, and spatial smoothing to accommodate inter-individual differences in neuroanatomy. The fMRI data were modeled voxel-wise, using a general linear model, in which the following events were included as regressors: successful stop-signal trials, failed stop-signal trials, and go-signal trials with stop-signal probability > 0%. For go-signal trials, we also included a parametric regressor modeling stop-signal probability level. The fMRI data were high-pass filtered and a first-order autoregressive model was used to model the remaining serial correlations. This task measures contextual cue-processing subdivided into a proactive and reactive component. For each participant, we computed four contrast images, to assess proactive inhibitory control: 1) activation during correct go-signal trials versus go-signal trials in the 0% stop-signal probability context, and 2) the parametric effect of stop-signal probability on go-signal activation; to assess reactive inhibitory control: 3) activation during successful stop-signal trials versus go-signal trials in the 0% stop-signal probability context.

Second, mean activation levels (i.e. parameter estimates) were extracted from ROIs in the striatum for all three contrasts (*Supplementary Material 2*). Analyses were performed to investigate genotype effects (risk allele vs. no risk allele). We defined a significance level of $p \leq 0.05$.

Results

Behavioral results

Results are presented in *Table 1*. Analyses between risk allele carriers and non-carriers revealed no differences in average response latency ($t(43) = 0.67$, $p = 0.51$) nor in variability in response latency ($t(43) = 0.98$, $p = 0.34$), indicating that both groups performed at an equal level during baseline go trials (with a 0% stop-signal probability).

The amount of proactive inhibition, calculated as the degree of response time slowing on go trials as function of stop-signal probability, did not differ

between the groups ($t(43) = 0.34$, $p = 0.74$). Measures of reactive inhibition also did not differ between the groups (speed of inhibition, SSRT: $t(43) = 0.22$, $p = 0.82$; accuracy of inhibition: $t(43) = 0.35$, $p = 0.73$).

Imaging results

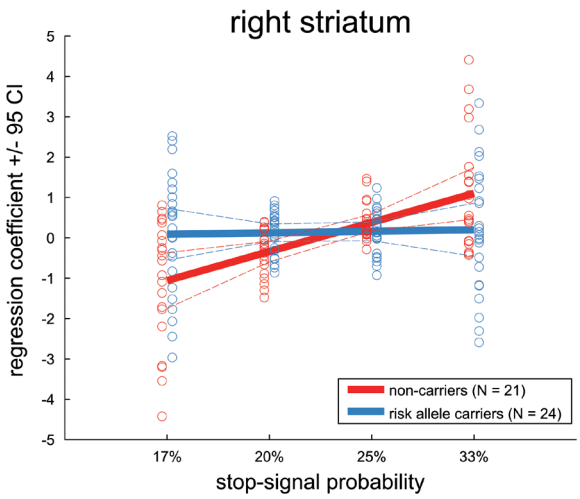
Proactive inhibitory control

Proactive inhibitory control results are shown in *Figure 2*. A regression analysis with stop-signal probability (4 levels: 17%, 20%, 25%, 33%) as within-subject and group (2 levels: risk allele carriers, non-carriers) as between-subject factor was performed on brain activation in the right striatum during proactive inhibitory control. The main effect of stop-signal probability was significant ($F(1,44) = 5.25$, $p = 0.027$), indicating that striatal activation increased with increasing stop-signal probability. However, the group by stop-signal probability interaction was also significant ($F(1,43) = 5.27$, $p = 0.027$), indicating that this effect was driven exclusively by the non-carriers. Indeed, post-hoc analyses revealed that non-carriers showed a significant proactive inhibition effect ($t(20) = 3.41$, $p = 0.003$), while siblings with the risk allele did not ($t(23) = 0.18$, $p = 0.89$). Finally, the main effect of group was significant ($F(1,43) = 6.18$, $p = 0.017$), indicating that siblings who carry the risk allele showed overall higher activation levels in the striatum during trials requiring proactive inhibitory control compared with siblings not carrying the risk allele.

Reactive inhibitory control

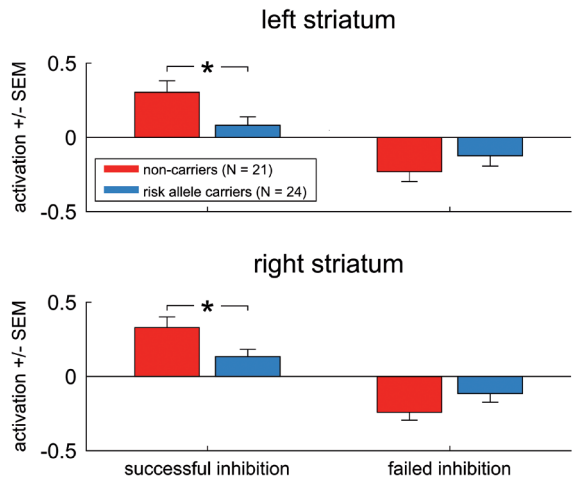
Reactive inhibitory control results are shown in *Figure 3*. Repeated-measures ANOVA's with condition (2 levels: successful inhibition, failed inhibition) as within-subject and group (2 levels: risk allele carriers, non-carriers) as between-subject factor were performed on activation in the left and right striatum. There was a main effect of condition (left: $F(1,43) = 32.33$, $p < 0.001$, right: $F(1,43) = 56.34$, $p < 0.001$), with activation in the striatum being higher after successful inhibition of a motor response as compared to failed inhibition (i.e. when a response was given when it should have been inhibited). However, the group by condition interaction was significant (left: $F(1,43) = 6.78$, $p = 0.01$, right: $F(1,43) = 9.15$, $p = 0.004$), indicating a smaller effect of inhibition success on striatal activation in risk allele carriers as compared to non-carriers. Post-hoc analyses revealed that this diminished effect in risk allele carriers was driven primarily by reduced activation in the striatum during successful inhibition (left: $t(43) = 2.350$; $p = 0.02$, right: $t(43) = 2.303$; $p = 0.03$) in the siblings carrying the risk allele compared with those who do not. Finally, the main effect of group was not significant (left: $F(1,43) = 0.63$, $p = 0.43$, right: $F(1,43) = 0.31$, $p = 0.58$).

f.2



Right striatal activation during proactive control in siblings carrying the risk allele (blue) and siblings who do not (red) plotted against stop-signal probability level (17–33%) with solid lines indicating the group effect and 95% confidence interval indicated by the dotted lines.

f.3



Left and right mean striatal activation during successful and failed inhibition in siblings carrying the risk allele (blue) and siblings who do not (red). Error bars indicate standard error of the mean.
* $p < 0.05$.

Discussion

Here, we investigated the impact of the strongest DRD2 schizophrenia-associated polymorphism to date (rs2514218) on striatal activation in 45 unaffected siblings of schizophrenia patients. These subjects share on average 50% of their genes with their ill relative, but do not take medication, thereby circumventing the confounding effects of antipsychotics on striatal activation. The 21 siblings carrying the risk allele did not differ from the 24 non-carriers on baseline task

performance (response speed, variability of response speed), reactive inhibition (speed and accuracy of inhibition), nor the amount of proactive control.

As expected, however, activation patterns in the striatum did differ between the groups. Risk allele carriers showed a diminished striatal response to increasing proactive inhibitory control demands (i.e. increasing levels of stop-signal probability), indicating that carriers failed to flexibly engage the striatum based on contextual cues. The overall level of striatal activation in carriers was elevated compared to non-carriers during proactive inhibition, possibly reflecting some sort of compensation for the loss of neural flexibility. Finally, risk allele carriers showed a blunted striatal response during successful reactive inhibition compared to the non-carriers.

This is the first study to demonstrate an association between striatal functioning and rs2514218. Our findings indicate that striatal functioning is impaired in carriers of the DRD2 risk allele, likely due to dopamine dysregulation at the DRD2 location. We observed this effect in siblings of schizophrenia patients, in the absence of confounding factors such as antipsychotic medication or secondary effects of the illness itself. Furthermore, both groups performed at an equal level, so that our finding of reduced striatal function cannot be attributed to poor task performance.

Proactive inhibitory control

Proactive inhibitory control is reflected by an increase in response latencies and is typically paralleled by an increase in striatal activation in healthy controls (van Belle et al., 2014; van Rooij et al., 2014; Vink et al., 2015a, 2014a, 2006, 2005; Zandbelt and Vink, 2010; Zandbelt et al., 2013). In the current study, we found such an increase in striatal activation only in the non-carriers of the risk allele. In contrast, DRD2 risk allele carriers did not show such an increase. These results are consistent with our previous reports of reduced striatal flexibility during proactive inhibition in both schizophrenia patients and siblings (Vink et al., 2006; Zandbelt et al., 2011). Therefore, the DRD2 risk allele might be involved in a diminished ability to flexibly engage the striatum in response to contextual cues (i.e. colors indicating stop-signal probability). This is consistent with the finding that striatal D2-like receptor function in humans plays a major role in the neural circuitry that mediates behavioral control, an ability that is essential for adaptive responding and is compromised in a variety of common neuropsychiatric disorders (Ghahremani et al., 2012). This may be the result of dopamine dysfunction at the DRD2 location. Another polymorphism within the DRD2 gene (rs1076560) shifts splicing of the two D2 isoforms, D2 short and D2 long, and has been associated with striatal dopamine signaling as well as

with cognitive processing (Sambataro et al., 2011). Interestingly, similar striatal deficits are observed during reward processing, where siblings (de Leeuw et al., 2014) and relatives of patients (Grimm et al., 2014) fail to engage the striatum in response to cues indicating a potential monetary reward. Moreover, this finding of diminished striatal flexibility mimics the effects of healthy aging on striatal activation during reward anticipation (Schott et al., 2007; Vink et al., 2015b). This is not surprising, given that healthy aging is associated with a decline in striatal dopamine availability (Dreher et al., 2008).

Despite this diminished striatal flexibility, the risk allele carriers did not perform worse than the non-carriers on any of the behavioral measures. This suggests some form of compensation in the risk allele carriers. Indeed, the carriers showed an increased overall level of activation in the striatum compared to non-carriers. This striatal activation increase may reflect an increase in the degree of effort that is invested (Pas et al., 2014). In other words, risk-allele carriers are less efficient in performing the task, since they need to invest more effort to compensate for the failure to flexibly engage the striatum. A similar striatal inefficiency is observed during healthy aging: we found that while performance remained intact in older subjects, striatal flexibility diminished and overall striatal activation levels increased (Vink et al., 2015b).

Reactive inhibitory control

Siblings carrying the DRD2 risk allele showed a blunted striatal response during reactive inhibition (*Figure 3*). Response inhibition is thought to be facilitated by fronto-striatal loops involving the right inferior frontal gyrus (rIFG), striatum, and supplementary motor area (SMA) (Cai et al., 2011; van Belle et al., 2014). Indeed, we have previously shown that repetitive transcranial magnetic stimulation (rTMS) of the rIFG as well as the SMA resulted in faster response inhibition, increased striatal activation, and reduced motor cortex activation (Zandbelt et al., 2013). Such inhibition may involve activation of the indirect pathway, a D2 based circuit within the basal ganglia that has a net inhibitory effect on the cortex (Alexander et al., 1986). In the current study, siblings carrying the DRD2 risk allele show diminished activation during successful response inhibition. Although at first glance this seems to be consistent with a role for the striatum in inhibition, there may be other factors at play. That is, if the height of striatal activation would reflect only inhibitory processing, then our results would suggest striatal hyperactivation in the non-carriers during successful inhibition, as they show a significantly higher level of striatal activation compared to risk allele carriers. However, the level of activation in the non-carriers is comparable with that of healthy volunteers (scanned with the same scanner and task; (Vink et al., 2014b;

Zandbelt et al., 2011)). Rather, striatal activation during successful inhibition may in part also reflect anticipatory processing triggered by contextual cues. This is in line with our current as well as previous proactive inhibition findings: we have shown repeatedly that striatal activation increases with increasing stop-signal probability in healthy volunteers (van Belle et al., 2014; van Rooij et al., 2014; Vink et al., 2015a, 2014a, 2006, 2005; Zandbelt and Vink, 2010; Zandbelt et al., 2013). In the case of successful inhibition, a stop-signal might have been anticipated already at the onset of the trial. In order to be successful, one then simply needs to refrain from responding, without there being the need for active inhibition. Such an interpretation also matches recent studies identifying increased striatal activation already at the onset of a trial in which the cue indicating stop-signal probability is being processed (Zandbelt et al., 2013). Moreover, we have linked the subjective anticipation of a stop-signal to higher striatum activation (Vink et al., 2015a). Unfortunately, the current task is not suited to differentiate between response inhibition and anticipation. Indeed, many processes overlap within the timeframe of a single trial: cue processing, motor preparation and execution or motor preparation and inhibition, outcome and feedback.

Our findings indicate striatal dysfunction during contextual cue-processing to be the functional consequence of carrying the schizophrenia risk allele rs2514218 polymorphism encoding DRD2. This is not surprising, given the role of DRD2 in striatal functioning. It may very well be that carrying the risk allele in siblings result in lower density of striatal DRD2, which in turn may lead to dysfunctional striatal dopamine transmission. Indeed, it has been shown that DRD2 polymorphisms affect DRD2 density in the basal ganglia (Bertolino et al., 2008). Dysfunctional dopamine neurotransmission in the striatum may prevent adequate signaling of cue information to prepare for upcoming events (Schultz, 2007).

Although rs2514218 is a non-functional variant, it lies in close proximity to DRD2 (at 47kb). As demonstrated by our SNAP results, this SNP is in strong LD with several common variants intronic in DRD2. How these SNPs or rs2514218 itself impact the functionality of DRD2 is currently unknown. Follow-up studies, e.g. targeted deep-coverage next-generation sequencing and preclinical studies, may hopefully shed light on this matter in the near future.

The association between brain activation during cognitive functioning and DRD2 genotype variation has been performed in one study in schizophrenia patients, although another DRD2 genotype was used (Vercammen et al., 2014). In this study, brain activity was measured during an emotional go/no-go task in schizophrenia patients and healthy controls genotyped for the DRD2 SNP rs2283265. No relationship between risk allele load and brain activation during

emotional response inhibition in schizophrenia patients was found. However, all patients in this study received antipsychotic medication blocking DRD2, interfering with striatal activation and obscuring the additive effects of the genetic polymorphism. Moreover, rs2283265 polymorphism was not associated with schizophrenia in the recent GWAS study (Ripke et al., 2014). Here, we extended this study by showing for the first time that a schizophrenia-risk allele encoding DRD2 influences striatal functioning in non-medicated unaffected siblings of schizophrenia patients who share 50% of their genes with their ill relative.

Limitations

Although we included 45 siblings, only six of them were homozygote for the risk allele. In the analyses, we combined heterozygote and homozygote carriers. In this way, we could only differentiate between risk allele carriers and non-carriers, but could not determine the additional effect of homozygosity. Furthermore, we included siblings of schizophrenia patients instead of patients. Since these siblings are not ill, the effects we observed may have been dampened by compensatory mechanisms available to these siblings but no longer to actual patients. Also, it is generally assumed that there are multiple genes involved in schizophrenia, with complex gene-gene and gene-environmental interactions underlying the heterogeneous phenotype and the effect the rs2283265 polymorphism has on striatal functioning. We investigated only a single polymorphism, there preventing us from investigating such interactions in siblings. However, the fact that we did observe striatal deficits in these siblings who do not take any antipsychotic medication adds to the strength of our finding.

Summary and conclusion

Our findings suggest a causal mechanism linking the schizophrenia risk allele rs2514218 polymorphism encoding DRD2 to diminished striatal functioning. We observed this effect in non-medicated siblings of schizophrenia patients, thereby circumventing illness-specific confounders. This finding is consistent with the observation of striatal deficits in schizophrenia patients and their first-degree relatives. Future studies should focus on genetic at risk groups such as offspring of patients, to evaluate the impact of this polymorphism on the functional development of the striatum. Moreover, such studies may uncover epigenetic and environmental factors that play a role in determining the effective impact of this polymorphism on the striatum and the development of psychopathology.

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Supplemental material 1

Imputation procedures

Genotypes were collected using the two platforms listed below. Numbers of SNPs and subjects can also be found below.

