Genetic & Environmental risk factors for Obsessive-Compulsive Symptoms: Do they affect the same brain?

Anouk den Braber

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door

Anouk den Braber

geboren te Haarlem

promotoren:	prof.dr. J.C.N. de Geus
	prof.dr. D.I. Boomsma
copromotor:	dr. D. van ‘t Ent

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1 General introduction and outline of thesis

I am waiting for my participants to arrive, an identical female twin pair, 28 years old. They are late and I start feeling a bit nervous, because there are only 3.5 hours left before another researcher has to use the MRI scanner. My cell phone is ringing, finally they have arrived. After introducing myself and my research assistant I ask the twins to take a seat. They are both holding a tissue in their hand and simultaneously start cleaning their chair before they sit down. When explaining the goal of my research project I am interrupted by one of the twins. She asks her sister whether they carefully locked the door of their car. They both begin to worry about the car and what could happen if they did not lock it. Then one stands up and says: I am sorry but I have to check it. I am looking at my research assistant, she looks at me, and we both know it; this twin pair is perfect for my study, but finishing the MRI protocol in time will be a challenge.

Obsessive-compulsive symptoms/disorder

The recurrent, persistent and intrusive anxiety provoking thoughts the twin pair experienced (is that chair really clean or do I get contaminated when sitting on it/did I lock the door, worse things will happen if I did not) are examples of obsessions. The subsequent repetitive behaviors performed to reduce the anxiety or distress induced by the obsessions (cleaning the chair before sitting down, and checking the door of the car), are called compulsions. Together these are referred to as obsessive-compulsive (OC) symptoms. Other well known obsessions include, need for symmetry, and somatic, sexual and aggressive obsessions and other well known compulsions include counting, ordering/precision and hoarding behavior. When these obsessions and/or compulsions cause marked distress, are time consuming (e.g., they take more than 1 hour a day), and significantly interfere with the individuals normal routine, occupational functioning, usual social activities or relationships with others, a person qualifies for a diagnosis of obsessive-compulsive disorder (OCD) [(American Psychiatric Association, 1994); for a complete overview of diagnostic criteria for OCD following the DSM-IV, see **table 1.1**]. The life-time prevalence of OCD is 0.5-2% (American Psychiatric Association, 1994; Grabe et al., 2000) but obsessions are much more prevalent in the general population – as high as 72% (Rachman and de Silva, 1978; Salkovskis and Harrison, 1984) and the prevalence of OC symptomatology reaches 20% (Fullana et al., 2009).

Neuroanatomical model of obsessive-compulsive disorder

Although the exact etiology and pathogenesis of OCD is unknown converging lines of evidence from neurological, neurosurgical, neuroimaging, pharmacological

Table 1.1. Diagnostic criteria for obsessive-compulsive disorder (DSM-IV)

A. Either obsessions or compulsions:

Obsessions as defined by (1), (2), (3), and (4):

(1) recurrent and persistent thoughts, impulses, or images that are experienced, at some time during the disturbance, as intrusive and inappropriate and that cause marked anxiety or distress

(2) the thoughts, impulses, or images are not simply excessive worries about real-life problems

(3) the person attempts to ignore or suppress such thoughts, impulses, or images, or to neutralize them with some other thought or action

(4) the person recognizes that the obsessional thoughts, impulses, or images are a product of his or her own mind (not imposed from without as in thought insertion)

Compulsions as defined by (1) and (2):

(1) repetitive behaviors (e.g., hand washing, ordering, checking) or mental acts (e.g., praying, counting, repeating words silently) that the person feels driven to perform in response to an obsession, or according to rules that must be applied rigidly

(2) the behaviors or mental acts are aimed at preventing or reducing distress or preventing some dreaded event or situation; however, these behaviors or mental acts either are not connected in a realistic way with what they are designed to neutralize or prevent or are clearly excessive

B. At some point during the course of the disorder, the person has recognized that the obsessions or compulsions are excessive or unreasonable. Note: This does not apply to children.

C. The obsessions or compulsions cause marked distress, are time consuming (take more than 1 hour a day), or significantly interfere with the person's normal routine, occupational (or academic) functioning, or usual social activities or relationships.

D. If another Axis I disorder is present, the content of the obsessions or compulsions is not restricted to it (e.g, preoccupation with food in the presence of an Eating Disorder; hair pulling in the presence of Trichotillomania; concern with appearance in the presence of Body Dysmorphic Disorder; preoccupation with drugs in the presence of a Substance Use Disorder; preoccupation with having a serious illness in the presence of Hypochondriasis; preoccupation with sexual urges or fantasies in the presence of a Paraphilia; or guilty ruminations in the presence of Major Depressive Disorder).

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

and neuropsychological studies point to a biological basis. These studies have contributed to the widely accepted neuroanatomical model of OCD involving the direct and indirect loops of the dorsolateral prefrontal and ventral medial prefrontral cortico–striato–thalamo–cortical (CSTC) circuits (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). In the direct loop, the prefrontal cortex (PFC) sends an excitatory glutamergic signal to the striatum, which in turn sends an inhibitory gamma-amminobutric acid (GABA)-ergic signal to the globus pallidus (GP) interna, resulting in decreased inhibition (disinhibition) of the thalamus and increased excitation of the PFC. In the indirect loop the striatum projects an inhibitory signal to the GP externa and subthalamic nucleus, that in turn sends an excitatory signal to the GP interna, resulting in increased inhibition of the thalamus and decreased excitation of the PFC. The (excitatory) direct loop is thought to function as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors, whereas the indirect (inhibitory) loop is thought to function as a negative feedback loop important for inhibiting and switching between behaviors. It has been hypothesized that an imbalance between these loops, with a stronger excitatory dopamine$_1$ influence on the direct loop of the ventromedial frontal-sriatal circuit and a stronger inhibitory dopamine$_2$ influence on the indirect loop of the dorsolateral frontal-striatal circuit, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate OC behavior (**figure 1.1**) (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). In addition to abnormalities in brain regions implicated in this model, disturbances in brain regions that are functionally connected to brain regions implicated in the ventral and dorsal frontal-striatal network (e.g., anterior cingulate cortex, amygdala, premotor cortex, parietal and temporal cortices), have been reported as well, which have led to an extension of the neuroanatomical model for OCD (Menzies et al., 2008a).

Although a disturbance in the CSTC loops, or its functional connections, seems to be the neurological basis for OCD, there are considerable inconsistencies across studies regarding the brain areas involved and the direction of anatomical and functional changes. These inconsistencies have been explained by methodological differences between studies (e.g., sample size, analysis methods). Another possible explanation lies in the extremely heterogeneous presentation of the OCD phenotype, in which symptoms can vary across patients as well as within patients over time. An approach that uses more homogeneous disease dimensions, such as only cases with early onset or with only one symptom dimension has been suggested to lead to more consistent results (Mataix-Cols et al., 2004; Miguel et al., 2005; van den Heuvel et al., 2009). However, we hypothesize that the observed inconsistencies might also relate to the differential impact of genetic and environmental risk factors for OCD on neurobiological pathways underlying this behavior.

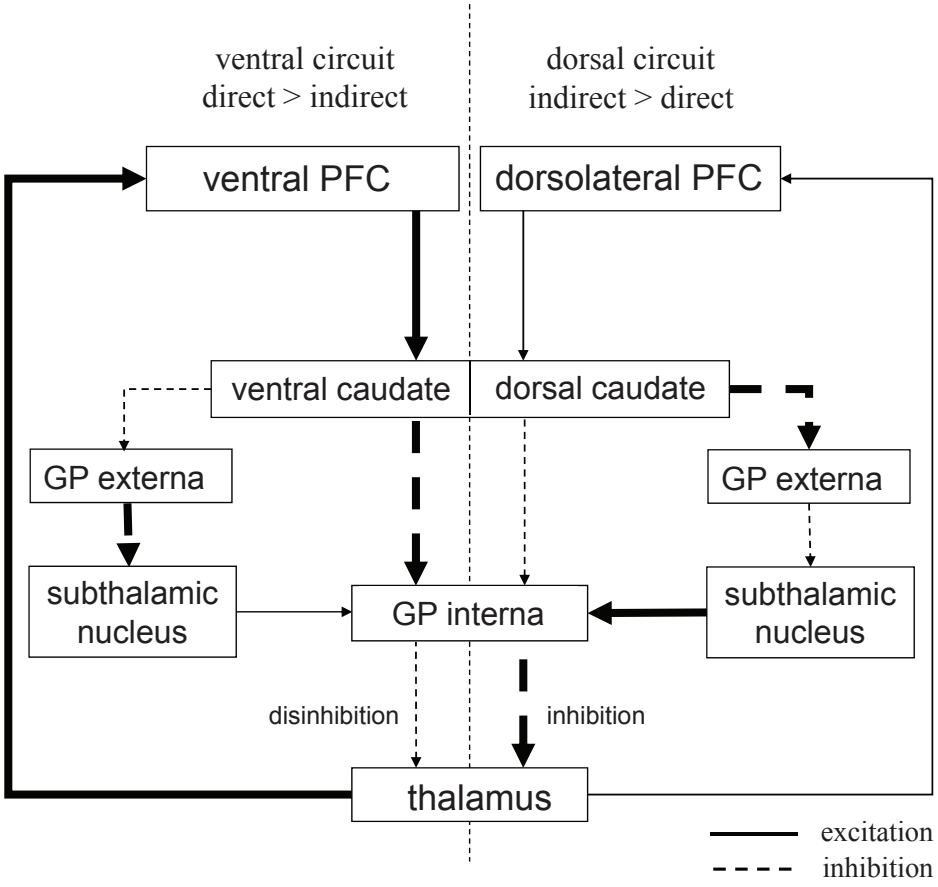

Figure 1.1. *A widely accepted neuroanatomical model of OCD involving the direct and indirect CSTC loops. It is hypothesized that an imbalance between these loops, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate OC symptoms (adapted from Mataix-Cols and van den Heuvel, 2006).*

Genetic and environmental risk factors for obsessive-compulsive disorder

Family studies and twin studies have indicated the importance of genetic as well as environmental risk factors with regard to the etiology of OCD. The disorder runs in families, especially the early onset type (Nestadt et al., 2000; van Grootheest et al., 2007), and heritability for OC symptomatology has been estimated between 27% and 47% in adults and between 45% and 65% in children (Eley et al., 2003; Hudziak et al., 2004; Jonnal et al., 2000; van Grootheest et al., 2007). In addition, linkage and association studies have indicated a number of vulnerability genes for OCD, with most of these studies pointing towards functional deficits of genes involved in serotonergic, glutamatergic and dopaminergic neural signalling (Bengel et al., 1999; Billett et al., 1998; Enoch et al., 2001; Nicolini et al., 2009).