St.1

	GROUP (07-07-09, Groningen)	WTCCC
Genotype platform	Illumina Human Hap 550k V3	Affymetrix 6.0
# SNPs	547,383	694,673
# Het. Haploid	--	--
# Mendel errors	--	~151k
# Subjects	1,434	1,914
Cases/controls/missing	758/676/--	392/1015/507
Males/females	905/529	937/977
Families	All founders	1,395 founders 519 non-founders *(contains pairs, trios, unrelated)*

We used IMPUTE2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) against the backbone of the 1000 Genomes 2012-July release (www.1000genomes.org). The imputation was done separately for the abovementioned platforms. Pre-imputation QC (quality control) was performed per dataset. Imputation was performed in three steps: (I) pre-imputation QC of genotype data; (II) processing the data for imputation; and (III) imputation. The QC included the followings steps: (I) sex checks; (II) removal of subjects with > 10% genotype missingness; (III) removal of SNPS with missingness in > 10% of subjects; and (IV) exclusion of SNPs with MAF < 0.01.

Supplemental material 2

Regions of interest

Sf. 1

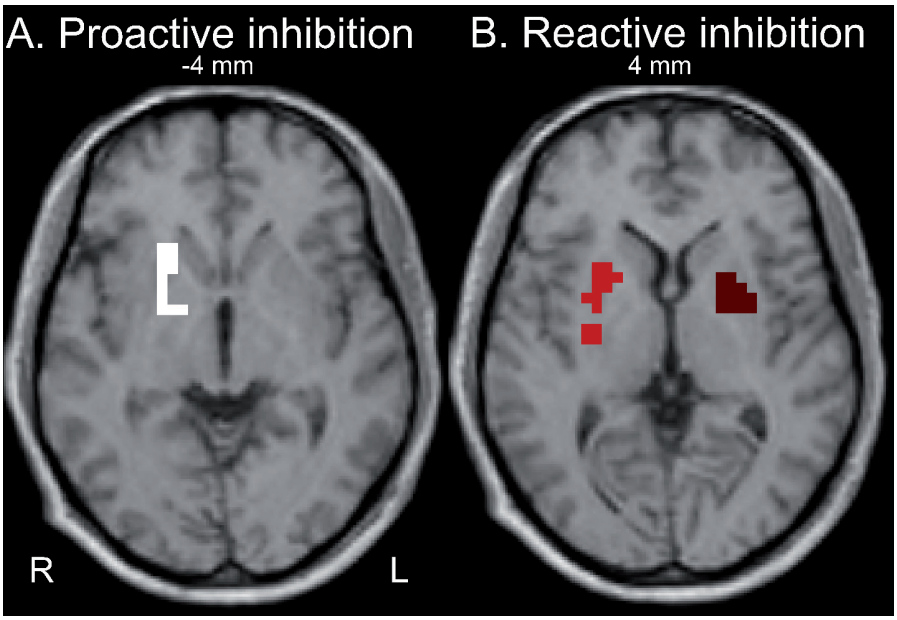

Figure showing the Regions of Interest (ROIs) used to select brain activation in the right striatum for proactive inhibition (panel A) and bilateral striatum for reactive inhibition. These ROIs were defined using an independent sample (see Zandbelt & Vink, 2010). This sample consisted of 24 healthy volunteers performing the same task. The ROI were defined using a cluster-level threshold (cluster-defining threshold $p < 0.001$, cluster probability of $p < 0.05$, family-wise error corrected for multiple comparisons).

PART III

Studies in offspring: neuro-imaging

Chapter 6

Impaired striatal function during reward anticipation in adolescent offspring of schizophrenia patients

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Submitted for publication

Abstract

Schizophrenia is a severe psychiatric disorder associated with impaired fronto-striatal functioning. Similar deficits are observed in unaffected siblings of patients, indicating that these deficits are linked to a familial risk for the disorder. Fronto-striatal deficits may arise during adolescence and precede clinical manifestation of the disorder. However, the development of the fronto-striatal network in adolescents at increased familial risk for schizophrenia is still poorly understood. In this cross-sectional study, we investigate the impact of familial risk on fronto-striatal functioning related to reward anticipation and receipt in 25 adolescent offspring of schizophrenia patients (SZ offspring) and 36 age-matched healthy controls (range 10-19 years). Subjects performed a reward task while being scanned with functional MRI. Overall response times and the amount of money won did not differ between the groups. Striatal activation during reward anticipation increased with age in the healthy controls, while it decreased in the SZ offspring. Activation in the orbitofrontal cortex during reward receipt did not differ between the groups. These results, taken together with data from adult state schizophrenia patients and their siblings, indicate striatal dysfunction to signify a familial vulnerability for schizophrenia.

Introduction

Schizophrenia is a highly heritable psychiatric disorder that is characterized by positive symptoms such as delusions and hallucinations, negative symptoms including affective flattening, as well as cognitive impairments (van Os and Kapur, 2009). Underlying these symptoms may be dysfunctions in the frontal lobe and the striatum (Hahn et al., 2012; McGuire et al., 2008; van Veelen et al., 2011, 2010; Waltz and Gold, 2007; Weinberger and Gallhofer, 1997; Zandbelt et al., 2011). Indeed, the striatum is the primary target of effective antipsychotics. Functional MRI studies in adult schizophrenia patients have consistently demonstrated abnormal fronto-striatal activity in the context of various cognitive tasks (Ehrlich et al., 2012; Koch et al., 2008; Murty et al., 2011; Quidé et al., 2013; Tu et al., 2006; van Veelen et al., 2011, 2010; Vink et al., 2006; Wolf et al., 2011; Zandbelt et al., 2011), in particular those that require the processing of rewards (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; Nielsen et al., 2012; Schlagenhauf et al., 2009). These studies report blunted ventral striatum activation during reward anticipation (Esslinger et al., 2012; Juckel et al., 2006; Morris et al., 2012; Murray et al., 2008; Nielsen et al., 2012; Schlagenhauf et al., 2009) and decreased orbitofrontal activation (Schlagenhauf et al., 2009) during reward receipt. Fronto-striatal deficits are also present in first-degree relatives (de Leeuw et al., 2015, 2014, 2013; Raemaekers et al., 2006; Vink et al., 2006; Zandbelt et al., 2011). Specifically, as in patients, we recently observed hypoactivation in the ventral striatum during reward anticipation in unaffected siblings of schizophrenia patients compared to matched healthy controls (de Leeuw et al., 2014), but found orbitofrontal cortex activation to be increased during receipt of reward. These findings are consistent with those of Grimm et al. (Grimm et al., 2014), who also identified reduced ventral striatum activation in relatives of patients. Taken together, these findings underscore the fact that impaired fronto-striatal function is associated with the familial vulnerability of the disorder.

It is, however, unclear when this familial vulnerability begins to impact fronto-striatal function. Neuropsychological studies report deficits in cognition and behavior (Keshavan et al., 2008; Rapoport et al., 2012) during adolescence in at-risk subjects prior to the clinical diagnosis. This is consistent with the idea that many psychiatric disorders begin to emerge during adolescence (Paus et al., 2008). However, almost no functional neuroimaging studies have been performed to investigate fronto-striatal functioning in young subjects at risk for schizophrenia. For the current study, we included adolescent offspring of schizophrenia patients (SZ offspring), as they are clearly at increased familial risk:

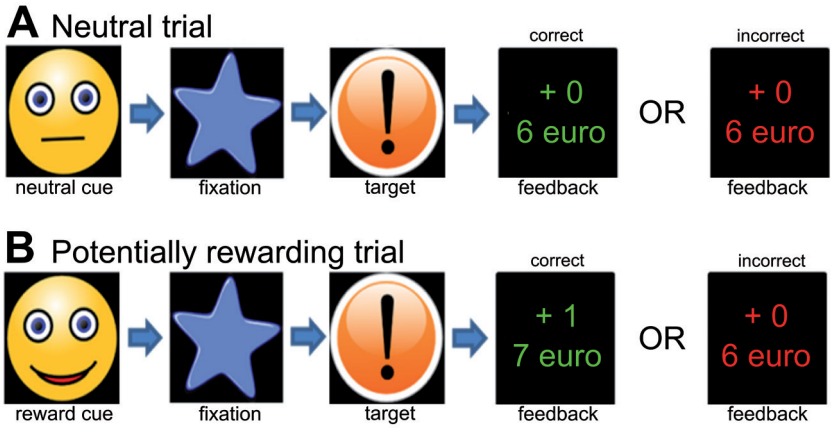
they have a tenfold increased incidence of schizophrenia-related or not otherwise specified psychosis in adulthood (Erlenmeyer-Kimling and Cornblatt, 1987). Importantly, and in contrast to clinical at-risk adolescents (i.e. at-risk mental state), these adolescents are selected based on familial risk alone and do not (yet) have a diagnosis of schizophrenia and consequently do not receive antipsychotic treatment.

In this cross-sectional study, we investigate the impact of familial risk on fronto-striatal functioning during adolescence. We obtained functional MRI data from 25 SZ offspring and 36 age-matched healthy controls (age 10-19 years). Subjects performed a modified version of the Monetary Incentive Delay task (Knutson et al., 2001) which was optimized to analyze changes in brain activation related to the anticipation and receipt of reward separately (Figee et al., 2011; van Hell et al., 2010; Vink et al., 2015). Two bilateral anatomical ROIs were a priori selected, based on their known involvement in the anticipation and outcome of reward (Haber and Knutson, 2010; Brian Knutson et al., 2001): the ventral striatum and orbitofrontal cortex. Based on findings of reduced ventral striatum activation levels in both adult schizophrenia patients and their siblings, combined with what is already known from normal adolescent development (Hoogendam et al., 2013), we hypothesized ventral striatum activation to be diminished in SZ offspring compared to that in healthy control adolescents. Formulating a hypothesis for the orbitofrontal cortex is less clear cut, as activation levels in the adult state differ between patients (reduced levels; (Schlagenhauf et al., 2009)) and their siblings (increased levels; (de Leeuw et al., 2014)) and this region has never been investigated in the context of reward processing in SZ offspring. Furthermore, the frontal cortex is one of the last regions to reach its mature state, so that the impact of the increased familial risk may be expressed only at the very end of adolescence (Casey et al., 2010).

Methods

Participants

Twenty-five SZ offspring (14.2 +/- 2.7 years, 9 males) and 36 unrelated healthy control subjects (13.1 +/- 1.9 years, 21 males) participated in this study. All subjects were right-handed. None of the participants received psychotropic medication, had any contraindications for MRI, suffered from alcohol or drug dependence, had a history of a neurological diagnosis, or psychotic disorder. Psychopathology in SZ offspring and controls was operationalized as current and past (lifetime) DSM-IV axis I disorders, assessed by the Schedule for Affective Disorders and



Schematic representation of the reward task, based on the monetary incentive delay task. There were two types of trials: a non-rewarding neutral (A) and a potentially rewarding trial (B). The inter-trial-interval ranged from 1029 to 6979 ms.

Schizophrenia for School Age Children Present and Lifetime Version (K-SADS-PL). Children and their parents were interviewed separately. None of the healthy controls had an affected first degree relative. DSM-IV axis I disorders and age of onset of parents in the SZ offspring group were ascertained during in-person interviews by the Structured Clinical Interview for DSM-IV Axis I Disorders (First, 1997) and further confirmed by the treating psychiatrist. Control parents were screened for psychiatric disorders by the MINI-SCAN (simplified version of the Schedules for Clinical Assessment in Neuropsychiatry) (Nienhuis et al., 2010), prior to participation.

Head motion parameters were investigated to ensure there were no differences in motion between the groups and that the maximum motion did not exceed predefined thresholds (scan-to-scan: > 3 mm) (Van Dijk et al., 2012).

Participants received monetary compensation for participation. Written informed consent was obtained from both parents or caregivers and offspring older than 12 years of age. The ethics committee of the University Medical Center of Utrecht approved this study.

Monetary Incentive Delay task

Participants performed a modified reward task (*Figure 1*) based on the Monetary Incentive Delay task. This task has been extensively described elsewhere (de Leeuw et al., 2014; Figee et al., 2013, 2011; Hoogendam et al., 2013; van Hell et

al., 2010). Trials were potentially rewarding (30 trials) or non-rewarding (30 trials), as indicated by a cue (smiling or neutral face, respectively) at the start of the trial. Following this cue and a fixation star, the target (exclamation mark) was presented. Participants were instructed to respond as fast as possible to the target by pressing a button, irrespective of cue type. Subjects could win €0.50 in a potentially rewarding trial when they responded within the time limit (duration of the target being presented on the screen). Subsequent feedback notified participants of their performance, indicating if they had earned money on that trial, as well as their cumulative total at that moment. Participants were informed that they would receive the total amount of reward (about €7.50) of the actual experiment in addition to the standard compensation for participation.

Target duration was individually adjusted to ensure that each participant could succeed in 50% of the trials. This adjustment was based on twenty practice trials, presented prior to the start of the experiment. From these practice data, the shortest reaction time to the target was used as individual response limit. In 50% of the trials, the target was presented for the duration of the individual response limit plus 200 ms, enabling participants to be successful. In the other trials, the response limit was decreased with 150 ms, making it virtually impossible to respond in time.

The task was designed in such a way that maximum statistical power concerning the fMRI analyses could be reached in a relatively short time period: only one level of reward was used (i.e. one amount of money) and there were no loss trials. Collinearity between the factors coding for anticipation (i.e. time between presentation of the cue and presentation of the target) and feedback was minimized by varying the duration of the anticipation time randomly (mean duration 3286 ms, range 779 - 6729 ms) and the inter-trial interval (mean duration 3535 ms, range 1029 - 6979 ms). In this way, the blood-oxygen level-dependent (BOLD) signal in response to reward anticipation could be modeled independently from that to reward outcome (Figee et al., 2011; Hoogendam et al., 2013; van Hell et al., 2010). The actual task consisted of 60 trials with a mean duration of 9571 ms (range 4946 - 16107 ms), resulting in a total task duration of 9 m 35 s.

Functional magnetic resonance imaging

Measurements

All imaging was performed on a Philips 3.0-T Achieva whole-body MRI scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a two dimensional echo planar imaging-sensitivity encoding (EPI-SENSE) sequence with the following parameters: voxel size 4 mm isotropic;

repetition time (TR) = 1600 ms; echo time (TE) = 23 ms; flip angle = 72.5°; 30-slice volume; SENSE-factor R = 2.4 (anterior-posterior). Three hundred twenty functional images were acquired during the task. In addition, a whole-brain three-dimensional fast field echo T1-weighted structural image was acquired for within-subject registration purpose. Scan parameters: voxel size 1 mm isotropic; TR = 25 ms; TE=2.4 ms; flip angle = 30°; 150 slices.

Image preprocessing

Image preprocessing and analyses were carried out with SPM5 (http://www.fil.ion.ucl.ac.uk/spm). After realignment, the structural scan was coregistered to the mean functional scan. Next, using unified segmentation, the structural scan was segmented, and normalization parameters were estimated. Subsequently, all scans were registered to a Montreal Neurological Institute T1-standard brain using these normalization parameters and a three-dimensional Gaussian smoothing kernel (8-mm full width at half maximum) was applied to all functional images.

Individual analyses

Individual datasets were analyzed using multiple-regression, to estimate brain activation time-locked to anticipation of reward, anticipation of non-reward, correct reward outcome, incorrect reward outcome, correct non-reward outcome, and incorrect non-reward outcome. To correct for head motion, the six realignment parameters were included in the design matrix as regressors of no interest. A high-pass filter was applied to the data with a cutoff frequency of 0.0058 Hz to correct for drifts in the signal. For each subject, we calculated brain activation related to reward anticipation (anticipation of reward versus anticipation of non-reward) and receipt of reward (correct reward outcome versus correct non-reward outcome).

Region of Interest analyses

A Region of Interest (ROI) analysis was applied to allow the investigation of the effect of group (two levels: SZ offspring, control) and age (and their interaction) on brain activation levels. ROIs were based on definitions of the Anatomic Automatic Labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002) and created using the WFU PickAtlas Toolbox implemented in SPM. The ventral striatum was defined as that part of the caudate nucleus below the z-coordinate of 0 mm. The orbitofrontal cortex ROI consisted of the medial part of the orbitofrontal cortex, entailing the bilateral gyrus rectus and medial orbital gyrus (Zald and Andreotti, 2010). For each participant, the mean activation level (expressed as percent signal change) during the two contrasts of interest (reward anticipation,

reward receipt) was calculated over all voxels in each ROI. Regression analyses were then performed for each ROI separately with activation level as dependent variable and group and age as predictors. We defined a significance level of $p \leq 0.05$.