Given the moderate heritability, as much as 35–73% of the risk for OCD should be accounted for by environmental stressors and/or adverse gene-environment interactions. Environmental risk factors found to be associated with OC symptomatology include perinatal problems (e.g., hypoxia), streptococcal infection, psychosocial stress, aspects of parenting (e.g., parental overprotection), emotional neglect, sexual abuse and several important life-events such as pregnancy, miscarriage and divorce (Albert et al., 2000; Alonso et al., 2004; Cath et al., 2008; Geller et al., 2008; Lin et al., 2007; Wilcox et al., 2008).

With the knowledge that only part of the variance in OC symptomatology can be explained by genetic factors and part by environmental factors and that both these factors have been shown to contribute substantially to individual differences in brain anatomy (Thompson et al., 2001; Toga and Thompson, 2005), we questioned ourselves if these two risk factors for OC symptomatology could affect the brain in different ways and whether that could explain part of the observed inconsistencies in literature on the neurobiology of OCD. Genetic risk factors for OCD might impact on slightly different brain regions than environmental risks do, but the affected brain regions might all be implicated in the neurological pathways involved in the regulation of anxiety and safety behaviors (e.g., genetic risk factors affect regions involved in the ventral frontal-striatal network, whereas environmental risk factors affect regions involved in the dorsal frontal-striatal network), so a disturbance in either one of these brain regions could mediate the observed OC behavior. This is what we wanted to investigate and thereby the main focus of this thesis. To answer these questions specific methodologies were necessary. First a method was needed to isolate OC symptoms mediated by environmental risk factors from OC symptoms mediated by genetic risk factors. Secondly, we needed a method to measure brain structure and function.

Isolate OC symptoms mediated by environmental risk factors from OC symptoms mediated by genetic risk factors: The discordant/concordant monozygotic twin design

A design that makes a distinction between genetically and environmentally mediated neurobiological changes that underlie the development of behavioral traits such as OCD, is the so-called discordant/concordant monozygotic (MZ) twin design. This design already has been proven useful in distinguishing between genetically and environmentally mediated neurobiological changes that underlie the development of depression and attention-deficit-hyperactivity disorder (de Geus et al., 2007; van 't Ent et al., 2009; Wolfensberger et al., 2008). Excluding post-twinning de novo mutations, all MZ twins begin life with identical

genomes. A discordance at the behavioral level, for example one twin scores very high on OC symptoms but the co-twin scores very low, is likely to arise from differential exposure to environmental influences. Consequently, neurobiological differences between the OC symptom high-scoring twin and the low-scoring co-twin from discordant pairs reflect environmental effects on the brain, rather than effects of genetic variation. In contrast, if a MZ twin pair is highly concordant with respect to their behavior, for example both twins are scoring very high or very low for OC symptoms, this similarity can either derive from their (near) complete sharing of genetic variants or from their sharing of the (family) environment. Previous studies, however, have shown that shared environmental factors do not play a significant role in OC symptomatology (Clifford et al., 1984; Jonnal et al., 2000; van Grootheest et al., 2007). Therefore, the similarity in OC symptomatology in MZ twin pairs likely reflects their genetic resemblance. Consequently, a comparison of neurobiological variables between groups of pairs of MZ twins that both score high (concordant-high) on OC symptoms with groups of pairs of MZ twins scoring concordantly low on OC symptoms will uncover the influence of genetic risk factors on these neurobiological variables.

How to explore the brain: structural and functional magnetic resonance imaging

A non-invasive technique that has been frequently used for obtaining information on brain structure and function is Magnetic Resonance Imaging (MRI). The physics behind MRI is complex. Basically, MRI involves imaging of the proton, the positively charged spinning nucleus of hydrogen atoms that are abundant in tissues containing water, proteins, lipids, and other macromolecules. An MRI scanner produces a powerful magnetic field, and when a person is placed in this magnetic field the protons within the body align with the direction of the magnetic field. When a radio frequency field is subsequently applied, the protons absorb the energy and change their spinning direction. The protons subsequently release the absorbed energy and turn back to the original alignment. The time it takes to return to the original alignment is referred to as relaxation time and depends on the physical and chemical characteristics of the tissue. There are three relaxation times that are of primary interest in MRI; T1, T2 and T2*. T1 is the "longitudinal" relaxation time and describes the time constant for the return of the magnetization to its equilibrium position aligned along the static magnetic field of the scanner whenever it is disturbed. T2 and T2*, the "transverse" relaxation times, are the time constants that describe how long the resonating protons remain coherent or rotate in phase following a radio frequency pulse (Brown and Semelka, 2010). The energy released by the protons during this relaxation process is received by a radio antenna, called a body coil, which in turn translates this information into an image of the scanned area of the body. By using magnetic field gradients

in different directions MRI makes it possible to obtain 2D images and 3D volumes in any arbitrary orientation. These 3D volumes are composed of voxels (volumetric pixels), the volume elements that contain information on the signal released by the protons from specific locations in the body.

In the early days, neuroimaging studies mainly used this technique to obtain information on anatomical features of the brain (e.g., gray matter density, volume or thickness). Nowadays, more specialized MRI scans, such as functional MRI (fMRI) and diffusion tensor imaging (DTI), are also frequently used.

With fMRI the functional properties of a brain region can be examined by measuring its level of neuronal activity during rest or during the performance of a cognitive task. The fMRI signal changes are dominated by the Blood Oxygenation Level Dependent (BOLD) mechanism, which implies that regional brain activations result in local excess of oxy-hemoglobin supply, which leads to an increase in the homogeneity of magnetic susceptibility, a decrease in T2*, and hence increased fMRI signal (Buxton, 2009). During an fMRI experiment this BOLD fMRI signal is continuously measured in all gray matter regions of the brain, and changes in this signal indirectly represent changes in the level of neuronal activity of these regions. For the analysis of fMRI scans obtained during the performance of a cognitive task, the recorded BOLD signal first needs to be aligned with the performed task in time, in order to know which brain regions are activated during the different conditions of the task (e.g., active or baseline condition).

DTI provides a measure of diffusion of water molecules within tissues, permitting the investigation of brain tissue microstructure. In structures with a highly coherent directional organization, e.g., white matter tracts in the brain, the dominant direction of diffusion is parallel to the fiber direction, so that diffusion becomes anisotropic. The most reported metric derived from DTI is fractional anisotropy (FA), which describes the degree of anisotropy within a voxel and can be interpreted as a proxy measure of white matter integrity (Beaulieu, 2002; Mori and Zhang, 2006). A reduction in FA may be interpreted as a reduced density of white matter fibers, less directional coherence of fibers, or a reduced degree of myelination of fibers, all indicative of damaged, disorganized or under-developed white matter.

Measuring differences in brain structure and function between groups of patients and controls using the above described techniques can provide us with valuable insights into the neurobiological features associated with the disease of interest. However, the outcomes of these comparisons may be confounded by several factors.

Most brain imaging studies, comparing patients with controls, mainly aim to explore abnormalities in brain structure and function that are related to the development of a disease. However, some of the brain abnormalities observed in these studies might actually be a consequence of the disease (e.g., neurobiological changes induced by the stress/anxiety the patient experiences), or of the medication used for treatment, rather than a cause. Comparing subjects at high risk for the disease of interest (e.g., subjects scoring very high for the disease symptoms), without clinical diagnoses or treatment history, with subjects at low risk for the disease may overcome part of this confounder and thereby provide us with better insights into the neurobiological factors associated with the development of the disease.

A second potential confounder lies in the fMRI technique. Obviously, fMRI is a very indirect measure of brain activity and, apart from the BOLD-effect, T2* is also influenced by other physiological factors such as respiratory and cardiac cycles which modulate blood oxygen levels and microvessel diameters (Birn et al., 2006; Glover et al., 2000; van Houdt et al., 2010; Windischberger et al., 2002). In paradigms where heart rate is modulated by the task, e.g., when there are different levels of task difficulty or emotional valence, it poses a serious threat to the interpretation of the data, since statistically significant differences between task conditions may then not be caused by the BOLD-effect alone, but also by non-neuronal responses of the vascular bed to heart rate variations. In order to correct for possible confounding, heart rate recorded during the fMRI experiment can be included in the fMRI analysis as a regressor of no interest.

Another potential source of heterogeneity observed between studies investigating brain structure and function in a sample of patients and controls relates to male-female differences in brain organization. Sex differences in the human brain are very evident. Males have approximately 9-12% larger brain volumes than females and apart from this global volume difference, regional sexual dimorphisms have also been reported, primarily for areas with high numbers of sex steroid receptors (amygdala and hypothalamus larger in males; hippocampus and caudate larger in females) (Cosgrove et al., 2007; Lenroot and Giedd, 2010). In studies investigating the neurobiology of neuropsychiatric disorders the number of males and females are generally not balanced and furthermore the distribution of males and females often differs between studies (e.g., more males than females in one study versus more females in another study). In particular for neuropsychiatric disorders that differ in prevalence and/or symptoms between males and females, like OCD, this may lead to different outcomes. In order to explore if neurobiological changes related to the disorder of interest differ between males and females, an interaction of the disorder by sex on brain structure or function needs exploration.

Outline of this thesis

The main aim of this thesis is to explore whether environmental or genetic risk factors for OC symptoms affect the structure and functioning of the brain in different ways, and if so, whether that could explain part of the observed inconsistencies between studies that compared OCD patients with controls. In order to investigate in what way environmental risk factors for OC symptoms affect the brain, anatomical brain images and functional brain changes during the performance of cognitive tasks, obtained using MRI, were compared within MZ twin pairs discordant for OC symptom scores. To explore neurobiological changes mediated by the genetic risk for OC symptoms, anatomical brain images and functional brain changes during the performance of cognitive tasks were compared between MZ twin pairs scoring both high for OC symptoms and MZ twin pairs scoring both low for OC symptoms.

In addition, within this thesis we explored whether heart rate, when modulated by the fMRI paradigm, could be a serious threat for the interpretation of the fMRI data. Furthermore, the interaction of OC symptoms by sex on gray matter volume was assessed in order to explore if OC symptom related changes in gray matter volume were different for males and females. For this analysis, an additional set of MRI scans was obtained from a sample of opposite-sex twin and sibling pairs scoring either both high or low for OC symptoms that were combined with MRI data obtained in the MZ (same-sex) twin sample. The participating twin and sibling pairs were all registered in the Netherlands Twin Register (Boomsma et al., 2006) and a complete description of the selection criteria, data collection and experimental procedures can be found in **chapter 2**.

Chapters 3 to 6 address the main aim of this thesis. In **chapter 3** task performance and brain activation during a planning paradigm are compared within MZ twin pairs discordant for OC symptoms, in order to investigate planning related functional brain changes mediated by the environmental risk for OC symptoms. **Chapter 4** describes regional brain changes for the same fMRI paradigm as used in chapter 3 but adds a comparison of MZ twin pairs who both scored high for OC symptoms with MZ twin pairs who both scored low for OC symptoms, in order to investigate planning related functional brain changes mediated by the genetic risk for OC symptoms. **Chapter 5** uses the MZ discordant/concordant twin design in order to examine the differential impact of non-shared environmental versus genetic risk factors for OC symptoms on inhibitory control related functional brain activation. In **Chapter 6** the differential impact of non-shared environmental versus genetic influences on white matter structure was investigated by comparing white matter volume as well as fractional anisotropy derived from DTI scans within MZ twin pairs discordant for

OC symptoms or between MZ twin pairs concordant-low and concordant-high for OC symptoms.

Chapters 7 to 9 are concerned with the possible impact of heart rate and sex differences in the interpretation of MRI data. **Chapter 7** explores the extent to which fMRI signal changes between cognitive task conditions are influenced by between-condition differences in heart rate. **Chapter 8** tries to create a more comprehensive picture of general sex differences in structural brain measures, by investigating differences in regional gray and white matter volume, white matter integrity and cortical thickness in carefully matched male-female pairs. **Chapter 9** investigates if sex could be a potential source of heterogeneity in the association of OC symptoms with structural brain imaging outcomes.

Finally, in **Chapter 10** the results of the performed studies are integrated and discussed.

2 Data collection: Sample selection and testing procedures

The data that form the basis of the studies described in this thesis were collected in two points in time. The first data collection took place from 2006-2009 and included structural and functional MRI and behavioral measurements in a sample of monozygotic (MZ) twin pairs selected to be either discordant or concordant for obsessive-compulsive (OC) symptoms. This first data set was mainly used to explore whether environmental or genetic risk factors for OC symptoms affect the brain in different ways. The second data collection took place from 2010-2011 and consisted of structural and functional MRI and behavioral measurements in a sample of opposite-sex twin and sibling pairs selected to be highly concordant for OC symptoms. MRI and behavioral measurements obtained in the opposite-sex twin and sibling pairs were combined with those obtained in the sample of MZ twin pairs in order to investigate OC symptom related sex differences in the brain. In this chapter, a detailed description of the complete selection and testing procedures will be given.

Sample selection: participating twins and siblings

All twins and siblings that participated in this study were recruited from the Netherlands Twin Register (Boomsma et al., 2006). In 2002/2003 and 2008/2009, surveys were sent to twin families including the 12-item Padua Inventory Abbreviated (PI-R-ABBR). The PI-R-ABBR is derived from the Padua Inventory-Revised version (PI-R), a widely used self-report inventory measuring OC symptoms (Sanavio, 1988; van Oppen et al., 1995). The PI-R consists of 41 items, that each have to be rated on a 5 point scale regarding degree of disturbance (0 = not at all disturbing – 4 = very much disturbing) (van Oppen et al., 1995). Reduction of the PI-R to 12 items was implemented by selecting two items of each of the five PI-R subcategories (washing, checking, rumination, precision and impulses) with highest factor loadings in a previous validation study (van Oppen et al., 1995), and adding another two items for each of the more equivocal obsession subscales: rumination and impulses. Examples of questions implemented in the PI-R-ABBR are shown in **table 2.1**.

Completed PI-R-ABBR questionnaires were returned by 20.204 subjects (mean PI-R-ABBR-score (SD): 7.27 (5.08)), including 9.512 twins and 2.403 siblings. From this sample we selected MZ twin pairs and opposite-sex twin and sibling pairs in the age range between 18 and 60 years who both scored very high, very low or very discordant for OC symptoms. A subject was classified as high-scoring for OC symptoms if the PI-R-ABBR score was ≥15. A subject was classified as low-scoring for OC symptoms if the PI-R-ABBR score was ≤7. These PI-R-ABBR cut-off scores were derived from sensitivity and specificity measurements in an independent sample of OCD patients when compared

Table 2.1. Examples of questions implemented in the 12-item PI-R-ABBR

Items	Category
In certain situations, I am afraid of losing my self-control and doing embarrassing things	Impulses
I check and recheck gas and water taps and light switches after turning them off	Checking
I feel obliged to follow a particular order in dressing, undressing and washing myself	Precision
Unpleasant thoughts come into my mind against my will and I cannot get rid of them	Rumination
If I touch something which I think is 'contaminated', I immediately have to wash or clean myself	Washing

to clinical controls (n=120; mean scores 20.7, SD 8.1; sensitivity 0.74 and specificity 0.72 at the best cut-off point of 16 (Cath et al., 2008)).

A total of 32 MZ twin pairs discordant for OC symptoms, 38 MZ twin pairs concordant-high for OC symptoms and 41 MZ twin pairs concordant-low for OC symptoms were invited by letter to participate in the first MRI study that investigated neurobiological changes mediated by environmental or genetic risk factors for OC symptoms.

An additional sample of 11 opposite-sex twin pairs scoring high for OC symptoms, 24 opposite-sex twin pairs scoring low for OC symptoms and 13 families, including at least one pair of opposite-sex siblings scoring high for OC symptoms (total of 31 subjects, including 14 high-scoring males, 14 high-scoring females and 3 low-scoring females), were invited to participate in the second MRI study that investigated OC symptom related sex differences in the brain.

Invitation procedures were the same for both samples. Approximately one week after an invitation letter **(Appendix I)** was sent, twins and siblings were contacted by phone and asked whether they were interested to participate in the study. In addition, twins and siblings were screened for possible exclusion criteria. Exclusion criteria included brain damage, neurological disease, color blindness and contraindications for MRI (e.g., pregnancy, ferromagnetic fragments, clips and devices in the body and claustrophobia). When interested, twins and siblings were sent additional information **(Appendix II)**, including a MRI brochure **(Appendix III)** and MRI questionnaire, were they could indicate possible contra-indications for MRI and use of medication **(Appendix IV)**. Approximately one week after the

additional information was sent, twins and siblings were again contacted by phone. When they agreed to participate they received, approximately 2-3 weeks in advance to their visit to the hospital for MRI scanning, a confirmation letter **(Appendix V)** that included: the date and time of the appointment made by phone, the route to the hospital, a self-report questionnaire and informed consent **(Appendix VI)** they were asked to fill out and sign at home and bring along when visiting the hospital, and a package containing an instruction brochure **(Appendix VII)** and required material for collecting buccal cell samples for DNA extraction, which they were also asked to perform at home and bring along when visiting the hospital.

In total, 20 MZ twin pairs discordant for OC symptoms (6 male/14 female pairs; mean age (SD): 35.60 (8.68)), 23 MZ twin pairs concordant-high for OC symptoms (6 male/17 female pairs; mean age (SD): 36.00 (10.55)) and 28 MZ twin pairs concordant-low for OC symptoms (8 male/20 female pairs; mean age (SD): 37.50 (8.79)) agreed to participate in the MRI study that investigated neurobiological changes mediated by environmental or genetic risk factors for OC symptoms, giving a response rate of 64%.

In the second study, that investigated OC symptom related sex differences in the brain, an additional sample of 5 opposite-sex twin pairs scoring high for OC symptoms (mean age (SD): 24.80 (9.27)), 19 opposite-sex twin pairs scoring low for OC symptoms (mean age (SD): 30.11 (9.64)) and 7 families including at least one pair of opposite-sex siblings scoring high for OC symptoms (total of 16 subjects, including 8 high-scoring males, 7 high-scoring females and one low-scoring female; mean age (SD): 32.13 (5.77)) agreed to participate, giving a response rate of 63%.

For the 57 twin pairs/families that did not participate in the MRI study, the most important reasons included; no time, too much effort (n=31), twins/siblings did not want to participate in MRI research (n=4), twins/siblings moved and new contact details could not be retrieved in time (n=5), twins/siblings moved to another country (n=3), or twins/siblings were excluded from the study due to neurological disease (n=1), pregnancy (n=6), claustrophobia (n=4) or ferromagnetic fragments, clips and devices in the body (n=3). The ethical review board of the VU University medical centre approved the study. All participants provided written informed consent.

Experimental procedures

 The data collection took place at the Academic Medical Centre (AMC) Amsterdam

and consisted of structural and functional MRI scans and the completion of questionnaires and diagnostic interviews. Twin pairs and siblings were always tested on the same day and a regular testing day took approximately 3.5 hours (for two subjects).

After the participants arrived at the AMC, they were first welcomed and testing procedures were explained. Thereafter, questionnaires, forms and buccal cell samples completed/collected by the participants at home were checked and some personal information was obtained (e.g., participant's name, date of birth, number of bank account for travel reimbursement). Then the participants' weight and height were measured and they were asked to fill out a questionnaire that measured state anxiety and state anger. Thereafter, the twins/siblings were separated for individual assessments. One of the participants first underwent the MRI protocol and thereafter was administered diagnostic interviews and questionnaires. The brother or sister had the protocol administered the other way around; first questionnaires and interviews followed by the MRI scan. The order in which the participants received the scan protocol or questionnaires/interviews was completely randomized. During the MRI session the participants had to perform a set of cognitive tasks. Prior to the performance of these tasks in the MRI scanner, participants were familiarized with the tasks during a practice session on a personal computer outside the scanner. In between the MRI session and the administration of questionnaires/interview, participants were provided with lunch, dinner or tea with cake, depending on the time the testing procedures took place. For the complete testing schedule and the approximate times see **table 2.2**. The different components of the testing protocol (interview/questionnaires and scan protocol) are described in more detail in the following sections.

Questionnaires and diagnostic interviews

Self-report questionnaire received at home

All twins and siblings that agreed to participate in our study received a self-report questionnaire at home that they were asked to fill out and bring to the hospital at the day of MRI scanning. This self-report questionnaire consisted of some general and demographic questions (e.g., questions on gender, health, number of siblings, birth weight, educational attainment), questions on experienced life events (e.g., death of a parent/sibling/partner/child, birth of a child, severe illness, marriage, burglary), the PI-R-ABBR, comparative twin rating questions (Reynolds et al., 2005), the 13-item Beck-Depression Inventory Short Form (Beck et al., 1961; Beck et al., 1974) and the 30-item Conners Adult Attention Deficit Hyperactivity Disorder (ADHD) Rating Scale (Conners et al., 1999).