Results

Behavioral results

Behavioral results are presented in *Figure 2*. All subjects were faster on potentially rewarding trials (296 +/- 4.5 ms) than on neutral trials (307 +/- 5.1 ms ; $F(1,59) = 16.10$; $p < 0.0001$). The main effect of group was significant ($F(1,57) = 5.58$, $p = 0.02$), with SZ offspring showing a larger effect of reward on reaction times (17ms +/- 18.3 ms) than controls (5.1 +/- 21.1 ms; $F(1,57) = 5.59$, $p = 0.02$). The main effect of age ($F(1,57) = 0.04$, $p = 0.85$) and the group by age interaction ($F(1,57) = 1.01$, $p = 0.31$) were not significant.

Additional analyses for reaction times of potentially rewarding and neutral trials separately did not reveal any significant main effects of group nor group by age interactions, indicating that there was no overall difference in performance between the groups. However, the main effect of age was significant for the speed of responding on potentially rewarding trials ($F(1,57) = 17.82$, $p < 0.001$) and of neutral trials ($F(1,57) = 13.62$, $p < 0.001$), with faster responses with increasing age.

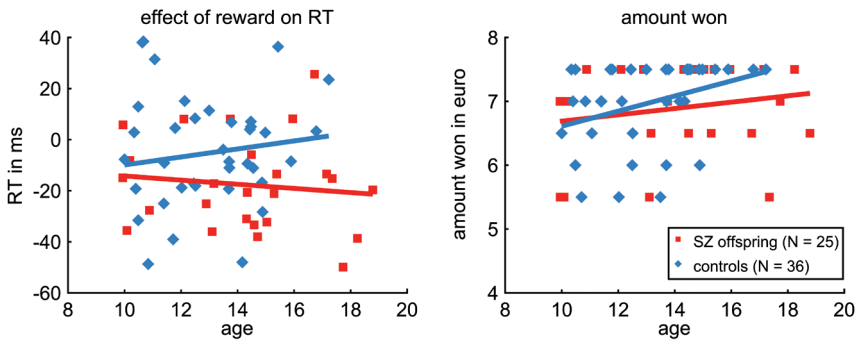
Both groups won the same amount of money (SZ offspring: 6.9 euro +/- 0.15, HC offspring: 6.9 euro +/- 0.67; main effect of group: $F(1,57) = 0.82$, $p = 0.37$). The main effect of age was significant ($F(1,57) = 4.07$, $p = 0.04$), with older subjects winning more money. The group by age interaction was not significant ($F(1,57) = 0.74$, $p = 0.39$), but explorative post-hoc analyses revealed that the age effect was only significant for the controls ($F(1,35) = 4.43$; $r = 0.34$; $p = 0.04$) but not the SZ offspring ($F(1,24) = 0.77$; $r = 0.17$; $p = 0.39$).

Additional analyses for accuracy of potentially rewarding and neutral trials separately did not reveal any significant main effects of group nor group by age interactions, indicating that there was no overall difference in performance between the groups. However, the main effect of age was significant for the level of accuracy on potentially rewarding trials ($F(1,57) = 4.07$, $p = 0.05$) and of neutral trials ($F(1,57) = 10.41$, $p < 0.001$), with higher accuracy with higher age.

Imaging results

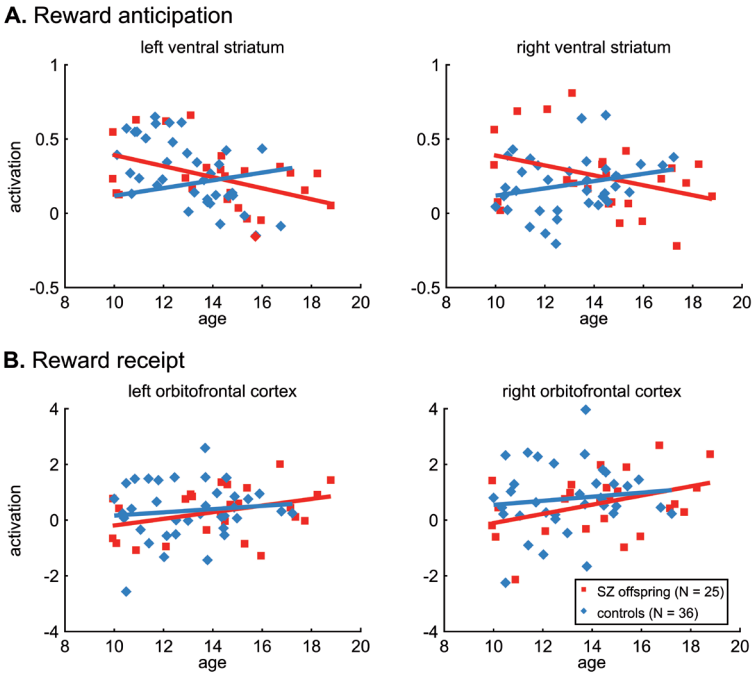
Brain imaging data are presented in *Figure 3*. Regression analyses showed a significant group by age interaction for reward anticipation in the left ($F(1,57) =$

f-2



Behavioral results. The effect of reward on reaction times (RT) and the amount of money won. Graphs show group trend lines (regression lines) and individual observations plotted against age for schizophrenia (SZ) offspring (in red) and controls (in blue).

f-3



Neuroimaging results. A. Activation during reward anticipation versus neutral anticipation in the left and right ventral striatum. B. Activation during reward receipt versus correct neutral outcome in the left and right orbitofrontal cortex. Graphs show group trend lines (regression lines) and individual observations plotted against age for schizophrenia (SZ) offspring (in red) and controls (in blue).

9.07, p = 0.004) and right ventral striatum ($F(1,57) = 5.70$, p = 0.02), with activation during reward anticipation declining across age in SZ offspring while increasing across age in controls. The main effects of group and age were not significant in left and right ventral striatum (all $p > 0.3$).

Additional analyses on activation levels during reward anticipation and neutral anticipation separately did not reveal any significant main effects of group nor group by age interactions, indicating that the effects we observed were not driven by an overall difference in activation levels between the groups.

Finally, we investigated the effect of gender across age and group for the left and right ventral striatum. There was no age by gender interaction effect for each group separately, nor was there a significant interaction between gender and group across age.

Regression analyses showed no significant main effect of group (all $p > 0.3$) nor a group by age interaction (all $p > 0.5$) during reward receipt in the left and right orbitofrontal cortex. The main effect of age showed a trend towards significance (left: $F(1,57) = 2.88$, p = 0.09, right: $F(1,57) = 3.57$, p = 0.06), pointing towards a general increase in activation across age for both groups combined.

Additional analyses on activation levels during reward receipt and correct neutral outcome separately did not reveal any significant main effects of group nor group by age interactions, indicating that the effects we observed were not driven by an overall difference in activation levels between the groups.

Finally, we investigated the effect of gender across age and group for the left and right orbitofrontal cortex. There was no age by gender interaction effect for each group separately, nor was there a significant interaction between gender and group across age.

Discussion

In this cross-sectional study, we show for the first time that activation in the ventral striatum during reward anticipation declines across age in SZ offspring (range 10 – 19 years), indicating impaired development of the striatum during adolescence in subjects at familial risk for schizophrenia. This pattern of decline across adolescence matches ventral striatum hypoactivation observed in both adult schizophrenia patients and their siblings (de Leeuw et al., 2014; Grimm et al., 2014; Juckel et al., 2006). In contrast, ventral striatum activation in controls increased across adolescence, replicating previous work (Hoogendam et al., 2013). We did not find a difference between SZ offspring and controls in orbitofrontal cortex activation. This may reflect the heterogeneity in adulthood,

with decreased activation in schizophrenia patients (Schlagenhauf et al., 2009) and increased activation in siblings (de Leeuw et al., 2014). It may very well be that only those subjects that will go on to develop schizophrenia show frontal deficits. SZ offspring, but not controls, showed a behavioral reward effect with faster responses on potentially rewarding trials compared to trials in which no money could be won. The absence of a behavioral reward effect in healthy control adolescents is consistent with previous reports (Hoogendam et al., 2013). There were no general differences in reaction times between groups, and both groups showed faster responses across increasing age. Also, both groups won the same amount of money, although only the controls showed an increase with age in the amount of money won. Finally, longitudinal follow-up of these subjects is needed to allow the association of specific activation patterns across age with clinical outcomes in adulthood.

Our finding of a decrease in ventral striatum activation across age in SZ offspring compared to control adolescents is consistent with striatal hypoactivation typically observed in adult schizophrenia patients and their siblings (de Leeuw et al., 2014; Grimm et al., 2014; Schlagenhauf et al., 2009). This decrease was observed in absence of a general difference in activation between the controls and SZ offspring, pointing towards a relative hyperactivation at the onset of adolescence and a hypoactivation at the end of adolescence in subjects at familial risk for schizophrenia. In fact, the level of striatal activation observed in SZ offspring at the onset of adolescence resembles levels typically seen in healthy adults (scanned with the same paradigm and scanner (de Leeuw et al., 2014)).

The relative hyperactivation in SZ offspring at the beginning of adolescence implies abnormal development already during childhood, prior to the onset of adolescence. This is consistent with the notion that genetic factors, in combination with environmental factors, may be at the base of this striatal deficit. For example, aberrations in the gene encoding the dopamine 2 receptor (DRD2), which is particularly prevalent in the striatum, may contribute to this striatal deficit in SZ offspring. (Davis et al., 1991; Haber et al., 2000). Indeed, recent data of a Genome-Wide Association Study (GWAS) show that rs2514218 polymorphism of the DRD2 is associated with schizophrenia (Ripke et al., 2014). In contrast to SZ offspring, we observed low levels of striatal activation during reward anticipation in young control adolescents, and this is consistent with most studies of reward processing in healthy adolescents (Bjork et al., 2010; Geier et al., 2010; Hoogendam et al., 2013).

The decline across age and subsequent relative hypoactivation in the older SZ offspring points to additional factors that come into play during adolescent development. Indeed, during adolescence, the brain undergoes major structural

and functional changes (Casey et al., 2010). These changes facilitate the fine-tuning of brain networks (Casey and Caudle, 2013), allowing/enabling higher-order cognitive functioning typical of adulthood. For example, we have previously shown an increase in functional connectivity between frontal and subcortical brain regions during response inhibition (Vink et al., 2014b) and emotion processing (Vink et al., 2014a), both of which are impaired in adult schizophrenia and their siblings (inhibition: (Vink et al., 2006; Zandbelt et al., 2011), emotion: (Baas et al., 2008; Phillips and Seidman, 2008; van Buuren et al., 2011)). With regard to reward processing, the typical pattern of functional development during adolescence is an gradual increase in striatal activation during reward anticipation (Bjork et al., 2010; Geier et al., 2010; Hoogendam et al., 2013). The decline in ventral striatum activation in SZ offspring may be related to the observation of abnormal volumetric changes of subcortical regions across adolescence (Dougherty et al., 2012).

We did not observe differences between SZ offspring and controls in orbitofrontal cortex activation during reward receipt. The orbitofrontal cortex is known to attribute subjective value to a reward and monitor outcome of current choices during decision making (Figee et al., 2011; Haber and Knutson, 2010; Vink et al., 2015). The orbitofrontal cortex is believed to have a function particularly during reward outcome, while its role during reward anticipation is more passive (Diekhof et al., 2012). While fMRI studies have shown blunted orbitofrontal activation during reward receipt in adult schizophrenia patients (Schlagenhauf et al., 2009), we did not find such an effect in adolescent SZ offspring. This lack of a difference in orbitofrontal activation may reflect the possible heterogeneous outcome in the current sample: 15% will develop schizophrenia in adulthood, 60% will develop psychopathology other than psychosis, and about 25% will remain relatively unaffected (Keshavan et al., 2008). Given that adult schizophrenia patients show hypofrontality during reward receipt (Schlagenhauf et al., 2009), it may very well be that only those subjects that will go on to develop schizophrenia show frontal deficits already during adolescence. Moreover, given that adult siblings of schizophrenia patients, despite sharing 50% of the genetic risk, show hyperfrontality rather than hypofrontality (de Leeuw et al., 2014), frontal activation may be elevated throughout development in part of the SZ offspring group (eg those that will not develop schizophrenia), obscuring possible hypoactivation in another subgroup (eg those that will later develop schizophrenia). It is also possibly that frontal deficits in SZ offspring emerge in a later stadium, being the end of adolescence or early adulthood. Lastly, we have shown frontal dysfunction to be present only in certain subgroups of patients, indicating that differences in orbitofrontal activation might exist even between

patients (van Veelen et al., 2011). Structural MRI studies are inconsistent as well. A study by Prasad et al. (Prasad et al., 2010), for example, showed reduced gyral surface area in frontal and parietal regions in SZ offspring (age 10 – 20), while another study did not find structural differences in the frontal lobe in adolescent SZ offspring (Sişmanlar et al., 2010). At this point, there is not enough data available to warrant the assumption of some form of linear relationship between the brain state during adolescence and adulthood. Longitudinal follow-up of all these subjects is required to determine developmental trajectories for clinical subgroups within the group of subjects at familial risk for schizophrenia.

SZ offspring but not control adolescents showed a significant behavioral reward effect, with faster responses when money could be won. Normally, such an effect is observed in healthy adults, but not in children and adolescents (Hoogendam et al., 2013). Importantly, there were no differences in reaction times across conditions, indicating that all subjects performed the task at an adequate level. Furthermore, reaction times decreased across age in both groups, which is consistent with the commonly observed pattern of faster responses in older subjects.

Control adolescents but not SZ offspring showed an increase in the amount of money they won across age. As such, this behavioral effect seems to parallel the change in ventral striatum activation: increase in controls but not in SZ offspring. It should be noted that the task was designed in such a way as to minimize individual differences in the amount of money that is won, to prevent biases in reward anticipation processing. It is possible that without such a manipulation, the difference between the groups may have been more pronounced.

There are several limitations. First, although our study is the first to provide an indication of developmental changes in the fronto-striatal network during adolescence in SZ offspring, these findings should be replicated in a within-subject longitudinal study. Second, we found no differences in age-related changes between males and females. Although such difference may be expected, the current study is likely underpowered to detect such gender effects. Third, related to the second point, the current studied included data of 25 SZ offspring. This is a relative small sample given the age-range (10-19) that is being examined. The size of the sample also limits potential subgroup analyses.

In conclusion, we show here for the first time a decline in ventral striatum activation across adolescent development in SZ offspring. Such a pattern, although established in a cross-sectional study, appears to be consistent with reduced ventral striatum activation in adult schizophrenia patients and their siblings. This finding provides additional evidence for the notion that impaired

ventral striatum activation signifies a familial vulnerability for schizophrenia. In contrast, we did not observe an effect of the familial risk for schizophrenia in the orbitofrontal cortex. Although more data is required, this finding seems to match the heterogeneity of findings in adulthood, with decreased activation in patients and increased activation in siblings of patients sharing the familial risk. Furthermore, since the frontal cortex is among the last brain regions to reach its mature state, effects may only become apparent later on in development, at the onset of adulthood. Long-term longitudinal studies covering adolescence and (early) adulthood are needed to link developmental trajectories to clinical outcomes.

These findings underscore the important role of the striatum in the development of schizophrenia. Given its role in reward processing, impaired striatal function may underlie depressive or negative symptoms that precede the clinical diagnosis of the disorder. Future longitudinal studies should investigate if this striatal deficit is specific for adolescents at increased familial risk for schizophrenia, and link developmental trajectories to clinical outcomes in adulthood.

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Chapter 7

Reduced fronto-striatal white-mater maturation in adolescent offspring of schizophrenia patients

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In preparation

Abstract

Schizophrenia is associated with fronto-striatal network impairments underlying clinical and cognitive symptoms. These impairments include disruptions in anatomical pathways, specifically the tract connecting the left nucleus accumbens and left dorsolateral prefrontal cortex (DLPFC). Similar deficits are observed in unaffected siblings of schizophrenia patients indicating that these deficits are linked to a genetic vulnerability for the disorder. Fronto-striatal tract disruptions may arise during adolescence, preceding the clinical manifestation of the disorder. However, to date no studies have been performed to investigate the development of fronto-striatal tract connections in adolescents who are at increased familial risk for schizophrenia. In this cross-sectional study, we investigate the impact of familial risk on fronto-striatal tract connections using diffusion tensor imaging in 21 adolescent offspring of schizophrenia patients and 30 matched typically developing adolescents, aged 10-18 years. Mean fractional anisotropy (FA) was calculated for the tract connecting the left nucleus accumbens and left DLPFC, as well as for the remaining tracts that connect the striatum with the frontal cortex. As expected, we found an impact of familial risk on fronto-striatal development: schizophrenia offspring showed no increase in FA across age in the tract connecting the left nucleus accumbens and left DLPFC, whereas healthy control adolescents exhibited an increase in FA. For the remaining fronto-striatal tract connections we did not find differences in FA across age between schizophrenia offspring and controls. These results indicate reduced white-mater maturation in subjects who are at familial risk for schizophrenia and match adult state schizophrenia patients and siblings showing reduced fronto-striatal white mater integrity.