Table 2.2. Data collected from twin/sibling pairs

At home	
MRI questionnaire and medication list (Appendix IV)	
Self-report questionnaire	
Informed consent (Appendix VI)	
Buccal cell samples for DNA extraction	
At the AMC	
Welcome of twin (sibling) pair and explanation of testing day (± 10 min)	
Checking data participants filled out/collected at home (± 5 min)	
Obtaining personal information (± 5 min)	
Measuring weight and height (± 5 min)	
Measuring state anxiety and state anger (± 10 min)	
Individual assessments	
twin (sibling) 1	twin (sibling) 2
Explaining and practising fMRI tasks on personal computer (± 15 min)	self-report questionnaires and interview (± 55 min)
Placement of electrodes for electrocardiography (± 10 min)	*lunch, dinner or tea with cake*
Structural MRI, functional MRI and DTI (± 60 min)	Explaining and practising fMRI tasks on personal computer (± 15 min)
lunch, dinner or tea with cake	Placement of electrodes for electrocardiography (± 10 min)
self-report questionnaires and interview (± 55 min)	Structural MRI, functional MRI and DTI (± 60 min)

Self-report questionnaires and diagnostic interviews obtained at the AMC

On the day of scanning the following diagnostic interviews and questionnaires were administered: (1) The state version of the State Trait Anxiety Inventory and the State Trait Anger Scale, to measure the participants state anxiety and state anger (Spielberger et al., 1970; Spielberger et al., 1983); (2) Tic screening: participants were screened for the eight most common tics (head shaking, eye blinking, other facial tics, shoulder raising, expressing swear words/foul language/dirty words, sound making, growling and throat clearing/coughing/sniffing) and were asked to

indicate whether they were familiar with one of these tics by answering 'yes' or 'no'; (3) An adapted form of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), to measure both lifetime and current obsessive-compulsive symptoms (Y-BOCS symptom checklist) and severity (Y-BOCS symptom severity) (Denys et al., 2004; Goodman et al., 1989b; Goodman et al., 1989a); (4) The Mini International Neuropsychiatric Interview to test for possible comorbidities (Sheehan et al., 1998). Comorbidities tested by the Mini International Neuropsychiatric Interview include depression, panic disorder, agoraphobia, social phobia, post-traumatic stress disorder and generalized anxiety disorder.

MRI scan protocol

MRI was performed on a 3.0 Tesla Intera MRI system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. For the selected sample of MZ twin pairs scoring discordant or concordant for OC symptoms who were scanned between 2006 and 2009, the MRI session consisted of a whole head anatomical scan, functional MRI scans obtained during the performance of three cognitive tasks and diffusion tensor imaging (DTI). Cognitive tasks performed while in the MRI scanner included the Tower of London planning paradigm, the cognitive and emotional Stroop and the Flanker task, which are all described in more detail below. For the sample of opposite-sex twin and sibling pairs, scanned in 2010/2011, the scan protocol was mainly the same, except for the fMRI scan obtained during the Flanker task which was replaced by two resting state scans that were followed by a resting state questionnaire (described in more detail below). See **table 2.3** for a summary of the scan protocol, including scan parameters and scan duration. During the MRI session, participants remained inside the scanner and were asked to minimize head movements during and between consecutive runs. The MRI protocol could not be completed by one of the twins from a concordant-low pair due to a metal artifact at the eyebrow level and by one of the twins from a concordant-high pair due to a panic attack. Furthermore, one MZ discordant pair could not complete the Tower of London, due to a technical problem on the day they were tested. Thus, structural MRI, DTI and fMRI during the Stroop paradigm were obtained in a total of 204 subjects, fMRI during the Tower of London in 202 subjects, fMRI during the Flanker in 140 subjects and resting state scans in 64 subjects.

Functional MRI

Cognitive paradigms, participants had to perform while in the MRI scanner, were projected on a screen at the end of the MRI scanner table and viewed by the

Table 2.3. Scan protocol, scan acquisition parameters and scan duration

MRI scan	Acquisition parameters	Duration
Survey scan	T1 weighted; repetition time (TR) = shortest; Echo Time (TE) = 4.60 ms	±1 min
Tower of London fMRI	Echo Planar Imaging Sequence; 440 volumes; 40 axial slices; 96x96 matrix; field of view (FOV) = 220 mm; TR = 2300 ms; TE = 30 ms; flip angle = 80°; slice thickness = 3.0 mm; 2.29 x 2.29 in plane resolution	±17 min
Stroop fMRI	Echo Planar Imaging Sequence; 260 volumes; 40 axial slices; 96x96 matrix; FOV = 220 mm; TR = 2300 ms; TE = 30 ms; flip angle = 80°; slice thickness = 3.0 mm; 2.29 x 2.29 in plane resolution	±10 min
T1-weighted anatomical scan	3D Gradient Echo T1 weighted sequence; 182 coronal slices; 256x256 matrix; FOV = 256; slice thickness = 1.2 mm; TR = 9.69 ms; TE = 4.60 ms; flip angle = 8°; voxel size = 1.00 mm x 1.00 mm x 1.20 mm	±6 min
Flanker fMRI*	Echo Planar Imaging Sequence; 250 volumes; 40 axial slices; 96x96 matrix; FOV = 220 mm; TR = 2300 ms; TE = 30 ms; flip angle = 80°; slice thickness = 3.0 mm; 2.29 x 2.29 in plane resolution	±10 min
DTI	Diffusion tensor images were obtained in 32 directions with singleshot echoplanar acquisition; 38 axial slices; 112x110 matrix; FOV = 230; slice thickness = 3.0 mm; TR = 4834 ms; TE = 94 ms; flip angle = 90°; voxel size = 2.00 mm x 2.00 mm x 3.00 mm; b-value = 1000 sec/mm2	±3 min
Resting state fMRI*	Echo Planar Imaging Sequence; 140 volumes; 38 axial slices; 80x78 matrix; FOV = 220 mm; TR = 2200 ms; TE = 30 ms; flip angle = 80°; slice thickness = 2.5 mm; 2.75 x 2.75 in plane resolution	±5 min

** For the sample of opposite-sex twin and sibling pairs the Flanker fMRI was replaced by two resting state scans, of which one was obtained immediately after the survey scan and the second before the DTI scan.*

subject through a mirror. Two MRI compatible response boxes were used to record the subject's responses. Before the experiment, the subjects practiced a number of trials on a computer outside the scanner and again inside the scanner, prior to the actual start of the session.

Tower of London

Stimuli for the Tower of London task consisted of images of three colored beads (red, blue and yellow) placed on three vertical rods of decreasing height **(figure 2.1)**. In each trial, a start configuration **(figure 2.1, bottom)** and final target configuration **(figure 2.1, top)** were simultaneously displayed. During planning trials **(figure 2.1A)**, subjects were requested to count the number of steps to get from the start to final target configuration, with the restrictions that only one bead could be moved at a time and that a bead could be moved only if there was no other bead on top. Five planning difficulty levels were included corresponding to the minimum number of moves (1-5) needed to achieve the target configuration. In addition, baseline stimuli were included **(figure 2.1B)** during which subjects only had to count the total number of yellow and blue beads. With each stimulus presentation, two possible answers (one correct and one incorrect) were presented at the bottom left and right of the screen. The correct answer had to be indicated by pressing the corresponding left or right hand button. No feedback regarding the correct answer was provided. The stimuli were presented in an event-related design of approximately 17 minutes with self-paced stimulus timing, i.e., a subsequent trial was presented on the screen immediately after the response on a previous trial, or directly after the maximum reaction time limit of 60 seconds. Presentation order of the stimuli was pseudo-random with distribution frequency of the six stimulus types similar to van den Heuvel (2005a). The stimulus presentation order was the same for all subjects, however, the total number of trials completed by each subject depended on the subject's reaction times.

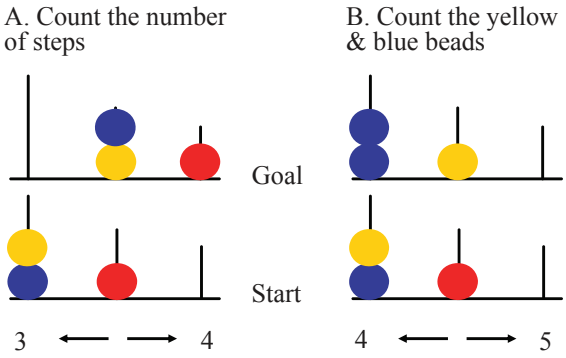

Figure 2.1. *Examples of Tower of London stimuli; (A) Planning condition; (B) baseline condition.*

Cognitive and emotional Stroop

The Stroop paradigm which was implemented in this study was developed by Dr. O.A. van den Heuvel (2005b) and consisted of 6 conditions: congruent color-words (e.g., the word "green" written in green), incongruent color-words (e.g., the word "red" written in blue), OC symptom related negative words (e.g., dirty, mess, uncertain), panic-related negative words (e.g., heart attack, cancer, panic), and two conditions with neutral words (e.g., table, world, guitar). The task was administered in 18 blocks of similar stimulus types (3 blocks of each condition). In each individual block 16 words were presented for 2 seconds separated by small intervals of 200 milliseconds. During the task participants were asked to report the ink color of the words that were written in the color "red", "yellow", "blue" or "green". The correct answer had to be indicated by pressing buttons: left middle finger for ink color yellow, left forefinger for green, right forefinger for red and right middle finger for blue. The subjects were asked to respond to the stimuli as fast and accurate as possible. The onset of each individual stimulus together with the subject's response was recorded, such that the data could be analyzed in an event-related manner. Total task duration was ±10 minutes.

Flanker

In the flanker task subjects had to indicate, as quickly as possible, the direction of a central target arrow (i.e., "<" left hand press; ">" right hand press) which was surrounded by four task irrelevant flankers of the same size and shape. The direction of the flanker arrows could be either congruent ('< < < < <' or '> > > > >') or incongruent ('< < > < <' or '> > < > >') to the direction of the central target arrow. Flankers and targets were displayed simultaneously. The task was administered in an event-related design. During the task 120 congruent and 120 incongruent trials were presented in random order. Stimuli were shown for 200 ms and the interstimulus interval consisted of a period of gray screen after each stimulus (randomized between 600 and 1600 ms) and a subsequent fixation cross for 1000 ms before the next stimulus. Total task duration was ±10 minutes.

Resting state

Before the start of each resting state scan participants were instructed to relax as much as possible, close their eyes and try not to fall asleep. Immediately after each resting state scan subjects had to complete a resting state questionnaire (RSQ) that was projected on a screen at the end of the MRI scanner table and viewed by the subject through a mirror. The RSQ was developed (by K. Linkenkaer-Hansen) for rating feelings and thoughts during the resting state scan and consisted of 50 items. Examples of items included in the RSQ are; I felt comfortable, I was thinking about the future or I felt sleepy. These questions could be answered on a five point scale, including not, a little, moderately, fairly strong and strong. Switching between these five possible answers could be done by pressing the

response buttons under the right forefinger and right ring finger, and the answer could be confirmed by pressing the response button under the right middle finger. Total duration for completing the questionnaire was approximately 5 minutes.

Measuring heart rate and respiratory frequency during fMRI

During resting state scans and during the performance of the three cognitive tasks within the MRI scanner, heart rate was measured in all participating subjects. In addition, for most subjects, excluding 12 MZ discordant twin pairs, respiratory frequency during fMRI was measured. Heart rate was measured by means of electrocardiography (ECG), for which a total of four (MRI compatible) ECG electrodes (Philips) were attached to the subject's chest. The respiratory signal was measured by the pressure exerted on a balloon that was placed at the level of the abdomen and fastened using a band. During the MRI experiment, ECG and respiratory signals were written to a text file along with the output of the slice selecting gradient of the MRI scanner and were mainly used to investigate whether changes in brain activation were related to changes in these two measures.

3 An fMRI study in monozygotic twins discordant for obsessive-compulsive symptoms

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Abstract

To examine neurobiological changes underlying obsessive-compulsive symptoms (OCS) we examined intrapair differences in behavior and fMRI brain activation in monozygotic twins discordant for OCS, using a Tower of London planning paradigm. Despite only mild evidence for impairment at the behavioral level, twins with OCS showed significantly decreased brain activation during planning in dorsolateral prefrontal cortex, thalamus pulvinar, and inferior parietal cortex. These findings are consistent with the hypothesis of disturbed cortico-striato-thalamo-cortical (CSTC) circuitry underlying OCS. In contrast to previous studies in patients with obsessive-compulsive disorder (OCD) we did not find robust evidence for reduced responsiveness in striatal brain regions. Together, these findings suggests that neurobiological mechanisms underlying OCS of environmental origin partly overlap with neurobiological changes in patients with OCD, where the disorder is likely caused by a combination of genetic and environmental influences. A difference between genetical and environmental etiologies may relate to the amount of reduced striatal responsiveness.

Introduction

Obsessive-compulsive symptoms (OCS) are highly prevalent in the general population (70%-80%: Rachman and de Silva, 1978). They are characterized by recurrent, persistent, and intrusive anxiety-provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions) performed to reduce anxiety and/or distress caused by the obsessions (American Psychiatric Association, 1994). Well-known obsessions are fear of contamination, pathological doubt, need for symmetry, and somatic, sexual and aggressive obsessions. Compulsions include checking, washing, counting, symmetry/precision and hoarding behavior. When obsessions and/or compulsions are performed for more than one hour a day and significantly interfere with daily life, persons fulfill the criteria for obsessive-compulsive disorder (OCD). OCD affects about 2% of the population (Miguel et al., 2005) and is generally assessed by clinical interviews, e.g., Diagnostic and Statistical Manual of Mental Disorders 4th edition: DSM-IV (American Psychiatric Association, 1994). Questionnaires, such as the Padua Inventory (PI) (Sanavio, 1988) and quantitative versions of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) (Goodman et al., 1989a; Goodman et al., 1989b) can be utilized to explore OC symptomatology on a more quantitative scale.

There is limited information about the etiology of OCD. Genetic factors appear to be at least partly responsible. The disorder runs in families (Nestadt et al., 2000;

Hettema et al., 2001) and twin studies indicate a heritability ranging from 27% to 47% in adults and 45%-65% in children. (van Grootheest et al., 2005; Jonnal et al., 2000).

If genetic factors explain 27-65% of the variability in OC symptoms, as much as 35% to 73% should be accounted for by environmental stressors or adverse gene-environment interactions. Environmental risk factors suggested for OCD include streptococcal infection, perinatal problems, psychosocial stress, and familial factors such as perceived parental rearing style (Miguel et al., 2005; Alonso et al., 2004). Furthermore, several life events, including pregnancy and divorce, may trigger OCD in people genetically predisposed to the disorder (Karno et al., 1988). A recent twin study in MZ twin pairs concordant and discordant for OC symptoms identified the following risk factors: sexual assault in women, low birth weight, and low educational level (Cath et al., 2008).

Neuroimaging studies have indicated several brain changes in OCD patients compared to unaffected controls. Structural magnetic resonance imaging (sMRI) has indicated gray matter abnormalities in the prefrontal cortex (PFC), orbitofrontal cortex (OFC), caudate nucleus, thalamus and anterior cingulate cortex (ACC) (Pujol et al., 2004; Valente Jr. et al., 2005); in line with the hypothesis of a disturbed cortico-striato-thalamo-cortical (CSTC) circuitry. Consistent with the sMRI findings, functional MRI (fMRI) studies have reported increased activation of these brain structures in OCD patients during performance of cognitive tasks and after symptom provocation. For example, it was recently found that OCD patients show increased activation of frontal-striatal and medial temporal brain regions during presentation of OC related threat words in a Stroop color-word naming task (van den Heuvel et al., 2005b). In addition, in the Eriksen flanker task increased anterior cingulate activation has been observed in OCD patients (Fitzgerald et al., 2005), in agreement with the hypothesis that OCD involves overactive interference monitoring and error-processing. Besides these brain regions, increased functional activation has also been reported for parahippocampal and parietal structures (Schienle et al., 2005; Viard et al., 2005). There is also evidence for abnormally reduced activation of brain areas. A recent study indicated that OCD patients are impaired on the Tower of London (ToL) cognitive planning task (Purcell et al., 1998a) and that this planning in OCD patients is associated with decreased fMRI activation of the dorsolateral prefrontal cortex (dlPFC) and caudate nucleus (van den Heuvel et al., 2005a). In summary, the overall picture points to a deficit of CSTC processing, combined with dysfunction of midbrain and brainstem systems. However, there are considerable inconsistencies regarding the brain structures involved and the direction of anatomical and functional changes. It may therefore be concluded that, until now, neuroimaging studies have been only marginal successful in reducing the observed variability in OC problem behavior associated

with variations in anatomy and/or function of specific brain regions.

An important reason for the inconsistent findings might be 1) the heterogeneity of the OCD phenotype and 2) the differential impact of genetic and environmental risk factors in OC behavior that does not necessarily lead to identical neurobiological pathways underlying OC behavior. With respect to the first issue, an approach that uses more homogeneous disease dimensions, such as familial cases, cases with early onset or with only one symptom dimension, might lead to more consistent results (Miguel et al., 2005). With respect to the second issue, group analyses of affected individuals in whom OCD is caused by differences in relative contributions of genetic and environmental risk factors may produce inconsistent results.

The present study, using a monozygotic discordant twin design (Martin et al., 1997) to explore OCS-related neurobiological alterations, is a first attempt to overcome the second issue. This discordant twin design allows the investigation of between twin brain differences that are specifically due to influences of environmental risk factors. Because MZ twins begin life with identical genomes, within twin pair differences in behavior mostly reflect exposure to individual-specific environment (although these may ultimately act through modification of gene expression).

We assessed differences in functional brain activation using the Tower of London task that measures the capability of cognitive planning. We aimed to investigate whether individuals with OC symptoms due to adverse environmental influences exhibit similar changes in task performance and functional brain activation during planning as previously observed in OCD patients.

Methods

Participants

For this study twin pairs were recruited from the Netherlands Twin Register (NTR) (Boomsma et al., 2002). In 2002 surveys were sent to twin families including the Padua Inventory-R (PI-R) abbreviated (Sanavio, 1988; van Oppen and Arntz, 1994). Symptoms were chosen on basis of 2 items of each subscale with highest factor loadings in a previous validation study (van Oppen and Arntz, 1994), covering the symptom factors generally found in the PI-R dimensions of OCD, and with one additional item for each of the more equivocal obsession subscales rumination and impulses. For a detailed description of reliability and validity of the PI-R abbreviated as a screening instrument of OC behavior (see Cath et al., 2008). Complete PI data were returned by 419 MZ twin pairs (n = 113 males). From this sample we selected twin pairs in the age range between 18-60 years,

in which one twin scored high (≥ 18) and the co-twin scored low (≤ 7) on the PI-R. These cut-offs were derived from sensitivity and specificity measurements in a sample of OCD patients (n = 120; mean scores 20.7, SD 8.1; sensitivity .74 and specificity .72, when compared to clinical controls (Cath et al., 2008)). From the initial selection of 29 MZ twin pairs, 17 pairs had to be omitted: 5 pairs already participated in other studies of our department, 1 pair was found to be dizygotic, 1 pair used psychotropic medication, 2 pairs suffered from severe claustrophobia and 8 pairs declined for practical reasons. Consequently, our final sample consisted of 12 MZ twin pairs discordant for OCS (14 females and 10 males).

Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, comorbidity, OC symptoms and severity of OC symptoms, tics, state-anger and state-anxiety. All twins were asked to collect buccal swabs for DNA extraction to test zygosity. The ethical review board of the VU medical centre approved the study and all participants provided written informed consent.

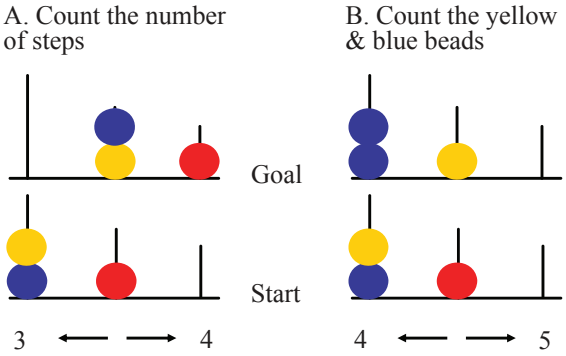


Figure 3.1. *Examples of Tower of London stimuli used in the present study. A. Planning condition; B. Baseline condition (adapted from van den Heuvel et al. (2005a)).*

Tower of London (ToL)

Stimuli for the ToL task consisted of images of 3 colored beads (red, blue, yellow), placed on 3 vertical rods of decreasing height (see **figure 3.1**). On each trial a start configuration (bottom) and final target configuration (top) were simultaneously depicted. During planning trials **(figure 3.1A)**, subjects were requested to count the number of steps from the starting configuration to reach the target configuration; with the restriction that only one bead could be moved at a time and that a bead could be moved only if there was no other bead on top.

Five planning difficulty levels were included that corresponded with the minimal number of moves (1 to 5) actually needed to achieve the target. In addition to stimuli that required planning, baseline stimuli were included **(figure 3.1B)** during which subjects only had to count the total number of yellow and blue beads. With each stimulus presentation, two possible answers (one correct and one incorrect) were presented at the bottom left and right of the screen, from which the correct one had to be chosen by pressing a corresponding left or right hand button. No feedback regarding the correct answer was provided during the task.

The stimuli were presented in an event-related design lasting 17 minutes with self-paced stimulus timing, i.e., a subsequent trial was presented on the screen immediately after the response on a previous trial, or directly after the maximum reaction time limit of 60 seconds. Presentation order of the stimuli was pseudo-random with a distribution frequency of the 6 stimulus types derived from van den Heuvel et al. (2005a). For all twins the stimulus presentation order was the same, however, the total number of trials completed by each twin depended on the twin's reaction times.

Stimuli were projected on a screen at the end of the MRI scanner table, viewed by the participant through a mirror. Two magnetic compatible response boxes were used to record the subject's performance. Prior to performance of the ToL task within the scanner, twins were made familiar with the task during a practice session on a personal computer outside the scanner. Furthermore, subjects performed a number of practice trials while being in the scanner, immediately before starting the actual task.

Image acquisition

The MRI session consisted of a structural part of about 6 minutes and a functional part of approximately 17 minutes. During the scan session the twins remained inside the scanner and were asked to minimize head-movement during and between consecutive runs. To reduce motion artifacts, the participant's head was immobilized using foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3D gradient-echo T1-weighted sequence (flip angle 8°; repetition time, TR = 9.69 ms; echo time, TE = 4.60 ms; matrix, 256x256 pixels; voxel size, 1.00 mm x 1.00 mm x 1.20mm). For fMRI, an echo planar imaging (EPI) sequence (flip angle 80°; TR = 2300 ms; TE = 30 ms; matrix, 96x96 pixels; field of view 220 mm x 220 mm) was used, covering the whole brain (40 axial slices; 2.29 mm x 2.29 mm in-plane resolution; 3.0 mm slice thickness). A total of 440 EPI volumes were scanned per subject.