Introduction

Underlying clinical and cognitive symptoms in schizophrenia may be dysfunctions of the frontal lobe and the striatum (Davis et al., 1991). First, functional neuroimaging studies have consistently demonstrated reduced fronto-striatal activity levels in schizophrenia patients (Morris et al., 2012; Nielsen et al., 2012; Quidé et al., 2013; van Veelen et al., 2011, 2010; Vink et al., 2006; Zandbelt et al., 2011). Second, patients show decreased frontal and striatal volumes (Buchanan et al., 1998; Harms et al., 2010; Keshavan et al., 1998; Oertel-Knöchel et al., 2012). Finally, diffusion tensor imaging (DTI) have shown reduced white matter integrity in tracts connecting frontal and striatal regions (Bracht et al., 2014; Quan et al., 2013). We recently observed such reductions also in unaffected siblings of schizophrenia patients (de Leeuw et al., 2015). Specifically, we found decreased fractional anisotropy (FA) in the tract connecting the left nucleus accumbens and left dorsolateral prefrontal cortex (DLPFC) for both patients and siblings. Consequently, reduced fronto-striatal white matter integrity may represent a vulnerability factor for schizophrenia. Such an interpretation is in line with other fronto-striatal deficits in first-degree relatives indicating genetic vulnerability for schizophrenia (de Leeuw et al., 2014, 2013; Grimm et al., 2014; Raemaekers et al., 2006; Vink et al., 2006; Zandbelt et al., 2011).

This genetic vulnerability may be at the basis of abnormal fronto-striatal development, preceding the overt manifestation of the illness (Paus et al., 2008; Rapoport et al., 2012). Deficits in fronto-striatal development may become apparent during adolescence, as during this time period the striatum is fully maturing, and the frontal cortex is developing (Casey et al., 2008, 1997). Indeed, healthy adolescents show an increase of white matter integrity in the brain across adolescence and this is reflected by increasing FA measures across age as shown in DTI studies (Cancelliere et al., 2013; Giorgio et al., 2008; Lebel et al., 2008; Peper et al., 2013; Peters et al., 2012). However, to date no DTI studies have been performed that investigated the impact of genetic vulnerability for schizophrenia on development of the tracts in the fronto-striatal network.

Therefore, we explored white matter integrity in fronto-striatal tract connections in a cohort of adolescent offspring of schizophrenia patients (SZ offspring) and matched healthy control adolescents using DTI. Support of fronto-striatal developmental abnormalities comes from a result of a DTI study reporting on increased FA values in the striatum (O'Hanlon et al., 2015), and neuropsychological studies showing deficits in cognition and behavior (Keshavan et al., 2008; Rapoport et al., 2012) in at-risk adolescents prior to the clinical diagnosis of schizophrenia.

Adolescent SZ offspring are considered to be at increased familial risk since environmental factors also play also an important role. Moreover, they have a tenfold increased incidence of schizophrenia-related or not otherwise specified psychosis in adulthood (Erlenmeyer-Kimling and Cornblatt, 1987). Importantly, and in contrast to clinical at-risk adolescents (Yung et al., 1998), these adolescents are selected based on familial risk and do not (yet) have a diagnosis of schizophrenia and consequently do not receive antipsychotic treatment.

Here, in this cross-sectional study we examined FA in fronto-striatal pathways using DTI in 21 SZ offspring and 30 matched typically developing adolescents, aged 10-18 years. FreeSurfer software (Fischl et al., 2004) was used to parcellate the gray matter regions used to trace the fiber bundles of interest. First, we investigated the tract that showed reduced FA in in both adult schizophrenia patients and siblings (de Leeuw et al., 2015), i.e. the pathway connecting the left nucleus accumbens and left DLPFC. We compared FA between SZ offspring and healthy control adolescents, and investigated FA across age in both groups. Finally, we compared mean FA among other fronto-striatal connections, including tracts from striatal subregions (caudate nucleus, putamen, and nucleus accumbens) directing to frontal cortex regions (DLPFC, medial orbital frontal cortex (mOFC) and inferior frontal gyrus (IFG)).

Given our report in schizophrenia patients and siblings (de Leeuw et al., 2015), we hypothesize abnormalities in the tract connecting the left nucleus accumbens and left DLPFC in SZ offspring who are at increased familial risk. Specifically, given the impact of this genetic vulnerability (Rapoport et al., 2012), we hypothesize an abnormal development of FA measure in SZ offspring across age. Finally, as in patients and siblings, we hypothesize no FA differences in the remaining fronto-striatal tracts.

Methods

Participants

Twenty-one SZ offspring (13.1 +/- 2.3 years, 6 males) and 30 typically developing control adolescents patients (13.2 +/- 1.9 years, 18 males) participated in this study. All subjects were right-handed. None of the participants received psychotropic medication, had any contraindications for MRI, suffered from alcohol or drug dependence, had a history of a neurological diagnosis, or a schizophrenia diagnosis (nor any psychosis) as verified by either the Mini International Neuropsychiatric Interview (20 items) (Sheehan et al., 1998) or the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1, 41 items) (Wing et al.,

1990). None of the healthy controls had a relative suffering from a mental disorder. DSM-IV axis I disorders and age of onset of parents in the SZ offspring group were ascertained during in-person interviews by the Structured Clinical Interview for DSM-IV Axis I Disorders (First, 1997) and further confirmed by the treating psychiatrist. Control parents were screened for psychiatric disorders by the MINI-SCAN (simplified version of the Schedules for Clinical Assessment in Neuropsychiatry) (Nienhuis et al., 2010), prior to participation, and excluded from study entry when any lifetime major psychiatric disorder was determined in one of the control parents.

Participants received monetary compensation for participation. Written informed consent was obtained from both parents or caregivers and offspring older than 12 years of age. The ethics committee of the University Medical Center of Utrecht approved this study.

Diffusion tensor imaging

Image acquisition and preprocessing

A T1-weighted structural MRI scan and a set of two diffusion-weighted scans were obtained from each subject using a 3.0 T Achieva scanner (Philips, Best, the Netherlands). One three-dimensional T1-weighted scan (185 slices; repetition time = 8.4 ms; echo time = 3.8 ms; flip angle = 8°; field of view, 252 x 185 x288 mm; voxel size: 1 mm isotropic) of the whole head was made for anatomical reference. The T1-weighted scans were used to extract anatomically delineated regions of interest (ROIs) of the caudate nucleus, putamen, nucleus accumbens, DLPFC (consisting of the rostral middle frontal gyrus (Kikinis et al., 2010)), mOFC and IFG (consisting of the Pars Opercularis, Pars Orbitalis and Pars Triangularis) (*Supplemental Figure 1*) in each hemisphere using the FreeSurfer 5.1.0 structural imaging pipeline (Fischl et al., 2004).

A set of two transverse diffusion-weighted (DWI) scans were acquired (30 diffusion-weighted volumes with different non-collinear diffusion directions with b- factor = 1000 s/mm^2 and 8 diffusion-unweighted volumes with b- factor = 0 s/mm^2; parallel imaging SENSE factor = 2.5; flip angle = 90°; 60 slices of 2.5 mm; no slice gap; 96 x 96 acquisition matrix; reconstruction matrix 128 x 128; FOV = 240 mm; TE = 88 ms; TR = 9822 ms; no cardiac gating; and total scan duration = 296 s). The second DWI scan is identical to the first except that the k-space readout is reversed which allows for correction of susceptibility artifacts during preprocessing. Preprocessing of the DWI scans was performed with the diffusion toolbox of Andersson et al. (Andersson and Skare, 2002; Andersson et al., 2003) and in-house developed software (Mandl et al., 2010). First, susceptibility artifacts were corrected by calculating a distortion map based on the two b = 0

images acquired with reversed k-space readout. Subsequently it was applied to all DWI volumes. This resulted in one corrected DWI set consisting of a single b = 0 volume (averaged over 8 b = 0 volumes) and 30 corrected weighted volumes (Andersson et al., 2003). Finally the DWI set was corrected for eddy-current distortions and small head movements (Andersson and Skare, 2002).

Fronto-striatal fiber tractography and diffusion parameter reconstruction

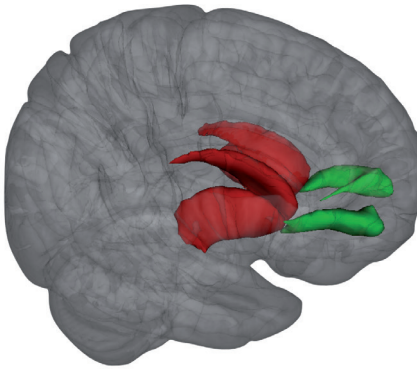
Diffusion modeling and probabilistic tractography were carried out using the FMRIB Diffusion Toolbox (FDT, version 2.0, http://fsl.fmrib.ox.ac.uk/fsl/fsl-4.1.9/fdt/fdt—probtrackx.html). This process involves generating connectivity distributions from user-specified seed voxels to target voxels. The three anatomical subregions of the striatum (nucleus accumbens, putamen and caudate nucleus) (*Supplemental Figure 2*) were used as seed masks and the three ROI's of the frontal cortex (DLPFC, mOFC and IFG) were defined as target ROI's (*Supplemental Figure 1*). In this way, a total of nine tracts were traced for and within each hemisphere between the frontal cortex and the striatum, leaving 18 fiber distributions for each subject in total. Each frontal ROI was specified as a waypoint and as a termination mask to ensure that only those streamlines running between the seed mask and target ROI were captured in the fiber distribution. The default parameters (5000 streamline samples, step length of 0.5 mm, and curvature threshold of 0.2) were used during the probabilistic fiber tracking procedure.

Because the seed points could be volumetrically dependent on individual or group differences, a group average fiber was reconstructed for each of the 18 fiber distributions. First, the Tract Based Spatial Statistics (TBSS) toolbox (version 1.2) (Smith et al., 2006) was applied to subjects' FA maps for warping into FMRIB58—FA standard space. This non-linear registration was also applied to each of the 18 individually obtained fiber distributions. By only selecting the top 1% of streamlines in each fiber distribution that overlapped in all participating subjects, a total of 18 group average tracts were reconstructed. The group average tracts were made binary and subsequently they were projected onto the warped FA-maps, allowing for the estimation of a mean FA measure per individual per tract.

Statistical analysis

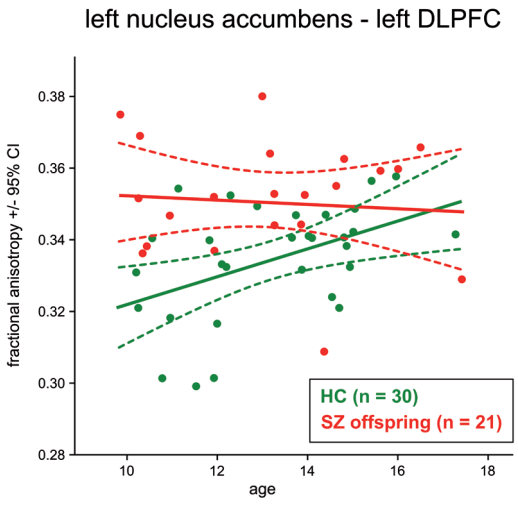
First, a regression analysis was performed to test for effects of group and age on FA in the tract that showed reduced FA in both schizophrenia patients and siblings (de Leeuw et al., 2015), i.e. the tract connecting the left nucleus accumbens and left DLPFC (*Figure 1*).

f.1



The striatum (red) and the tract connecting the nucleus accumbens and DLPFC bilaterally (green). Mean fractional anisotropy was compared along averaged fibers connecting the left nucleus accumbens and left DLPFC.

f.2



Fractional anisotropy in the tract connecting the left nucleus accumbens and left DLPFC. Mean FA increased across age in healthy control adolescents (in green) in this tract, whereas there was no such an increase in offspring of schizophrenia patients (in red). Graphs show group trend lines (regression lines) with 95% confidence interval indicated by the dotted lines, and individual observations plotted against age for offspring of schizophrenia patients (SZ offspring) and healthy controls adolescents (HC).

Next, explorative analyses were performed on FA of the other tracts connecting the three subregions of the striatum (caudatus, putamen and nucleus accumbens) with the frontal regions (DLPFC, mOFC and IFG). These results were Bonferroni corrected for multiple testing, resulting a critical p value of 0.0031.

Results

First, the regression analysis for mean FA in the tract connecting the left nucleus accumbens and left DLPFC showed a significant group by age interaction ($F(1,51) = 4.45$, $p = 0.04$), with mean FA increasing across age in healthy control adolescents ($r = 0.5$, $p = 0.01$), and no such an increase in SZ offspring ($r = -0.09$, $p = 0.71$) (*Figure 2*). There was a main effect of group in this tract showing increased mean FA in SZ offspring (FA: 0.35 ± 0.02) as compared to healthy control adolescents (FA: 0.33 ± 0.02) ($F(1,51) = 7.19$, $p = 0.01$). Next, an explorative analysis for mean FA in the tract connecting the right nucleus accumbens and right DLPFC revealed a main effect of group (FA in SZ offspring: 0.37 ± 0.02; FA in controls: 0.35 ± 0.02; $F(1,51) = 4.27$, $p = 0.04$), and a trend towards a significant group by age interaction ($F(1,51) = 2.31$, $p = 0.14$).

Finally, explorative analyses on mean FA in the remaining tracts projecting from subregions of the striatum directing to the frontal cortex regions did not reveal any significant main effects of group nor group by age interactions (*Supplemental Table*). The main effect of age was not significant.

These results match our previous finding in adult schizophrenia patients and siblings.

Discussion

This cross-sectional study investigated fronto-striatal white-matter tracts in 21 SZ offspring and 30 typically developing control adolescents aged 10-18 years using DTI. Here, we show for the first time developmental abnormalities in (fronto-striatal) white matter integrity in SZ offspring. Specifically, in SZ offspring mean FA did not increase across age in the tract connecting the left nucleus accumbens and left DLPFC, whereas healthy control adolescents showed a (linear) increase in FA. This result matches adult state reduced fronto-striatal white matter integrity observed in both schizophrenia patients and siblings (de Leeuw et al., 2015), and indicates abnormalities (e.g. reduced maturation) in the formation of white-matter tracts in subjects who are at familial risk for schizophrenia. We did

not find differences across age in FA between SZ offspring and controls in other fronto-striatal tracts, and this also resembles results from adult schizophrenia patients and siblings (de Leeuw et al., 2015). Longitudinal follow-up of these subjects is needed so that developmental changes of FA measures across age can be coupled with clinical outcomes in adulthood.

Our finding that FA does not increase in SZ offspring across age in the tract connecting the left nucleus accumbens and left DLPFC is consistent with decreased mean FA in this tract observed in adult schizophrenia patients and siblings (de Leeuw et al., 2015). In contrast, FA measures in healthy control adolescents increased across age, consistent with previous studies (Peper et al., 2013; Peters et al., 2012). As such, these findings provide evidence for the notion that genetic vulnerability for schizophrenia may result in fronto-striatal neurodevelopmental deficits (Paus et al., 2008; Rapoport et al., 2012).

However, (overall) mean FA for this tract in SZ offspring is increased as compared to healthy control adolescents and this is consistent with one study in SZ offspring (age range 9-18) reporting on increased volumetric measures of subcortical regions across age (Dougherty et al., 2012). High FA levels may indicate suboptimal adaptation to a premorbid process, serving as a marker for persistently elevated risk, or instead an effective compensation that is protective against psychosis (Dougherty et al., 2012). Furthermore, increased FA in SZ offspring is most pronounced at the onset of adolescence and therefore indicates abnormal development already during childhood, prior to the onset of adolescence. Together, this support further evidence for the notion that genetic factors, in combination with environmental factors, may be at the base of this deficit (Paus et al., 2008; Rapoport et al., 2012).

Interestingly, mean FA in SZ offspring is slightly higher as compared to adult SZ patients and siblings (we used the same analysis and scanner in our de Leeuw et al. paper (de Leeuw et al., 2015)), indicating that FA is likely to decrease after adolescence only in those subjects that will develop schizophrenia or become a sibling of a patient. In contrast, typically developing adolescents showed lower mean FA levels as compared to control adults, indicating an ongoing continuation of FA values after adolescence, and this is consistent with a neurodevelopment DTI study in young adults (aged 18-25) (Peper et al., 2013).

The results presented here match those in adult state schizophrenia patients and siblings (de Leeuw et al., 2015). This suggests that deficits specifically in the tract connecting the left nucleus accumbens and left DLPFC may be the result of genetic vulnerability for schizophrenia. Indeed, abnormalities in both DLPFC and nucleus accumbens may underlie clinical and cognitive symptoms in schizophrenia, and are consistently found in patients in neuro-imaging studies

(Meyer-Lindenberg et al., 2002; van Veelen et al., 2010). Explorative analyses in the tract connecting the right nucleus accumbens and right DLPFC revealed an increased FA in SZ offspring and no FA differences across age. However, FA values in this tract did not differ in adult state patients and siblings (de Leeuw et al., 2015). Finally, none of the other fronto-striatal tracts revealed a significant difference between SZ offspring and controls, although we found a trend towards significance in SZ offspring for increased FA in the tract connecting the left putamen and left DLPFC.

Since FA is used as an index for the microstructural integrity of white matter fiber bundles (Basser and Pierpaoli, 1996), our finding of increasing FA across age in healthy adolescents may indicate maturation of white matter tracts (Cancelliere et al., 2013; Giorgio et al., 2008; Lebel et al., 2008; Peper et al., 2013; Peters et al., 2012), whereas no such FA increase in SZ offspring may suggest stagnated white matter fiber maturation possibly as a result of genetic vulnerability for schizophrenia. Although the current study focused on fronto-striatal tracts, genetic vulnerability for schizophrenia may also affect the maturation of other white matter tracts. Indeed, white matter integrity abnormalities have been reported in patients and unaffected siblings throughout the brain (Boos et al., 2013; Camchong et al., 2009; Fitzsimmons et al., 2013; Hao et al., 2009; Hoptman et al., 2008; Kubicki et al., 2013; Moran et al., 2014; Muñoz Maniega et al., 2008; Wang et al., 2011).

Interestingly, a recent population-based case-control study showed that adolescent who reported subclinical psychotic symptoms, also showed increased FA in striatal regions (O'Hanlon et al., 2015). Although these individuals do not have a familial vulnerability, they are clearly at increased risk for developing schizophrenia since they display subclinical psychotic symptoms (Yung et al., 1998). This indicates that familial risk as well as clinical risk are both associated with increased mean FA in striatal regions. Whether clinically at-risk individuals also show a lack of FA increase across age should be further explored. What is known to date is that clinically at-risk adolescents do show white matter integrity abnormalities throughout the brain including corpus callosum, internal and external capsule, longitudinal fasiculus, fronto-occipital fasiculus, and posterior corona radiata (Carletti et al., 2012; von Hohenberg et al., 2014).

It is not surprising that we found abnormal FA values in the tract connecting the nucleus accumbens and the DLPFC, given that these regions are key components of the reward network (Maoz et al., 2013; McClure et al., 2004; Sesack and Grace, 2010). Indeed, our finding seems to be consistent with earlier reports on impaired fronto-striatal reward processing in schizophrenia patients (Morris et al., 2012; Nielsen et al., 2012) and siblings (de Leeuw et al., 2014; Grimm et al., 2014). It

may very well be that a genetic vulnerability for white-matter abnormalities together with risk alleles impacting dopamine function may together underlie our finding of abnormal FA values in this particular tract. Such a multifaceted origin may also explain why some white matter tracts are not (or less) affected than others.

Our study has several limitations which need to be addressed. First, although our study is the first to provide an indication of developmental changes in the fronto-striatal network during adolescence in SZ offspring, these findings should be replicated in a within-subject longitudinal study. Second, the current studied included data of 21 SZ offspring. This is a relative small sample given the age-range (10-18) that is being examined. The size of the sample also limits potential subgroup analyses.

Here, we show for the first time in SZ offspring that mean FA in the tract connecting the left nucleus accumbens and left DLPFC does not increase across age whereas healthy control adolescents showed a (linear) increase in FA. Such a pattern, although cross-sectional, appears to be consistent with decreased FA in adult schizophrenia patients and their siblings (de Leeuw et al., 2015) and indicates reduced maturation of white matter integrity. Moreover, no differences in FA across age were found between SZ offspring and controls in other fronto-striatal tracts, and this is also consistent with schizophrenia patients and siblings. Future longitudinal studies are needed to associate developmental trajectories to clinical outcomes in adulthood. These findings underscore the vulnerability of SZ offspring, who show brain abnormalities even in the absence of clear psychopathology or cognitive impairments.

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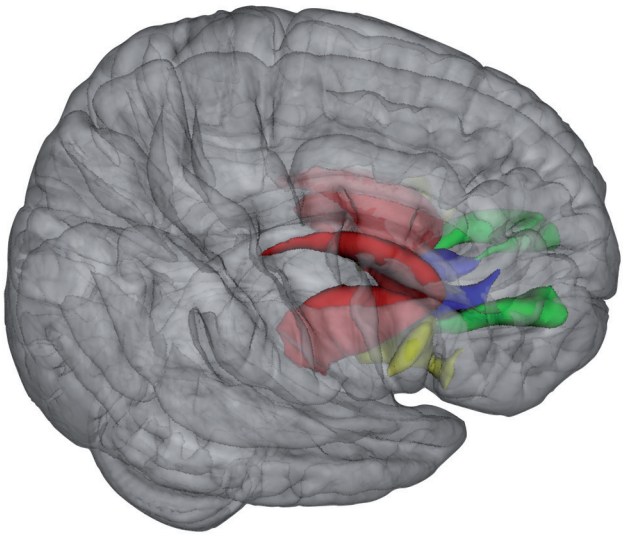
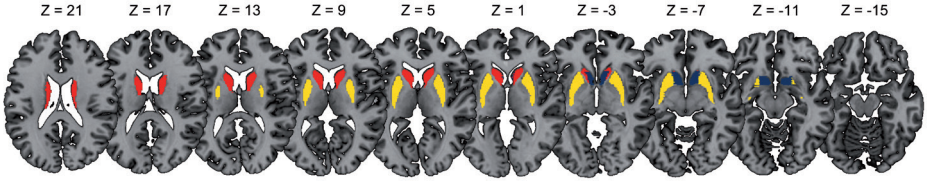
Supplemental material

Sf.1



Mean fractional anisotropy was compared along averaged fibers connecting the striatum (red) with the with frontal cortex regions: striatum - DLPFC (green), striatum - mOFC (blue), and striatum - IFG (yellow). Right = right.

Sf.2



Mean fractional anisotropy was compared along averaged fibers connecting the striatum (red) with the with frontal cortex regions: striatum - DLPFC (green), striatum - mOFC (blue), and striatum - IFG (yellow). Right = right.

		HC ($n = 30$)	SO ($n = 21$)	p (group)	p (age x group)
Nucleus Accumbens - mOFC	R	0.21 ± 0.01	0.21 ± 0.01	0.28	0.22
	L	0.19 ± 0.02	0.19 ± 0.01	0.07	0.05
Nucleus Accumbens - IFG	R	0.30 ± 0.02	0.32 ± 0.02	0.08	0.16
	L	0.29 ± 0.02	0.30 ± 0.01	0.08	0.13
Caudate Nucleus - DLPFC	R	0.33 ± 0.01	0.34 ± 0.01	0.05	0.14
	L	0.33 ± 0.02	0.34 ± 0.02	0.01	0.02
Caudate Nucleus - mOFC	R	0.29 ± 0.01	0.29 ± 0.01	0.10	0.13
	L	0.26 ± 0.01	0.26 ± 0.01	0.01	0.01
Caudate Nucleus - IFG	R	0.29 ± 0.02	0.30 ± 0.01	0.03	0.07
	L	0.32 ± 0.02	0.33 ± 0.01	0.01	0.01
Putamen - DLPFC	R	0.36 ± 0.02	0.38 ± 0.01	0.05	0.14
	L	0.36 ± 0.02	0.38 ± 0.01	0.004	0.01
Putamen - mOFC	R	0.30 ± 0.01	0.30 ± 0.01	0.09	0.17
	L	0.26 ± 0.01	0.27 ± 0.01	0.01	0.03
Putamen - IFG	R	0.26 ± 0.01	0.27 ± 0.01	0.08	0.16
	L	0.25 ± 0.02	0.26 ± 0.01	0.005	0.01

Fractional anisotropy for tracts connecting subregions of the striatum with the frontal cortex regions, other than Nucleus Accumbens - DLPFC.

Abbreviations: HC, healthy controls; SO, schizophrenia offspring; L, left; R, right. Values represent mean ± SD. Note that no result survived Bonferroni correction for multiple testing.

Chapter 8

Discussion

The objectives of the research presented in this thesis were: 1) to investigate whether functional and structural abnormalities in the fronto-striatal network, as these are shown in schizophrenia patients, are related to a genetic vulnerability for schizophrenia by examining this network in non-affected siblings (Part I, Chapter 2, 3 and 4), 2) to examine the underlying genetic underpinnings of abnormal fronto-striatal activation in siblings (Part II, Chapter 5), and 3) to explore the impact of genetic vulnerability on fronto-striatal network development in offspring of schizophrenia patients (Part III, Chapter 6 and 7).

Part I studies in siblings: neuro-imaging

The research described in **Chapter 2-4** centers on fronto-striatal network function in unaffected siblings of schizophrenia patients. These siblings share on average 50% of their genes with their ill relative. Further, although they are not (yet) ill, siblings show cognitive impairments intermediate between patients and controls (Barrantes-Vidal et al., 2007; Brahmbhatt et al., 2006; Chen et al., 2009; Delawalla et al., 2008; Snitz et al., 2006) which may be related to fronto-striatal deficits. To measure fronto-striatal function in siblings, a working memory task and a reward task were used. Activation in siblings was compared to that of matched healthy controls. Importantly, task-performance was matched across group in order to eliminate cognitive impairements in siblings from confounding the imaging results. In addition to measuring task related activation, fronto-striatal white-matters tracts were investigated in schizophrenia patients and siblings as compared to healthy controls.

Fronto-striatal functional deficits in siblings

In **Chapter 2** working memory (WM) and default-mode network (DMN) brain activity was investigated with functional Magnetic Resonance Imaging (fMRI) in 23 non-medicated unaffected siblings of schizophrenia patients and 24 matched healthy controls using a Sternberg WM task. WM load was determined individually so that each participant performed at a 90% accuracy level.

During the encoding phase of WM, siblings showed hyperactivity in the medial prefrontal cortex (MPFC). The MPFC is the anterior part of the DMN and is normally suppressed during WM processing (Mayer et al., 2010; Raichle et al., 2001). The result presented in this thesis suggest that siblings fail to deactivate DMN. Only one study has reported DMN dysfunction in schizophrenia patients and siblings during WM processing (Whitfield-Gabrieli et al., 2009). Consistent with the result in this thesis, they found that patients and siblings showed

MPFC hyperactivation compared to controls. However, in their study overall performance (reaction time and accuracy) was lower in siblings compared to healthy controls.

During the maintenance phase, there were no differences in activation between siblings and controls.

Finally, during the retrieval phase, siblings showed hyperactivation in regions of the WM network such as the striatum, the inferior parietal cortex and the dorsolateral prefrontal cortex (DLPFC). This latter finding is consistent with previous fMRI studies in unaffected siblings during the Sternberg task (Karlsgodt et al., 2007; Meda et al., 2008), the N-back task (Brahmbhatt et al., 2006; Callicott et al., 2003) and the AX-CPT task (Delawalla et al., 2008; Thermenos et al., 2004). The results of hyperactivation in the frontal cortex (e.g. DLPFC) and the striatum may reflect abnormalities in dopamine transmission in siblings.

Regression analysis showed that the amount of activation in the striatum and the DLPFC during retrieval was related to the amount of activation in the MPFC during the encoding phase. Specifically, siblings who failed to suppress the MPFC during encoding showed hyperactivity of the DLPFC and the striatum during retrieval. The finding of striatal hyperactivation in siblings may therefore be causally related to their inability to suppress the DMN. These findings are in line with connectivity data of Whitfield et al. (Whitfield-Gabrieli et al., 2009) showing reduced anti-correlations (negative connectivity during rest and n-back task) between MPFC and DLPFC in siblings. Since adequate DMN deactivation is related to dopamine activation in the striatum (Braskie et al., 2011), this finding may also reflect abnormalities in dopamine transmission. Indeed, in healthy controls, there was not such a relation between DMN suppression and DLPFC activation, suggesting that if DMN activity is adequately suppressed during encoding, DLPFC activity does not need to be enhanced during the retrieval phase of WM.

In **Chapter 3** reward processing was explored in the same cohort of 27 unaffected siblings and 29 matched controls using fMRI. Task performance was manipulated online so that all subjects won the same amount of money.

During reward anticipation, siblings showed reduced activation in the left ventral striatum as compared to healthy controls. The finding of blunted ventral striatal activation during reward anticipation is consistent with findings in medication-free (Juckel et al., 2006; Schlagenhauf et al., 2009) and medication-naïve patients (Esslinger et al., 2012; M. Ø. Nielsen et al., 2012). Moreover, a recent study revealed decreased ventral striatal activity in first-degree relatives of schizophrenia, including parents, siblings and adolescent offspring of

schizophrenia patients (Grimm et al., 2014). The attenuated striatal activation during reward anticipation has been suggested to represent a dysfunction in dopamine transmission.

The decreased ventral striatal activity was correlated with the degree of subclinical negative symptoms in siblings. This finding is consistent with results from Simon et al. who reported similar correlations between decreased ventral striatal activity during reward anticipation and apathy in schizophrenia patients (Simon et al., 2010). Moreover, apathy is associated with dysfunctional dopamine activation in the reward network (Bressan and Crippa, 2005).

During the receipt of reward siblings showed increased activation in the bilateral ventral striatum. This finding is consistent with one study showing increased ventral striatal activity in schizophrenia patients during successful reward feedback (Schlagenhauf et al., 2009), but not with one other study (Simon et al., 2010). This discrepancy is likely caused by the fact that all patients received antipsychotic medication which has a direct effect on activation of the fronto-striatal network (Abler et al., 2008; Juckel et al., 2006; Kirsch et al., 2007; M. O. Nielsen et al., 2012; Schlagenhauf et al., 2008).

Furthermore, the increase in ventral striatal activation during the receipt of reward was related to the reduced activation during reward anticipation in siblings. This may indicate impaired cue processing in siblings (Schultz, 2007): a failure to trigger activation in the ventral striatum in the context of cues signaling potential reward and, as a result striatal hyperactivity during receipt of reward. Since cue processing rely on adequate dopaminergic transmission (Shohamy et al., 2008), these results in siblings may provide further evidence for impairments in dopamine transmission.

Finally, increased activity in the orbitofrontal cortex during the receipt of reward was found in siblings. This finding is consistent with one study showing increased orbitofrontal cortex activation during reward feedback in schizophrenia patients (Schlagenhauf et al., 2009). However, they contrasted reward outcome with no-reward outcome (i.e. incorrect reward trial). In fact, schizophrenia patients were found to show decreased activation during successful reward receipt (Schlagenhauf et al., 2009). The specifics of orbitofrontal activation during the outcome of reward in schizophrenia patients and siblings should be further explored.

Fronto-striatal structural deficits in siblings

In **Chapter 4** fronto-striatal pathways in 24 schizophrenia patients, 30 unaffected siblings and 58 healthy controls using diffusion tensor imaging (DTI) were investigated.

No differences between patients, siblings and controls in mean fractional anisotropy (FA) were found when taking the whole striatum as a seed region. This is in line with data from Quan et al. (2013), who also did not find differences between schizophrenia patients and controls in FA values between tracts connecting DLPFC and striatum (Quan et al., 2013). However, subregion analyses showed reduced FA in the tract connecting the left nucleus accumbens and left DLPFC in schizophrenia patients as well as their unaffected siblings compared to controls. This finding is consistent with findings from Bracht et al. (Bracht et al., 2014) who compared fronto-striatal tracts between schizophrenia patients and healthy controls, although they investigated only patients and computed a measure representing spatial extension of fiber tracts (PIBI: probability indices forming part of a bundle of interest). The finding of decreased mean FA in both schizophrenia patients and siblings may indicate decreased white matter integrity since FA is used as an index for the microstructural integrity of white matter fiber bundles (Basser and Pierpaoli, 1996).