Data analysis

MRI data were analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK). EPI scans were slice time corrected, realigned and normalized to the standard MNI (Montreal Neurological Institute) brain of SPM. Subsequently, data were resliced to 3 mm x 3 mm x 3 mm voxels and spatially smoothed using an 8 mm isotropic Gaussian kernel. After high-pass filtering (cut-off 128 seconds), functional scans were analyzed in the context of the general linear model using delta functions convolved with a canonical hemodynamic response function. Event duration, computed as the time between stimulus and response onset, was included in the model to account for hemodynamic responses of varying lengths to each type of stimulus. Error trials and head-movement parameters were modeled as regressors of no interest. For each subject, a 'planning vs. baseline' main effect was computed in which brain activation during all planning trials was compared with brain activation during baseline trials. In addition, a main effect of 'task load' was computed using a linear contrast to identify brain regions that show MR signal intensity variation correlated with task difficulty (van den Heuvel et al., 2005a).

Differences in questionnaire- and interview data between high and low-scoring twins were tested using paired sampled t-tests available in SPSS software (SPSS Inc, Chicago, Illinois), with significance level $p < 0.05$. For analysis of ToL task performance, reaction times and reaction accuracy (percentage of correct responses) were evaluated statistically by means of a paired MANOVA design with main variables 'task load' (the 5 planning difficulty levels) and 'twin OCS status' (twins scoring high on OCS vs. twins scoring low on OCS). When applicable, degrees of freedom were adjusted conforming to the method of Geisser and Greenhouse (Geisser and Greenhouse, 1958). Uncorrected degrees of freedom are reported, however, to facilitate interpretation of the statistical design.

Functional MRI contrast estimates for 'planning vs. baseline' and 'task load' were entered into a second-level analysis. Main effects across twins for both contrasts were obtained by one-way ANOVA and reported at an individual voxel threshold of $p < 0.05$, corrected for multiple comparisons (false discovery rate: FDR), with minimal cluster extent of 10 voxels. Differences in contrast estimates between OCS high twins and their OCS low-scoring co-twins were investigated by paired sample t-test, masked with the appropriate contrast main effect (mask thresholded at $p < 0.005$, uncorrected), and reported at an uncorrected individual voxel threshold of $p < 0.001$.

Results

Questionnaire and interview data

Demographics and data on OC symptoms of our twin sample are summarized in **table 3.1**. In line with the initial selection criteria, scores on the PI-R abbreviated obtained in 2002 differed significantly between OCS high and low twins ($t = 8.89$, $df = 11$, $p < 0.001$). Re-administration of this interview at the time of MRI data collection (in 2006) indicated that within twin pairs OCS differences had slightly diminished over time: mean PI-R score of the OCS high twins was decreased by 6.75 points while mean PI-R score of the OCS low group was increased by 2.66 points. Despite this, presumably reflecting an influence of current state dependence, PI-R scores remained significantly elevated in OCS high twins ($t = 2.23$, $df = 11$, $p = 0.047$). Y-BOCS scores obtained at the time of MRI on current OCS severity were also higher in OCS high compared with low twins ($t = 2.157$, $df = 11$, $p = 0.054$). Together, these findings indicate that within-twin pair OCS discordance was stable and also present when neuroimaging was performed.

One of the OCS high twins (female) met the criterion for OCD according to the Mini-International Neuropsychiatric Interview (MINI) at the time of MRI examination. To clarify in further detail the OC symptomatology of the persons scanned, we decided to analyze the data using severity scores of current Y-BOCS data, following the definitions used in the family study on OCD (Pauls et al., 1995), and in best estimate processes by the Tourette Syndrome Association (TSA) genetic consortium and the Obsessive Compulsive Foundation (OCF) genetic collaboration on OCD. In this method, following DSM-IV criteria, OCD is established using the Y-BOCS severity criteria, as follows: OCD is diagnosed when: OC symptoms take more than 1 hour a day and persons experience distress/ interference from the symptoms; subthreshold OCD is diagnosed when persons experience either distress from their OC symptoms but spend less than 1 hour on the symptoms, or experience no distress from the symptoms but spend more than 1 hour on the symptoms. After analysing the data using these criteria, there were 3 persons in the high-scoring group who fulfilled criteria of OCD (among whom the person who met OCD criteria using the MINI), and 2 persons fulfilled criteria of subthreshold OCD as a consequence of the time (>1 hour) spent on symptoms. In the low-scoring group no subjects fulfilled criteria of OCD but 2 persons fulfilled criteria of subthreshold OCD as a consequence of the time (>1 hour) spent on the symptoms.

Comorbidity, according to the MINI and at the time of MRI, tended to be more prevalent in the OCS high twins (see **table 3.1**: last column). However, statistical analysis did not reveal any significant within pair differences ($t = 1.42$, $df = 11$, $p = 0.184$). Separate screening for tics ($t = .90$, $df = 11$, $p = 0.389$), symptoms of

Table 3.1. Twin sample demographics

Twin pair	Age	Sex	OCS scores	Padua 2002	Padua current	Y-BOCS severity	Comorbidity	
1	35	F	high/low	20/5	16/15	0/2	--	/ --
2	31	F	high/low	34/0	36/3	23/0	DE; PD+AP; SP; PTSD; GAD	/ --
3	26	F	high/low	22/6	19/16	3/0	PD+AP; GAD	/ --
4	34	F	high/low	18/5	15/1	9/0	--	/ --
5	23	M	high/low	18/7	9/10	1/0	--	/ --
6	42	M	high/low	18/3	6/6	9/1	--	/ --
7	28	M	high/low	18/5	6/7	0/0	--	/ --
8	25	F	high/low	18/5	5/8	0/0	--	/ --
9	25	M	high/low	18/0	14/2	1/0	DE	/ --
10	36	M	high/low	24/5	15/3	2/0	--	/ --
11	38	F	high/low	18/6	9/6	2/2	--	/ --
12	50	F	high/low	18/6	13/8	12/6	--	/ --
mean	32.8	7F/5M		20.3/4.4	13.6/7.1	5.2/0.9		
standard deviation	8.1			4.7/2.3	8.4/4.8	6.7/1.8		

Twin sample demographics. Twin pair: twin pair number; age: age at time of MRI exam; sex: M = male, F = female; OCS scores: obsessive and compulsive symptoms measured with the 12-item Padua Inventory; high = score ≥18 ; low = score ≤7; Padua 2002: Padua score in 2002; Padua current: Padua score at time of MRI; Y-BOCS severity: Y-BOCS severity score at time of MRI; Comorbidities: comorbidities at the time of MRI; DE = depressive episode, PD = panic disorder, AP = agoraphobia, SP = social phobia, PTSD = post-traumatic stress disorder, GAD = generalized anxiety disorder. Data for OCS high and low twins within cells are separated by a forward slash ('/').

depression (Beck's Depression Inventory Revised (BDI-R): t = 0.73, df = 11, p = 0.481), or state anxiety- and state anger (State Trait Anxiety Inventory (STAI): t = 0.73, df = 11, p = 0.482; State Trait Anger Scale (STAS): t = 1.00, df = 11, p = 0.339) also did not reveal significant differences between OCS high and low twins.

Task performance

Figure 3.2 shows measures of response latency (top) and response accuracy (bottom) as a function of task load. Significant main effects of the variable 'task load' (response latency: F (4, 44) = 118.58, p<0.001; response accuracy: F (4, 44) = 30.04, p<0.001) indicated that reaction times increased and reaction accuracy decreased with increasing task difficulty. There were no significant differences between the OCS high and low twins in response latencies and accuracies, neither for the baseline condition (response latency: t = 0.68, df = 11, p = 0.514; response accuracy: t = -0.36, df = 11, p = 0.725) nor during planning ('OCS status' main effect - response latency: F (1, 11) = 1.16, p = 0.305; response accuracy: F (1, 11) = 0.00, p = 0.981; 'OCS status' by 'task load' interaction - response latency: F (4, 44) = 1.07, p = 0.380; response accuracy: F (4, 44) = 1.42, p = 0.262). When comparing task performance for the two highest levels of task load (4 and 5 steps), we did find an indication of decreased response accuracy in OCS high twins for the most difficult planning condition (5 steps) ('OCS status' by 'task load' interaction: F (1, 11) = 3.61; p = 0.084).

Functional Imaging

Main effect

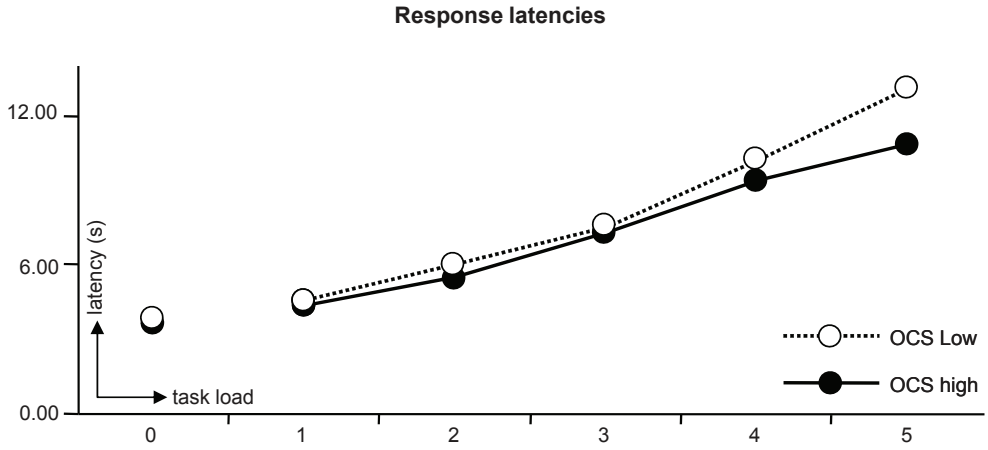
Regions showing increased BOLD signal for 'task vs. baseline' and 'task load' contrasts are summarized in the top and bottom panels of **figure 3.3** (glass brain projections) and **tables 3.2** and **3.3**, respectively. For both contrasts, clusters of increased brain activation associated with ToL planning were noted, bilaterally, in parietal cortex (Brodmann areas 7 and 40), premotor cortex (BA 6 and 8), anterior prefrontal cortex (BA 10), dorsolateral prefrontal cortex (BA 9) and cerebellum. For the 'task vs. baseline' contrast also robust task related activation was found in regions of the basal ganglia (see for example the selected anatomical overlay in the top right of **figure 3.3**). Basal ganglia activation was virtually absent for the 'task load' contrast (bottom right of **figure 3.3**).