Moreover, the decrease in white matter integrity in schizophrenia patients was not related with symptom severity as measured with the PANSS. This was anticipated given that Bracht et al. also did not find such a relationship (Bracht et al., 2014). Therefore, this finding is consistent with the notion that the finding presented in this thesis of reduced white matter integrity in the tract connecting left nucleus accumbens and left DLPFC is related to the genetic vulnerability for schizophrenia rather than to the clinical manifestations.

Part II studies in siblings: imaging genetics

Given the notion that the findings presented in Part I suggest dopamine dysfunction in schizophrenia (siblings), it is likely that genes involved in dopamine function may contribute to these fronto-striatal abnormalities. Therefore, in **Chapter 5** the impact of the strongest dopamine receptor D2 (DRD2) schizophrenia-associated polymorphism to date (rs2514218) on striatal activation in 45 unaffected siblings of schizophrenia patients was explored, using a stop-signal anticipation task (Zandbelt & Vink, 2010). Twenty-one siblings were risk-allele carriers.

Despite of equal task performance, risk allele carriers showed a diminished striatal response to increasing proactive inhibitory control demands (i.e. increasing levels of stop-signal probability). This result indicates that risk allele carriers failed to flexibly engage the striatum based on contextual cues (i.e. colors indicating stop-signal probability). Similar deficits are observed during

reward processing, where siblings (Chapter 3) and relatives of patients (Grimm et al., 2014) fail to engage the striatum in response to cues indicating a potential monetary reward. Despite this diminished striatal flexibility, the risk allele carriers did not perform worse than the non-carriers on any of the behavioral measures. This suggests some form of compensation in the risk allele carriers. Indeed, the overall level of striatal activation in carriers was elevated compared to non-carriers during proactive inhibition. This striatal activation increase may reflect an increase in the degree of effort that is invested (Pas et al. 2014).

Finally, risk allele carriers showed a blunted striatal response during reactive inhibition compared to the non-carriers. Although this seems to be consistent with a role for the striatum in inhibition, striatal activation during successful inhibition may in part also reflect anticipatory processing triggered by contextual cues (Vink et al., 2015).

The findings presented in this thesis indicate striatal dysfunction during contextual cue-processing to be the functional consequence of carrying the schizophrenia risk allele rs2514218 polymorphism encoding DRD2. Indeed, it has been shown that DRD2 polymorphisms affect DRD2 receptor density in the basal ganglia (Bertolino et al., 2008). Dysfunctional dopamine neurotransmission in the striatum may prevent adequate signaling of cue information to prepare for upcoming events (Chapter 3; Schultz, 2007).

Part III studies in offspring: neuro-imaging

The research in **Chapter 6** and **Chapter 7** examined fronto-striatal network dysfunction in adolescent offspring of schizophrenia patients (SZ offspring), with the aim to unravel the impact of increased genetic vulnerability for schizophrenia on fronto-striatal network development. Since environmental factors also play an important role, these offspring are considered to be at increased familial risk.

Fronto-striatal functional deficits in offspring

In **Chapter 6** reward processing was investigated in 25 SZ offspring and 36 age-matched healthy controls (age 10-19 years).

Activation in the ventral striatum declined across age in SZ offspring. This pattern of decline across adolescence matches the adult state ventral striatum hypoactivation observed in both schizophrenia patients and siblings (Chapter 3; Grimm et al., 2014; Juckel et al., 2006). The decline in ventral striatum activation in SZ offspring may be related to the observation of abnormal volumetric changes of subcortical regions across age (Dougherty et al., 2012). This decrease

was observed in absence of a general difference in activation between the controls and SZ offspring, suggesting a relative hyperactivation at the onset of adolescence and a hypoactivation at the end of adolescence in subjects at familial risk for schizophrenia. The early relative hyperactivation in SZ offspring implies abnormal development already during childhood, prior to the onset of adolescence. This is consistent with the notion that genetic factors, in combination with environmental factors, may be at the base of this striatal deficit. For example the rs2514218 polymorphism of DRD2, which is 1) particularly distributed in the striatum, 2) highly associated with schizophrenia (Ripke et al., 2014) and 3) has an impact of on striatal activation in 45 unaffected siblings (Chapter 5), may contribute to this striatal deficit in SZ offspring (Davis et al., 1991; Haber et al., 2000). In contrast to SZ offspring, low levels of striatal activation during reward anticipation in young control adolescents were observed, and this is consistent with most studies of reward processing in healthy adolescents (Bjork et al., 2010; Geier et al., 2010; Hoogendam et al., 2013).

No difference between SZ offspring and controls in orbitofrontal cortex activation was found. The failure to detect an effect of familial risk for schizophrenia on orbitofrontal activation may not be consistent with a structural MRI by Prasad et al. which showed reduced gyral surface area in frontal and parietal regions in SZ offspring (Prasad et al., 2010). However, another structural MRI study did not find structural differences in the frontal lobe in adolescent SZ offspring (Sişmanlar et al., 2010). The lack of a difference in orbitofrontal activation may reflect the heterogeneity of the sample: 15% will develop schizophrenia in adulthood, 60% will develop psychopathology other than psychosis, and about 25% will remain relatively unaffected (Keshavan et al., 2008). Given that adult schizophrenia patients show hypofrontality during reward receipt (Schlagenhauf et al., 2009), it may very well be that only those subjects that will go on to develop schizophrenia show frontal deficits already during adolescence. Moreover, given the fact that adult siblings of schizophrenia patients, albeit sharing the familial risk, show hyperfrontality (Chapter 2; Chapter 3) rather than hypofrontality, frontal activations may be elevated throughout development in at least part of the SZ offspring group. At this point, however, there is not enough data available to warrant the assumption of some form of linear relationship between the brain state during adolescence and adulthood.

Fronto-striatal structural deficits in offspring

In **Chapter 7** fronto-striatal white-matter tracts were investigated in 21 SZ offspring and 30 typically developing control adolescents aged 10-18 years using DTI.

In SZ offspring, mean FA did not increase across age in the tract connecting the left nucleus accumbens and left DLPFC, whereas healthy control adolescents showed a (linear) increase in FA. The results in this tract are consistent with findings of decreased mean FA observed in adult schizophrenia patients and siblings (Chapter 4) as well as increasing FA measures in healthy control adolescents across age (Peper et al., 2013; Peters et al., 2012). Since FA is used as an index for the microstructural integrity of white matter fiber bundles (Basser and Pierpaoli, 1996), the finding of increasing FA across age in healthy adolescents may indicate maturation of white matter tracts (Cancelliere et al., 2013; Giorgio et al., 2008; Lebel et al., 2008; Peper et al., 2013; Peters et al., 2012), whereas no such FA increase in SZ offspring may suggest stagnated white matter fiber maturation possibly as a result of genetic vulnerability for schizophrenia.

Mean FA for this tract in SZ offspring is increased as compared to healthy control adolescents and this is consistent with one study in adolescent SZ offspring (age range 9-18) reporting on increased volumetric measures of subcortical regions across age (Dougherty et al., 2012). High FA levels may indicate suboptimal adaptation to a premorbid process, serving as a marker for persistently elevated risk, or instead an effective compensation that is protective against psychosis (Dougherty et al., 2012). Interestingly, mean FA in SZ offspring is slightly higher as compared to adult SZ patients and siblings (Chapter 4), indicating that FA is likely to decrease after adolescence only in those subjects that will develop schizophrenia or become a sibling of a patient. In contrast, control adolescents showed lower mean FA levels as compared to control adults, indicating an ongoing continuation of FA values after adolescence, and this is consistent with a neurodevelopment DTI study in young adults (aged 18-25) (Peper et al., 2013).

No differences in FA across age were found between SZ offspring and controls in other fronto-striatal tracts. This matches results in adult state schizophrenia patients and siblings, in whom differences were found in mean FA only in the tract connecting the left nucleus accumbens and left DLPFC. These findings combined suggest that deficits specifically in this tract may be the result of genetic vulnerability for schizophrenia. Further, abnormalities in both DLPFC and nucleus accumbens may underlie clinical and cognitive symptoms in schizophrenia, and are consistently found in patients in neuro-imaging studies (Meyer-Lindenberg et al., 2002; van Veelen et al., 2010).

Implications

The finding of fronto-striatal impairments in both unaffected siblings and offspring of schizophrenia patients underscores the point that family matters: increased familial risk leads to brain abnormalities even in the absence of clear psychopathology. The results presented in this thesis may provide clues for the development of novel early intervention strategies, aimed at minimizing the impact of the familial risk on the development of brain function. An optimistic outcome could be delaying disease development and expression as well as mitigating the social burden for patients and those around them. Specifically, drugs that target the striatum during development may be of therapeutically consideration. For example pharmacological inhibition of PDE10A has already been proven to be effective in preclinical models of psychotic, cognitive, and negative symptoms of schizophrenia (Charych et al., 2010; Grauer et al., 2009). PDE10A is predominantly expressed in the putamen, caudate nucleus, exclusively in medium spiny neurons (Simpson et al., 2010; Xie et al., 2006).

In addition to early intervention, the data in this thesis may provide stepping-stones for the development of new diagnostic strategies of at-risk individuals. To date, neurocognitive, clinical, and behavioral data have not revealed specific impairments to determine who has the highest chance of actually developing schizophrenia. Combining these data with neuroimaging measurements and genetic information may increase sensitivity, which in turn could lead to a better identification of those participants at the highest risk for abnormal development in an early stage. It could be that future help-seeking individuals who are at increased familial risk will perform a blood DNA test examining schizophrenia-associated polymorphisms, as well as undergo fMRI scanning while performing tasks that evoke fronto-striatal activity. If these individuals show a particular blueprint of deficits across multiple domains, then this information could aid risk assessment and subsequent interventions. Such risk assessment has already been used for myocardial infarction (http://cvdrisk.nhlbi.nih.gov/).

Finally, if siblings and offspring are aware of their vulnerability, this may lead to a better compliance to strategies focused on the prevention of psychosis.

Future research

Common environmental factors cannot be ruled out. First, in order to fully investigate the genetic determinants of fronto-striatal impairments, future studies should include monozygotic and dizygotic twins who are discordant for

schizophrenia. For example a discordant twin-design would be most suitable. Indeed, during spatial memory it has been shown that unaffected monozygotic co-twins show the greatest impairments compared to unaffected dizygotic co-twins and healthy control twin pairs, indicating a strong effect of genetic loading (Bachman et al., 2009; Cannon et al., 2000; Glahn et al., 2003).

Second, whether abnormal striatal activity in siblings and offspring reflects dopamine dysfunction should be further explored by PET studies. For example, using PET, Brunelin et al. reported increased ventral striatal dopamine release in siblings compared to healthy controls in response to metabolic stress (Brunelin et al., 2010).

Finally, longitudinal follow-up studies of adolescent offspring of schizophrenia patients are needed to allow the association of specific activation patterns during reward processing across age with clinical outcomes in adulthood. In addition, investigating FA measures of the fronto-striatal network in a longitudinal follow-up study would elaborate the specifics of these structural abnormalities across age in offspring of schizophrenia patients. Moreover, these studies are required to determine developmental trajectories for clinical subgroups within the group of subjects at familial risk for schizophrenia.

Summary and conclusion

PART 1 Siblings of schizophrenia patients, like their ill family member, show fronto-striatal impairments.

PART 2 Siblings carrying the strongest schizophrenia-associated polymorphism encoding dopamine D2 receptor show the most pronounced impairments in striatal functioning.

PART 3 Adolescent offspring of schizophrenia patients show fronto-striatal deficits.

In short, family matters!

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Chapter 9

Nederlandse samenvatting

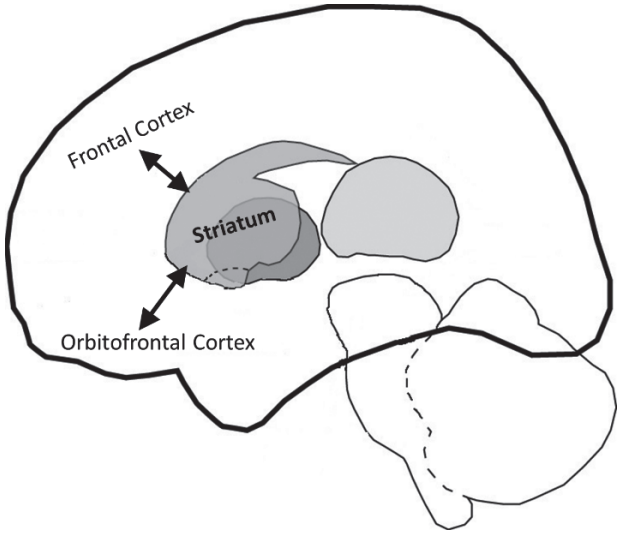
Achtergrond

Schizofrenie is een psychiatrische ziekte die wordt gekenmerkt door positieve symptomen zoals wanen en hallucinaties, negatieve symptomen waaronder vervlakt affect en terugtrekgedrag, alsook cognitieve defecten. Deze symptomen worden veroorzaakt door disfunctioneren van de frontaal cortex en het striatum: dopamine hyperactiviteit in het striatum en dopamine hypoactiviteit in de frontaal cortex. Striatale hyperactiviteit wordt geassocieerd met psychose (positieve symptomen). Behandeling van psychose bestaat uit medicatie die hun werk doen door de verhoogde dopamine activiteit in het striatum te blokkeren. Frontale hypoactiviteit wordt geassocieerd met negatieve symptomen. De frontale cortex en het striatum vormen tezamen een netwerk, het zogeheten fronto-striatale netwerk (*Figuur 1*). Naast de klinische symptomen, wordt verondersteld dat stoornissen in dit netwerk, ook verantwoordelijk zijn voor de typerende cognitieve stoornissen van schizofrenie.

Schizofrenie is een overerfbare aandoening. Wanneer iemand van een eeneiige tweeling schizofrenie heeft, dan is meer dan 40% van de andere helft van de tweeling ook aangedaan. Ongeveer 13% van de kinderen is aangedaan wanneer één van de ouders schizofrenie heeft en dit risico wordt verhoogd naar ongeveer 50% wanneer beide ouders de diagnose schizofrenie hebben. Broers en zussen van schizofreniepatiënten hebben een 10 keer zo hoge kans om schizofrenie te ontwikkelen. Hoewel deze familieleden (nog) niet ziek zijn, vertonen zij weldegelijk cognitieve gebreken in een mate van ernst tussen patiënten en gezonde controles in. Dit zou op een disfunctionerend fronto-striataal netwerk kunnen duiden, in afwezigheid van de daadwerkelijke ziekte.

Het doel van het onderzoek in dit proefschrift was dan ook om in deze eerstegraads familieleden de functie van het fronto-striatale netwerk te meten door middel van verschillende neuro-imaging technieken. Niet-aangedane broers en zussen alsook kinderen van schizofreniepatiënten werden onderzocht. Broers en zussen delen ongeveer 50% van de genen met hun zieke familielid waardoor het mogelijk is de genetische kwetsbaarheid voor schizofrenie in volwassenheid te onderzoeken (Deel 1). Daarnaast werd ook de associatie tussen fronto-striatale functie en de genetische predispositie voor schizofrenie in deze broers en zussen onderzocht (Deel 2). Tenslotte werden kinderen van schizofreniepatiënten onderzocht met als gevolg dat ook de impact van genetische kwetsbaarheid op de ontwikkeling van het fronto-striatale netwerk kon worden ontrafeld (Deel 3).

f.1



Schematische weergave van fronto-striatale circuits.

Deel I studies in broers en zussen: neuroimaging

Fronto-striatale netwerk disfunctie in broers en zussen

In **Hoofdstuk 2** werd breinactiviteit gemeten in werkgeheugen- en default mode netwerken met functionele MRI in 23 niet-aangedane, medicatievrije broers en zussen van schizofreniepatiënten versus 24 gezonde controles tijdens het uitvoeren van de Sternberg taak. Het werkgeheugenniveau werd individueel vóór het scannen vastgesteld, opdat alle participanten op 90% accuratesse presteerden.

Ondanks deze gelijke accuratesse, vertoonden broers en zussen tijdens de encodering fase (opslaan) van het werkgeheugen een verhoogde activiteit in de mediale prefrontale cortex (MPFC). De MPFC is het anterieure deel van het default mode netwerk en activiteit wordt normaliter onderdrukt tijdens werkgeheugenprocessen. De resultaten van deze studie suggereren dat bij broers en zussen het deactiveren van het default mode netwerk faalt. Eén andere studie rapporteerde, evenals de resultaten in dit proefschrift, een disfunctie van het default mode netwerk (MPFC hyperactivatie) tijdens werkgeheugen in schizofreniepatiënten alsook in broers en zussen.

Tijdens de retentiefase (vasthouden) van het werkgeheugen waren er geen verschillen in activatie tussen broers en zussen ten opzichte van gezonde controles. Ten slotte, tijdens de retrievalfase (ophalen) van het werkgeheugen, vertoonden

broers en zussen hyperactivatie in de gebieden die geassocieerd zijn met het werkgeheugennetwerk waaronder het striatum, de inferieure parietale cortex en de dorsolaterale prefrontale cortex (DLPFC). Deze bevinding is consistent met eerdere fMRI studies in broers en zussen tijdens de Sternberg taak, de N-back taak en de AX-CPT taak. Hyperactiviteit in de frontaal cortex (d.w.z. DLPFC) en striatum representeren mogelijk abnormaliteiten in dopaminetransmissie bij broers en zussen van patiënten met schizofrenie.

Regressieanalyse toonde dat de hoeveelheid activatie in het striatum en de DLPFC tijdens de retrievalfase waren gerelateerd aan de hoeveelheid activatie in de MPFC tijdens de encodering fase. Broers en zussen die faalden de MPFC te deactiveren tijdens encodering, vertoonden hyperactiviteit van de DLPFC en striatum tijdens retrieval. Striatale hyperactivatie zou daarom een resultaat kunnen zijn van het onvermogen om de default mode netwerk te onderdrukken. Deze veronderstelling wordt gesteund door connectiviteitsdata van Whitfield et al. die verminderde anticorrelaties (negatieve connectiviteit tijdens rust en n-back taak) aantoonde tussen de MPFC en DLPFC in broers en zussen. Omdat deactivatie van het default mode netwerk gerelateerd is aan dopamine activiteit in het striatum, zou de bevinding zoals gepresenteerd in dit proefschrift abnormaliteiten in dopamine transmissie kunnen representeren. In gezonde controles werd namelijk geen relatie tussen default mode netwerk suppressie en DLPFC activatie gevonden. Hierbij kan worden gesuggereerd dat indien default mode netwerk activiteit voldoende wordt onderdrukt tijdens encodering, DLPFC activiteit niet verhoogd hoeft te zijn tijdens de retrievalfase van het werkgeheugen.

Hoofdstuk 3 beschrijft een onderzoek waarin beloningsverwerking met fMRI wordt onderzocht in hetzelfde cohort van 27 niet-aangedane broers en zussen versus 29 gezonde controles. Taakaccuratesse werd tijdens het scannen individueel aangepast zodat alle participanten eenzelfde bedrag wonnen.

Tijdens de anticipatie van beloning vertoonden broers en zussen verminderde activiteit in het linker striatum ten opzichte van gezonde controles. Deze bevinding is consistent met resultaten in medicatie-vrije schizofreniepatiënten. Bovendien liet een recente studie verminderde ventraal striatale activiteit zien in eerstegraads familieleden van schizofreniepatiënten. Onder deze groep behoorden ouders, broers en zussen alsook kinderen van patiënten. Verminderde striatale activiteit tijdens de anticipatie van beloning suggereert dat er sprake is van een disfunctie in dopamine transmissie.

Verminderde ventraal striatale activiteit bleek gecorreleerd te zijn met de mate van subklinische negatieve symptomen in broers en zussen. Deze bevinding is consistent met een studie van Simon et al. die vergelijkbare correlaties vond tussen verminderde ventraal striatale activiteit en apathie in broers en zussen. Apathie is tevens geassocieerd met een disfunctie in dopamine transmissie tijdens beloningsverwerking.

Tijdens het ontvangen van beloning vertoonden broers en zussen verhoogde activiteit in het ventraal striatum beiderzijds. Deze bevinding is consistent met één studie die verhoogde ventraal striatale activatie in schizofreniepatiënten tijdens succesvolle beloningsfeedback vond, maar inconsistent met een andere studie. Deze discrepantie kan worden verklaard door het gegeven dat alle patiënten in deze studies antipsychotische medicatie gebruikten welke een direct effect op fronto-striatale activiteit kunnen hebben.

Verhoogde ventraal striatale activatie tijdens het ontvangen van beloning was gerelateerd aan verminderde activatie tijdens anticipatie van beloning in broers en zussen. Dit zou kunnen wijzen op een gestoorde verwerking van cues: het ventraal striatum wordt niet voldoende geactiveerd op cues die beloning aanduiden en daarop volgt striatale hyperactivatie tijdens het ontvangen van beloning. Omdat het verwerken van cues afhankelijk is van juiste dopamine transmissie, zouden ook deze resultaten erop kunnen wijzen dat er in broers en zussen sprake is van abnormaliteiten in dopamine transmissie.

Tenslotte werd verhoogde activiteit gevonden in de orbitofrontale cortex tijdens het ontvangen van beloning in broers en zussen. Deze bevinding is consistent met een studie waarin verhoogde orbitofrontale frontale cortex activatie werd gevonden tijdens beloningsfeedback in schizofreniepatiënten, alhoewel in deze studie de uitkomst van beloning werd gecontrasteerd met uitkomst zonder beloning (d.w.z. incorrecte beloningstrial). Tijdens het daadwerkelijk succesvol ontvangen van beloning vertoonden patiënten in deze studie verminderde activiteit. De specifieke kenmerken van orbitofrontale activatie tijdens de uitkomst van beloning in schizofreniepatiënten alsook in broers en zussen, zou in toekomstige studies verder moeten worden onderzocht.

In **Hoofdstuk 4** worden fronto-striatale banen onderzocht door middel van diffusion tensor imaging (DTI) in 24 schizofreniepatiënten, 30 niet-aangedane broers en zussen én 58 gezonde controles.

Wanneer het gehele striatum als uitgangspunt werd genomen werden er geen verschillen in fractionele anisotropie (FA) tussen patiënten, broers en zussen en controles gevonden. Deze bevinding is overeenkomstig met een studie van Quan et al. die ook geen FA verschil vond in schizofreniepatiënten ten opzicht van

controles in de banen die de DLPFC verbinden met het gehele striatum. De in hoofdstuk 4 beschreven analyse in de sub-regio's van het striatum liet zien dat schizofreniepatiënten alsook broers en zussen verlaagde FA waarden vertoonden in de baan die de linker nucleus accumbens verbindt met de linker DLPFC. Deze bevinding is consistent met een studie van Bracht et al. die fronto-striatale banen onderzocht in schizofreniepatiënten en controles, alhoewel een afgeleide maat van de spatiele extensie van de vezelverbindingen werd gebruikt (zogenaamde PIBI maat: probability indices forming part of a bundle of interest). De bevinding zoals in dit proefschrift gepresenteerd van verminderde FA in zowel schizofreniepatiënten als broers en zussen kunnen duiden op verminderde witte-stof integriteit aangezien FA wordt gebruikt als een index voor de integriteit van micro-structurele witte-stof vezelbundels.

Naast bovengenoemde bevinding, bleek bij nadere analyse dat de verminderde witte-stof integriteit in schizofreniepatiënten niet gerelateerd was aan de symptoomernst, gemeten met de PANSS. Deze bevinding was verwacht aangezien in de studie van Bracht et al. dergelijke relatie niet werden gevonden. Deze bevinding voert bewijs aan dat witte-stof integriteit in deze baan is gerelateerd aan genetische kwetsbaarheid voor schizofrenie en niet aan klinische symptomen.

Deel II studies in broers en zussen: genetica in beeld

Omdat de bevindingen zoals gepresenteerd in Deel I impliceren dat er een dopamine disfunctie bestaat in schizofreniepatiënten alsook in broers en zussen, zou het aannemelijk zijn dat onderliggende genen die betrokken zijn bij dopaminefunctie, bijdragen aan deze fronto-striatale afwijkingen. Om deze reden werd in **Hoofdstuk 5** de impact van het tot op heden sterkst schizofrenie-geassocieerde polymorfisme (rs2514218) van de dopamine D2 receptor (DRD2) op striatale activatie onderzocht in 45 niet-aangedane broers en zussen van schizofreniepatiënten door middel van een stopsignaal anticipatie taak. Eénentwintig broers en zussen waren drager van het risico allel.

Ondanks gelijke taakaccuratesse, vertoonden dragers van het risico allel een verminderde striatale activatie respons op toenemende proactieve inhibitie (d.w.z. op toenemende kans-niveaus op basis van waarschijnlijkheid van het stopsignaal). Dit resultaat impliceert dat het de dragers van het risico allel niet lukt om het striatum te modificeren aan de hand van contextuele cues (d.w.z. kleuren die waarschijnlijkheid van stopsignaal aangeven). Vergelijkbare afwijkingen werden gevonden tijdens beloningsverwerking: broers en zussen (Hoofdstuk 3) als ook andere eerstegraads familieleden lukken het niet om het

striatum te modificeren aan de hand van cues die in potentie financiële beloning inhouden. Ondanks deze verminderde striatale "rek", presteerden de dragers van het risico allel op gedragsmaten niet slechter dan de niet-dragers. Dit suggereert dat er een soort compensatie bestaat in dragers van het risico allel. Het algehele niveau van striatale activatie in dragers van het risico allel was inderdaad gemiddeld verhoogd vergeleken met niet-dragers tijdens proactieve inhibitie. Deze toegenomen striatale activiteit zou mogelijk toegenomen inspanning kunnen representeren.

Tenslotte vertoonden dragers van het risico allel een verminderde striatale respons tijdens reactieve inhibitie vergeleken met niet-dragers. Alhoewel er voor het striatum een belangrijke rol in inhibitie is weggelegd, zou deze striatale activiteit tijdens succesvolle inhibitie ook voor een deel verklaard kunnen worden door anticipatieverwerking, een en ander getriggerd door contextuele cues.

De bevindingen gepresenteerd in dit proefschrift wijzen erop dat striatale disfunctie tijdens contextuele cue-verwerking een consequentie is van het dragen van het schizofrenie-geassocieerde risico allel van polymorfisme (rs2514218) welke DRD2 encodeert. Het is inderdaad aangetoond dat in het algemeen DRD2-polymorfismen de DRD2 dichtheid in de basale ganglia (negatief) beïnvloedt.

Deel III studies in kinderen: neuroimaging

Hoofdstuk 6 beschrijft een studie over beloningsverwerking in 25 adolescente kinderen van schizofreniepatiënten en 36 gezonde controle adolescenten (leeftijd 10-19 jaar).

Activiteit in het ventrale striatum nam af over leeftijd in kinderen van schizofreniepatiënten. Dit afnamepatroon tijdens adolescentie komt overeen met ventraal striatale hypoactiviteit ten tijde van volwassenheid in zowel schizofreniepatiënten als broers en zussen (Hoofdstuk 3). De afname in ventraal striatale activatie in kinderen van schizofreniepatiënten zou gerelateerd kunnen zijn aan abnormale volumeveranderingen van subcorticale gebieden over leeftijd. Er werd geen algeheel verschil gevonden in activiteit tussen controle en schizofreniekinderen en dit suggereert een relatieve hyperactiviteit bij aanvang, en een hypoactivatie aan het einde van adolescentie in kinderen met familiair risico voor schizofrenie. De vroege hyperactiviteit in kinderen van schizofreniepatiënten impliceert dat er tijdens de kindertijd, dus reeds vóór adolescentie, abnormale ontwikkeling plaatsvindt. Deze bewering is overeenkomstig met de gedachte dat genetische factoren, in combinatie met omgevingsfactoren, de basis vormen

van dit deficit in het striatum. Het rs2514218 polymorfisme van DRD2 zou kunnen bijdragen aan dit striatale deficit in deze kinderen omdat 1) DRD2 zich voornamelijk in het striatum bevindt, 2) het polymorfisme geassocieerd is met schizofrenie, en 3) het polymorfisme een impact heeft op striatale activiteit in 45 broers en zussen van patiënten (Hoofdstuk 5). In tegenstelling tot kinderen van schizofreniepatiënten, werden bij jonge controle adolescenten lage waarden van striatale activiteit tijdens anticipatie van beloning gevonden en dit is overeenkomstig met studies over beloningsverwerking in gezonde adolescenten.

Er werden geen activatieverschillen in de orbitofrontale cortex gevonden tussen kinderen van schizofreniepatiënten en controles. Dit zou niet in overeenstemming zijn met een structurele MRI studie van Prasad et al, die verminderde gyrale oppervlakte vond in frontale en pariëtale gebieden in deze kinderen. Echter een andere structurele MRI studie vond geen structurele verschillen in de frontaalkwab in adolescente kinderen van schizofreniepatiënten. Deze inconsistenties zouden verklaard kunnen worden door de heterogeniteit van de groep kinderen: 15% ontwikkelt schizofrenie tijdens volwassenheid, 60% ontwikkelt psychopathologie anders dan psychose en ongeveer 25% blijkt relatief onaangedaan. Aangezien is aangetoond dat volwassen schizofreniepatiënten hypofrontaliteit vertonen tijdens het ontvangen van beloning, zou het kunnen zijn dat alleen diegene die schizofrenie ontwikkelen tijdens adolescentie vergelijkbare frontale afwijkingen vertonen. Bovendien, aangezien volwassen broers en zussen van schizofreniepatiënten, alhoewel zij familiair risico dragen, hyperfrontaliteit vertonen (Hoofdstuk 2; Hoofdstuk 3) in plaats van hypofrontaliteit, zou in tenminste een deel van deze kinderen gedurende de adolescentie frontale activiteit verhoogd kunnen zijn.

In **Hoofdstuk 7** werden fronto-striatale witte-stof banen onderzocht in 21 adolescente kinderen van schizofreniepatiënten en 30 gezonde controle adolescenten tussen 10 en 18 jaar door middel van DTI.

In kinderen van schizofreniepatiënten werd geen verhoging van FA over leeftijd gevonden in de baan die de linker nucleus accumbens verbindt met de linker DLPFC, terwijl gezonde controle adolescenten wel een (lineaire) toename vertoonden in FA waarde. De gevonden resultaten in deze baan zijn in overeenstemming met 1) de bevindingen van verminderde FA die bij volwassen schizofreniepatiënten alsook bij broers en zussen werden gevonden (Hoofdstuk 4), maar ook met 2) toenemende FA waarden in gezonde controle adolescenten over leeftijd. Omdat FA wordt gebruikt als een index voor microstructurele integriteit van witte-stof vezelbundels, zou de bevinding van toenemende FA over leeftijd in gezonde adolescenten kunnen duiden op (gezonde) maturatie van

witte-stof banen. Geen toename in FA in kinderen van schizofreniepatiënten zou gestagneerde witte-stof vezel maturatie kunnen betekenen mogelijk als resultaat van genetische kwetsbaarheid.

De gemiddelde FA waarde van bovengenoemde baan is in kinderen van schizofreniepatiënten verhoogd vergeleken gezonde controle adolescenten en dit is in overeenstemming met een studie die verhoogde volumewaarden van subcorticale gebieden over leeftijd in kinderen van schizofreniepatiënten rapporteerde. Interessant is dat de gemiddelde FA waarde in kinderen van schizofreniepatiënten enigszins hoger is vergeleken met volwassen schizofreniepatiënten en broers en zussen (Hoofdstuk 4). Het zou goed kunnen zijn dat FA vermindert na adolescentie alleen in diegene die daadwerkelijk schizofrenie ontwikkelt of een broer of zus wordt. Daarentegen vertoonden controle adolescenten een lager gemiddeld FA vergeleken volwassen controles, en dit zou kunnen duiden op continuatie van stijging in FA waarden na adolescentie, in overeenstemming met eeen neuro-ontwikkeling studie middels DTI in jongvolwassenen (18-25 jaar).

Er werden geen verschillen in FA over leeftijd gevonden tussen kinderen van schizofreniepatiënten en controles in andere fronto-striatale banen. Dit is overeenkomstig met volwassen schizofreniepatiënten alsook broers en zussen, bij wie louter verschillen werden gevonden in de baan die de linker nucleus accumbens verbindt met de linker DLPFC gemiddelde FA. Deze bevindingen suggereren dat afwijkingen specifiek in deze baan het resultaat is van genetische kwetsbaarheid voor schizofrenie. Er worden bij patiënten in neuroimaging studies consistent afwijkingen in de DLPFC en nucleus accumbens gevonden en mogelijk zouden abnormaliteiten in deze gebeden ten grondslag liggen aan klinische en cognitieve symptomen.