OCS high versus low within twin pair differences

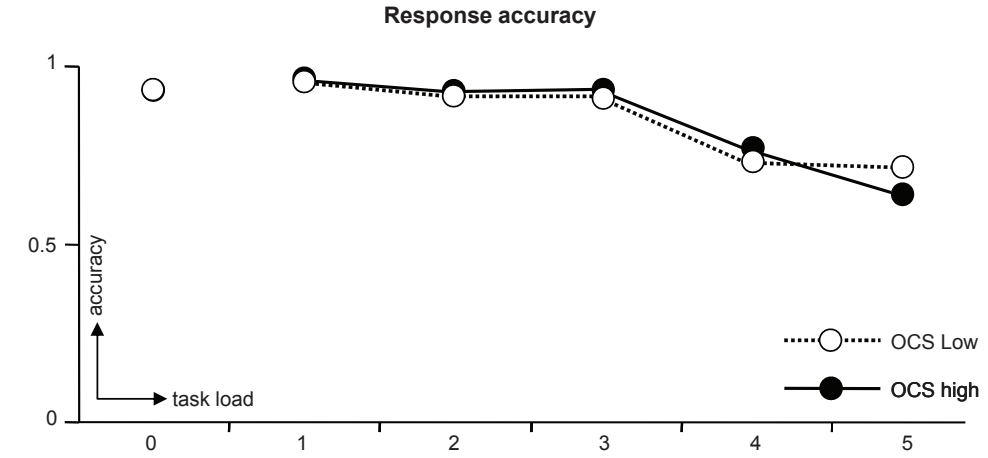
'Planning vs. baseline'

Table 3.4 and **figure 3.4** summarize the OCS high versus low within twin pair comparison results for the 'planning vs. baseline' contrast. Relative to their low-scoring co-twins, twins who scored high on OCS exhibited clusters of decreased brain activation in the right and left premotor gyrus (clusters labeled A, B and C

in **table 3.4** and **figure 3.4**), left dorsolateral prefrontal cortex (cluster labeled E) and left inferior parietal gyrus (cluster D). Increased brain activation for the OCS high twins was observed in the left precentral gyrus (cluster F), right postcentral gyrus (cluster G), right supramarginal gyrus (cluster H) and left inferior temporal gyrus (cluster I).



Low	3.86 (0.77)	4.59 (0.67)	6.01 (1.16)	7.55 (1.08)	10.24 (3.09)	13.07 (4.48)
High	3.65 (1.07)	4.34 (0.82)	5.48 (0.99)	7.27 (1.61)	9.40 (1.96)	10.87 (2.78)



Low	0.94 (0.02)	0.95 (0.04)	0.91 (0.11)	0.92 (0.08)	0.73 (0.08)	0.72 (0.16)
High	0.94 (0.03)	0.96 (0.03)	0.93 (0.07)	0.94 (0.09)	0.77 (0.12)	0.65 (0.22)

Figure 3.2. *ToL task performance. Top panel: mean latencies (ms) of correct responses as a function of task load levels 1, 2, 3, 4 and 5 (task load 0 = baseline condition); bottom panel: response accuracy (between 0 and 1) as a function of task load. Data for OCS high and low twins are indicated by filled and open circles, respectively.*

Table 3.2. Brain regions showing significant BOLD signal increase for the 'planning vs. baseline' contrast

	Anatomical location	BA	OCS low (n = 12)						OCS high (n = 12)					
			MNI coordinates			Z score	p-value	# voxels	MNI coordinates			Z score	p-value	# voxels
			x	y	z				x	y	z			
PC	Left parietal cortex	7/40	0	-66	54	5.98	<0.001	2363	-6	-60	57	5.78	<0.001	2416
	Right parietal cortex	7/40	33	-78	42	4.73			9	-63	63	5.51		
PM	Left premotor cortex	6	-30	3	57	5.53	<0.001	471	-24	3	63	5.00	<0.001	470
	Right premotor cortex	6	33	15	60	5.63	<0.001	855	33	15	60	5.78	<0.001	612
PFC	Left anterior prefrontal cortex	10	-30	60	6	4.61	<0.001	158	-39	54	-6	3.89	<0.001	93
	Left DLPFC	9	-36	21	30	4.89	<0.001	106	-39	33	33	4.43	<0.001	107
	Right anterior prefrontal cortex	10	39	60	6	3.81	0.676	20	36	57	15	3.48	<0.001	612
	Right DLPFC	9	45	30	36	4.71	<0.001	855	21	33	30	4.80	0.946	13
CBL	Left cerebellum	--	-30	-60	-36	4.13	0.009	62	-30	-66	-36	3.97	0.143	34
	Right cerebellum	--	39	-51	-39	4.05	0.020	54	39	-51	-39	4.46	0.834	16
BG	Left caudate nucleus	--	-9	15	0	4.33	<0.001	109	-9	15	-3	3.99	<0.001	128
	Left globus pallidus	--	-15	0	3	3.29			--	--	--	--	--	--
	Right caudate nucleus	--	12	9	3	4.35	<0.001	151	12	21	6	3.56	0.002	75

Brain regions showing significant BOLD signal increase after applying the 'planning vs. baseline' contrast for OCS low (left) and OCS high (right) twins. Anatomical location: location of cluster (PC = parietal cortex; TC = temporal cortex; PM = premotor cortex; PFC = prefrontal cortex; CBL = cerebellum; BG = basal ganglia); BA: Brodmann area; MNI coordinates (mm): location of voxel with largest effect size; Z score: z-value of voxel with largest effect size; p-value: cluster p-value; # voxels: number of voxels in cluster. For cases where regional differences correspond to local maxima within a large interconnected cluster, cluster p-value and number of voxels are displayed in single, merged, cells.

Table 3.3. Brain regions showing significant BOLD signal increase for the 'task load' contrast

	Anatomical location	BA	OCS low (n = 12)						OCS high (n = 12)					
			MNI coordinates			Z score	p -value	# voxels	MNI coordinates			Z score	p -value	# voxels
			x	y	z				x	y	z			
PC	Left parietal cortex	7/40	-45	-57	51	4.83	<0.001	795	-27	-81	45	3.82	<0.001	123
	Right parietal cortex	7/40	54	-51	48	3.89	<0.001	299	24	-54	69	3.93	<0.001	384
PM	Left premotor cortex	6	-27	3	66	5.41	<0.001	2255	-27	3	69	5.28	<0.001	693
	Right premotor cortex	6	21	3	66	4.72			27	0	54	4.41	<0.001	471
PFC	Left anterior prefrontal cortex	10	-36	57	15	4.78			-36	54	15	4.26	<0.001	242
	Left DLPFC	9	-45	27	36	4.63			-30	36	39	4.81		
	Right anterior prefrontal cortex	10	30	63	9	4.42			36	57	15	4.10	<0.001	471
	Right DLPFC	9	45	33	33	4.08			24	33	36	4.24		
CBL	Left cerebellum	--	-33	-57	-39	4.10	0.001	97	-42	-57	-36	3.63	0.132	27
	Right cerebellum	--	27	-66	-30	4.15	<0.001	160	33	-66	-27	3.56	0.014	45
BG	Left putamen	--	-18	9	12	3.52	0.179	36	--	--	--	--	--	--

Brain regions showing significant BOLD signal increase after applying the 'task load' contrast for OCS low (left) and OCS high (right) twins.

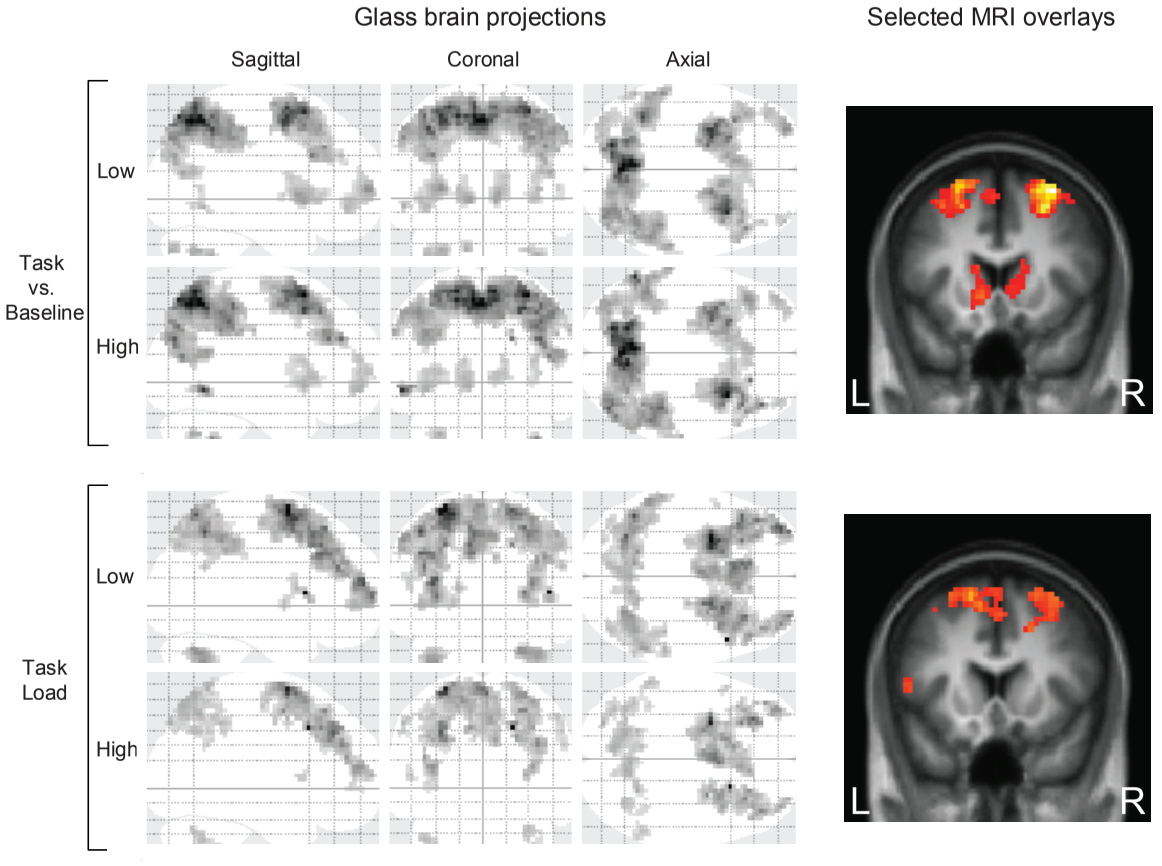


Figure 3.3. *Brain regions showing increased BOLD signal during ToL cognitive planning. Glass brain overviews on the left depict brain activity patterns for 'task vs. baseline' (top) and 'task load' (bottom) contrasts in OCS low (Low) and high (High) scoring twins. Selected anatomical overlays on the right illustrate the difference in functional activation of basal ganglia structures between 'task versus baseline' (top) and 'task load' (bottom) contrasts. Functional activations for OCS high twins are shown, overlaid on an averaged structural MRI across all twins.*

Table 3.4. Clusters with differences in brain activity between OCS high and low twins: 'planning vs. baseline' contrast

Test	Cluster label	Anatomical location	BA	MNI coordinates			Z score	*p* -value	# voxels
				x	y	z			
high < low	A	Right frontal gyrus	6	15	-3	63	4.17	<0.001	29
	B		8	15	24	48	3.07	0.001	9
	C	Left frontal gyrus	6	-30	0	51	3.01	0.001	15
	E		9	-36	21	30	3.14	0.001	4
	D	Left inferior parietal gyrus	40	-39	-51	48	3.32	<0.001	17
high > low	F	Left precentral gyrus	6	-21	-15	69	3.10	0.001	6
	G	Right postcentral gyrus	1	36	-39	69	3.32	<0.001	6
	H	Right supramarginal gyrus	40	51	-54	21	3.35	<0.001	22
	I	Left inferior temporal gyrus	37	-48	-57	-3	3.64	<0.001	16

Clusters with regional brain activity differences between OCS high and low-scoring twins for the 'planning vs. baseline' contrast. Test: test for significant increases / decreases in OCS high relative to OCS low twins; Cluster label: alphabetical cluster label as displayed in anatomical overlays of figure 3.4.