Implicaties en vervolgonderzoek

De resultaten gepresenteerd in dit proefschrift leveren aangrijpingspunten voor mogelijke nieuwe vroege-interventie behandelingen. Bijvoorbeeld medicatie dat tijdens de ontwikkeling van het brein specifiek aangrijpt op het striatum zou overwogen kunnen voor therapeutische doeleinden. De farmacologische inhibitie van PDE10A bleek reeds effectief te zijn tijdens preklinische (dier)modellen van psychose. PDE10A komt met name tot expressie in de medium spiny neuron in het putamen en de nucleus caudatus.

Naast behandeling zou dit proefschrift ook kunnen bijdragen aan de ontwikkeling van nieuwe diagnostische methoden. In de toekomst zou een

hulpzoekend persoon, die een verhoogd familiair risico op schizofrenie heeft, een DNA onderzoek kunnen doen naar schizofrenie-geassocieerde polymorfismen samen met een fMRI scan tijdens een fronto-striatale taak. Wanneer hij of zij voldoet aan een bepaald (nader te bepalen) blauwdruk, zou het wellicht mogelijk kunnen zijn om een voorspelling te doen over het risico op toekomstige psychose en zouden interventies al dan niet kunnen worden toegepast.

Longitudinaal vervolgonderzoek in kinderen van schizofreniepatiënten is dan wel nodig om de associatie te onderzoeken tussen specifieke fronto-striatale patronen tijdens de adolescentie en klinische uitkomstmaten tijdens volwassenheid. Naast longitudinaal onderzoek zou onderzoek met mono- en dizygote tweelingen discordant voor schizofrenie nodig zijn om de volledige genetische invloed op fronto-striatale afwijkingen te onderzoeken.

Conclusies

DEEL 1 Broers en zussen van schizofreniepatiënten vertonen, net als hun zieke familielid, fronto-striatale afwijkingen.

DEEL 2 Broers en zussen die drager zijn van het sterkst schizofrenie-geassocieerde polymorfisme van de dopamine D2 receptor vertonen de meeste afwijkingen tijdens het functioneren van het striatum.

DEEL 3 Adolescente kinderen van schizofreniepatiënten vertonen fronto-striatale afwijkingen.

Kortom, van je familie moet je het hebben!

Chapter 10

Dankwoord

Family matters, maar collega's en vrienden zeker ook! Zonder hen was dit proefschrift nooit tot stand gekomen. In dit dankwoord wil ik dan ook naast mijn familie ook mijn collega's en vrienden bedanken voor alle steun, geduld en vertrouwen die zij mij de afgelopen jaren hebben gegeven.

Ten eerste wil ik alle proefpersonen bedanken voor hun deelname aan het onderzoek.

Geachte professor Kahn, beste René. Ik wil je graag bedanken voor je vertrouwen, je duidelijkheid, je kritiek maar ook je loyaliteit. Ik heb onze meetings altijd als leerzaam ervaren maar ik was ook blij met ons gedeelde gevoel voor humor en interesse voor elektronica (Apple, red.). Je kritiek op papers was altijd raak. Ook vond ik onze niet werk-gerelateerde gesprekken, bijvoorbeeld tijdens congressen, altijd prettig en onderhoudend. Ik hoop dat ik in de toekomst nog veel met je kan samenwerken.

Beste copromotor, Hi Matthijs. Ik ben ongelofelijk blij dat jij mijn copromotor bent geweest. Naast dat je me veel geleerd hebt over fMRI, statistiek en artikelen schrijven, is jouw manier van werken een voorbeeld voor mij: duidelijkheid, logica en to-the-point staan bij jou voorop. Tijdens mijn promotietraject heb ik je vader zien worden van twee prachtige dochters. Ik vond het leuk om dat met jou mee te maken. Naast onderzoek hebben we ook ontzettend veel gelachen, ik waardeer je droge humor enorm. Ook wil ik je bedanken voor de analyses van de Tour de France. Laten we in de toekomt nog veel onderzoek gaan doen!

Geachte professor Hulshoff Pol, professor Sommer, professor Veltman, professor Kappelle en dr. Ruhé, dank dat u wilde plaatsnemen in de beoordelingscommissie.

De collega onderzoekers wil ik graag bedanken voor al hun hulp met scannen, analyses draaien, input geven, wetenschappelijke discussies en gezelligheid tijdens congressen. Mariët van Buuren, Bram Zandbelt en Christian Widschwendter, bedankt voor het meewerken aan de inclusies en al het voorwerk. Zonder jullie was dit proefschrift nooit tot stand gekomen. Stefan du Plessis, baie dankie vir die maak van die plaatjes! Marc Bohlken, bedankt voor je DTI analyses en prettige samenwerking. René Mandl, bedankt voor je snelle DTI-commentaar op de papers. Ook wil ik de flowmanagers Mirjam Bloemendaal, Fiona van Heiligenberg en Pascal Pas graag bedanken voor de hulp bij het scannen. Jurjen Luykx en Kristel van Eijk wil ik bedanken voor al hun hulp bij de geneticazoektochten, analyses en input op de papers. Wiepke Cahn en Manon Hillegers, dank dat ik mocht participeren in de GROUP en BRIDGE studies. Ruby Pouwels, dank voor je inclusies en input op

de papers. Hanna van den Munkhof, dank voor je nuttige bijdrage aan de papers. Yumas el Hankouri, bedankt voor het netwerkbeheer. Bas Neggers, bedankt voor onze gesprekken over fMRI en TMS.

Ook wil ik alle arts-assistenten en oud arts-assistenten (inmiddels psychiater) bedanken voor hun collegialiteit, geduld en begrip. Veel stages heb ik gecombineerd met onderzoek (50/50) en altijd kon ik in mijn afwezigheid op jullie waarneming en loyaliteit rekenen, superveel dank daarvoor. Ik heb een geweldige opleidingsperiode met jullie gehad, niet in de laatste plaats tijdens het Voorjaarscongres en de assistentenuitjes. In het bijzonder dank ik alle arts-onderzoekers die eenzelfde traject als ik ondergaan of hebben ondergaan. Christiaan Vinkers en Jurjen Luykx, dank voor jullie scherpe meningen, ongekend enthousiasme voor het vak en de projecten die we samen doen (waaronder de Jonge Psychiater). Dank ook, samen met Gerben van de Kraats, voor de intervisie-avonden met de daarbij horende gezelligheid, lekker eten, goede wijn maar natuurlijk ook de leermomenten die daaruit voortvloeiden, zij het in 020 of 030. Ook arts-onderzoekers Annet van Bergen, Lot de Witte, Kim Maijer, Chris Schubart, Joeri Tijdink, Edwin van Dellen en Sander Haijma wil ik bedanken voor de levendige wetenschappelijke maar ook zeker niet-wetenschappelijke discussies.

Ook wil ik alle psychiaters van het UMC Utrecht waarmee ik heb gewerkt enorm bedanken voor de opleidingsmomenten en begrip wanneer ik afwezig was voor het onderzoek. In het bijzonder mijn opleiders en supervisoren: Ine Klijn, Rob van Ojen, Wiepke Cahn, Hilgo Bruining, René Kahn, Jaap Wijkstra, Nicoletta van Veelen, Iris Sommer, Paulien Gritters en Arnold Franken. Mede door jullie supervisie kan ik per oktober met vertrouwen het avontuur als psychiater aangaan.

Veel dank gaat uit naar de (ex-)secretaresses van de staf: Elly Schreurs, Emmy Drost, Jeanette Sopacua, Xandra Schram, Joyce van Baaren, Paula Ywema en Mirjam van Male. Bedankt voor al jullie hulp, adviezen en ondersteuning. Maar ook alle secretaresses van de zorglijnen en MGGZ wil ik graag bedanken voor alle hulp en gezellige gesprekken.

Naast alle (!) psychiatrie afdelingen in het UMC Utrecht heb ik ook enkele stages buiten Utrecht afgelegd. Ik zou alle (ex-)psychiaters van het Tergooi met wie ik heb gewerkt willen bedanken, in het bijzonder Tjeerd Wouters, Dieneke Bloemkolk en Duuk Sierink. Ook wil ik graag de verpleging van A14 en het secretariaat van Tergooi bedanken. Dank voor het fantastische jaar, ik heb er veel geleerd. Ook wil ik graag alle arts-assistenten en de psychiaters van GGZ inGeest locatie Zuiderpoort

bedanken, in het bijzonder Marijke Jitta, Jan-Martijn Bakker en Célestine Engel. Het secretariaat en ook de vaste werknemers van de crisisdienst, allemaal bedankt voor de fascinerende stage bij de crisisdienst.

Ik wil de familie Janssen bedanken dat zij wilden meewerken aan de bijzondere foto op deze cover. Door jullie hulp is het thema van het proefschrift volledig tot zijn recht gekomen. Dim Balsem (de fotograaf), bedankt dat je deze prachtige foto wilde maken en je flexibiliteit hierin.

Ik ben veel dank verschuldigd aan al mijn vrienden. Niet zozeer vanwege de hoog wetenschappelijke input maar juist het bezig houden met andere dagelijkse en weekendse zaken houden mijn leven dynamisch. Jullie weten wat ik bedoel.

Dan ben ik aangekomen bij mijn paranimfen. Arija, Arie, wat hebben wij veel meegemaakt gedurende onze studie geneeskunde in het AMC (cowboylaarzen) en opleiding/promotie in het UMC. Wij sleepten elkaar erdoor heen, ik zou de finish nooit gehaald hebben zonder onze levendige gesprekken waarbij we altijd op één lijn zaten. Helaas werken we niet meer samen nu, maar ik weet zeker dat dit in de toekomst wel gaat gebeuren.

Bob, family matters en dat is zeker het geval! Wat bof ik toch met zo'n (overigens unaffected) sibling. Je bent iemand op wie ik echt altijd en onvoorwaardelijk kan rekenen en dat betekent veel voor me. Ik hoop dat we samen in de toekomst nog vele momenten zullen meemaken, in het bijzonder tijdens de belangrijkste bijzaak van het leven.

Graag wil ik ook mijn gehele schoonfamilie bedanken, in het bijzonder Josée en Flip, voor hun steun en waardering bij de voltooiing van dit proefschrift. Maar ook het begrip en aanpassingsvermogen wanneer ik, al dan niet werk-gerelateerd, verstek heb moeten laten gaan, heb ik enorm gewaardeerd.

Mijn ouders, Peter en Mieke, wil ik bedanken voor hun hulp, adviezen en betrokkenheid. Ik weet dat jullie apetrots op me zijn. Peet, de scherpe discussies die wij hadden over de invloed van de psychologie op ontstaan en ontwikkeling van psychiatrie vond ik verhelderend en relativerend. Mieks, je hebt altijd eindeloos in mij geloofd, van peuter tot nu, en zie hier het resultaat!

Caroline, ik heb zo veel belangrijke momenten met je gedeeld en ik weet zeker dat er nog vele gaan volgen.

Chapter 11

List of publications

de Leeuw M., Bohlken M.M., Mandl R.C.W., Hillegers M.H.J., Kahn R.S., Vink M. Reduced fronto-striatal white-mater maturation in adolescent offspring of schizophrenia patients. In preparation.

Vink M.*, **de Leeuw M.***, Luykx J.J., van Eijk K.R., van den Munkhof H., van Buuren M., Kahn R.S. DRD2 schizophrenia-risk allele is associated with impaired striatal functioning in unaffected siblings of schizophrenia patients. Submitted for publication.

Vink M.*, **de Leeuw M.***, Pouwels R., van den Munkhof H., Kahn R.S., Hillegers M.H.J. Impaired striatal function during reward anticipation in adolescent offspring of schizophrenia patients. Submitted for publication.

Kleerekooper I., van Rooij S.J.H., van den Wildenberg W.P.M., **de Leeuw M.**, Kahn R.S., Vink M. The effect of aging on reactive and proactive inhibitory control. Submitted for publication.

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de Leeuw M., Bohlken M.M., Mandl R.C.W., Kahn R.S., Vink M. Reduced fronto-striatal white matter integrity in schizophrenia patients and unaffected siblings: a DTI study. npj Schizophrenia, 2015 April; 1: 15001.

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* both authors contributed equally

Chapter 12

Curriculum Vitae

De auteur van dit proefschrift werd geboren op 4 maart 1983 te Amsterdam. In 2001 behaalde hij het eindexamen gymnasium aan het Stedelijk Gymnasium te Haarlem. Datzelfde jaar begon hij aan de studie Geneeskunde bij de Universiteit van Amsterdam. In 2003 behaalde hij enkele propedeuse vakken in de Psychologie. Het doctoraalexamen Geneeskunde werd afgelegd in 2005. Gedurende de doctoraalfase verrichtte hij onderzoek bij het Nederlands Instituut voor Hersenonderzoek te Amsterdam, onder begeleiding van professor D.F. Swaab. Hij behaalde het artsexamen in 2008. In het kader van het artsenexamen volgde hij een keuze coschap op de afdeling Interne Geneeskunde van het Tygerberg Ziekenhuis te Kaapstad. Zijn oudste coschap voltooide hij op de afdeling Neurologie van het Onze Lieve Vrouwen Gasthuis te Amsterdam. In de periode rondom het artsexamen deed hij functioneel MRI onderzoek op de afdeling Psychiatrie Stemmingsstoornissen in het Academisch Medisch Centrum. Hij participeerde in de DELPHI studie onder begeleiding van dr. H.G. Ruhé en professor A.H. Schene. In 2008 startte hij de opleiding tot psychiater aan het Universitair Medisch Centrum Utrecht (UMC Utrecht) met als opleiders professor R.S. Kahn, dr. J. Wijkstra en dr. N.M.J. van Veelen. In het kader van de opleiding deed hij naast stages in het UMC Utrecht een stage ziekenhuispsychiatrie in het Tergooi Ziekenhuis te Blaricum en een stage sociale psychiatrie bij GGZ inGeest te Haarlem. In 2010 startte hij met het promotieonderzoek met professor R.S. Kahn als promotor en dr. M. Vink als copromotor. Hij gaf lezingen op verschillende internationale congressen, won in 2012 de eerste prijs voor zijn poster tijdens het congres van Schizophrenia International Research Society (SIRS) te Florence en won in 2013 een Travel Award tijdens de workshop van European College of Neuropsychopharmacology (ECNP) te Nice. Op 1 oktober 2015 zal hij zijn proefschrift verdedigen en op dezelfde dag de opleiding tot psychiater afronden. Vanaf 1 november 2015 is hij als psychiater en onderzoeker verbonden aan GGZ Rivierduinen en het Leids Universitair Medisch Centrum.

The author of this thesis was born on March 4th, 1983 in Amsterdam. In 2001, he passed his gymnasium exam at the Stedelijk Gymnasium in Haarlem. That same year he started his study Medicine at the University of Amsterdam. In 2003 he passed several bachelor courses in Psychology. He obtained his master's degree in Medicine in 2005. During the final master year, he performed research in the Netherlands Institute for Brain Research under supervision of professor D.F. Swaab. He passed the medical exam in 2008. As part of the medical exam he took his last internships at the department of Internal Medicine at the Tygerberg Hospital in Cape Town (South Africa) and the department of Neurology at Onze Lieve Vrouwen Gasthuis in Amsterdam. During the period of his medical exam he also performed research in functional MRI at the department of Psychiatry Mood Disorders at the Academic Medical Centre. He participated in the DELPHI study under supervision of dr. H.G. Ruhé and professor A.H. Schene. In 2008 he started his psychiatry residencies at the University Medical Center Utrecht (UMC Utrecht), his supervisors were consecutively professor R.S. Kahn, dr. J. Wijkstra and dr. N.M.J. van Veelen. In addition to clinical residencies at the UMC Utrecht, he was a resident in hospital psychiatry at Tergooi Hospital in Blaricum and a resident social psychiatry at GGZ inGeest in Haarlem. In 2010 he started his PhD trajectory with professor R.S. Kahn as supervisor and dr. M. Vink as co-supervisor. He was invited to give oral presentations at several international congresses, won the first prize in 2012 for his poster during the congress of Schizophrenia International Research Society (SIRS) in Florence (Italy), and won a Travel Award in 2013 during the workshop of European College of Neuropsychopharmacology (ECNP) in Nice (France). He will defend his thesis on the 1st of October 2015 and on the same day he will finish his psychiatry residency. As of the 1st of November 2015 he will work as a psychiatrist and researcher at GGZ Rivierduinen and Leiden University Medical Center.