Table 3.5. Clusters with differences in brain activity between OCS high and low twins: 'task load' contrast

Test	Cluster label	Anatomical location	BA	MNI coordinates			Z score	*p* -value	# voxels
				x	y	z			
high < low	A	Left frontal gyrus	46	-48	39	18	3.95	<0.001	14
	B	Right pulvinar	--	18	-36	9	3.07	0.001	6
high > low	C	Right medial frontal gyrus	8	21	30	36	3.24	0.001	11

Clusters with regional brain activity differences between OCS high and low twins for the 'task load' contrast. Cluster label: alphabetical cluster label as displayed in figure 3.5.

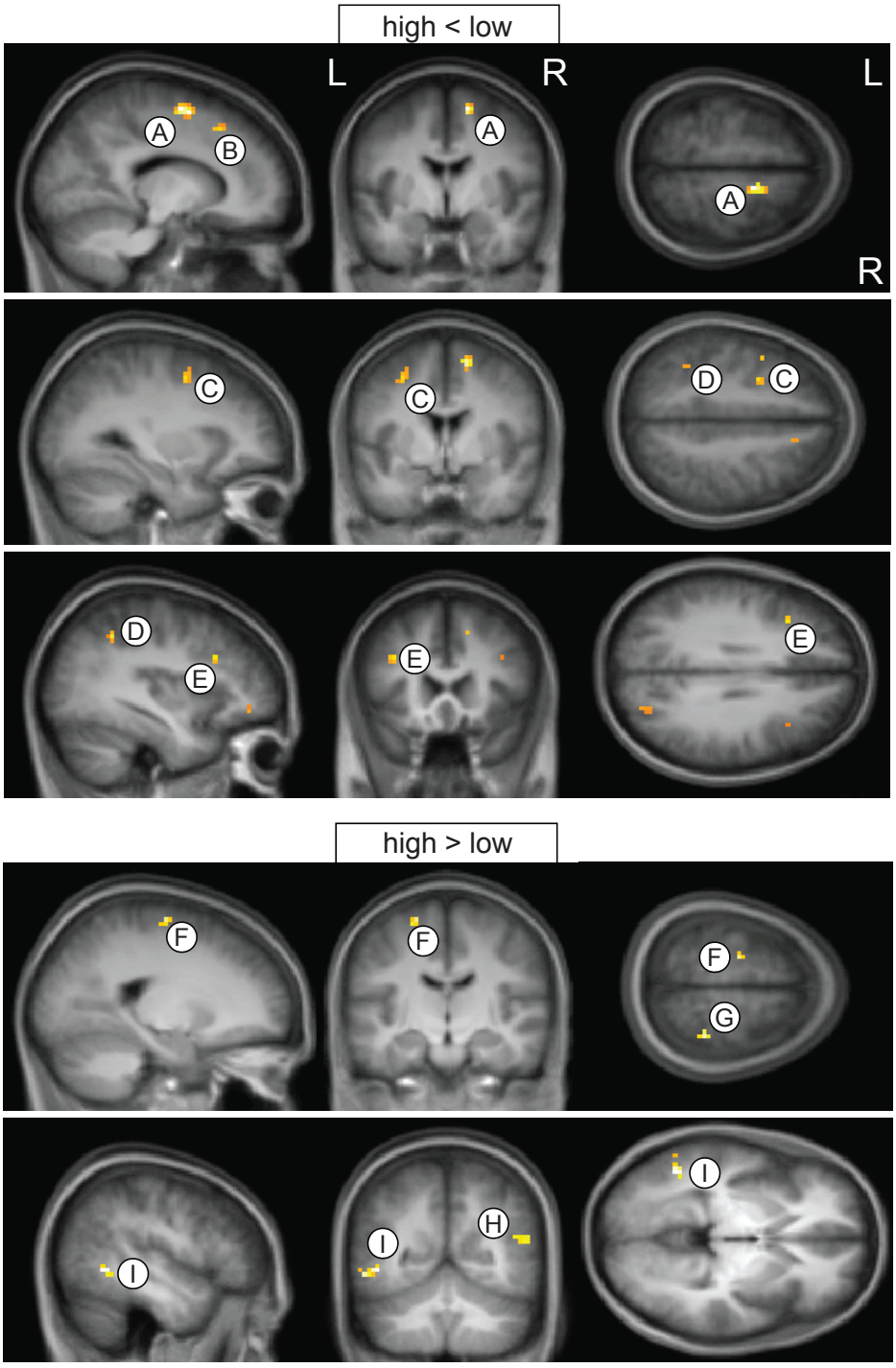


Figure 3.4. *Brain regions showing reduced (top panels) and increased (bottom panels) BOLD signal in OCS high versus low twins for the 'planning vs. baseline' contrast. Clusters of significant difference are overlaid on an averaged structural MRI across all twins.*

'Task load'

For the 'task load' contrast (**table 3.5** and **figure 3.5**), clusters of decreased brain activation in OCS high compared to OCS low-scoring twins were noted in the left dorsolateral prefrontal cortex (cluster labeled A in **table 3.5** and **figure 3.5**) and right pulvinar (cluster B). We found only a single cluster of relatively increased brain activation for the OCS high-scoring twins in a region of the right medial frontal gyrus (cluster C).

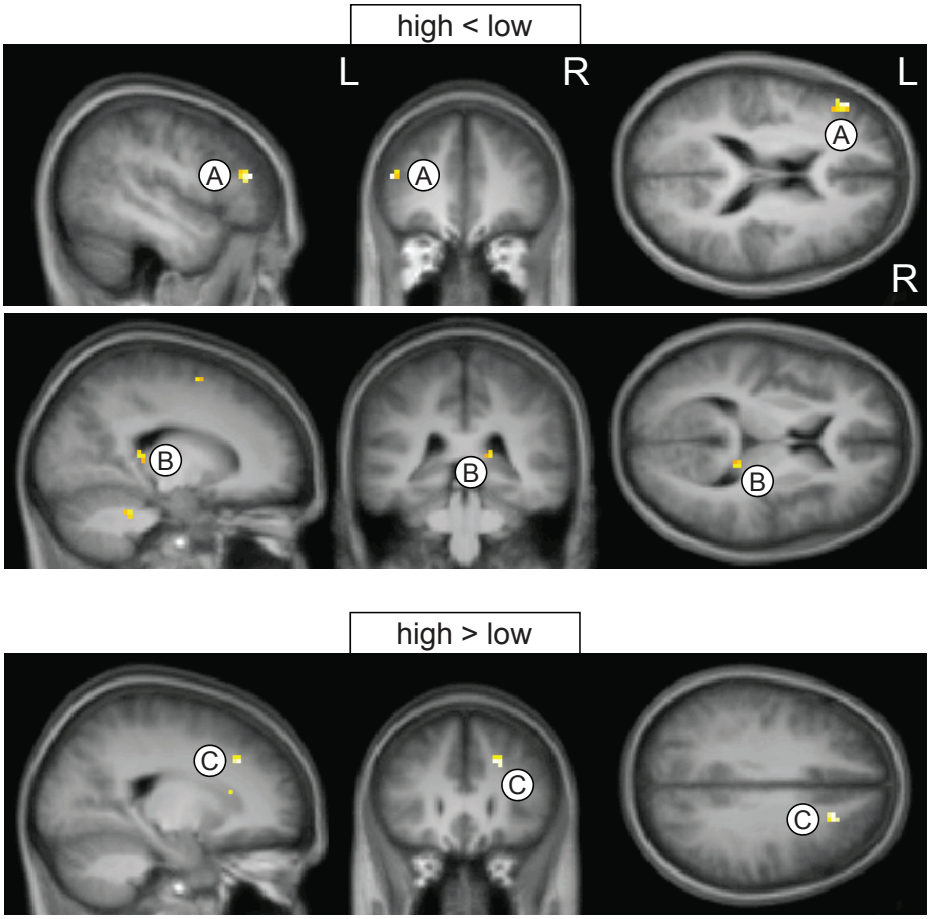


Figure 3.5. *Clusters with significantly reduced (top) and increased (bottom) BOLD signal in OCS high versus low twins, for the 'task load' contrast.*

Discussion

We examined behavioral performance and concurrent brain activation, measured with fMRI, during execution of the Tower of London cognitive planning task in genetically identical twins discordant for obsessive-compulsive symptoms. Differences in task performance and fMRI activation between twins scoring high and low on OCS were expected to be indicative of neurobiological changes related to the environmentally mediated risk for OCD.

Although impaired ToL planning at the behavioral level, as reported earlier in OCD patients (van den Heuvel et al., 2005a), was evident in our sample only by a tendency towards decreased reaction accuracy for the highest planning difficulty level (5 planning steps), comparison of fMRI data indicated several areas with decreased brain activation during ToL performance in OCS high-scoring twins.

In agreement with van den Heuvel et al. (2005a) we observed reduced brain activity in regions of the dlPFC for both 'task vs. baseline' and 'task load' contrasts. The dlPFC is importantly involved in executive functions including cognitive planning, inhibitory control and decision making (Faw, 2003; Newman et al., 2003; Remijnse et al., 2006; Rosenberg and Keshavan, 1998). Furthermore, decreased dlPFC activity is compatible with the neuroanatomical model of OCD that proposes a disturbance of cortico-striato-thalamo-cortical circuitry (Mataix-Cols and van den Heuvel, 2006; Singer and Minzer, 2003; Rosenberg and Keshavan, 1998).

For the contrast 'task load', an additional area of reduced activation was found in the pulvinar of the right thalamus. Although decreased responsiveness of thalamic regions was absent in the study by van den Heuvel et al. (2005a), OCD related changes for the pulvinar (Viard et al., 2005) as well as other regions of the thalamus have been found in several other neuroimaging studies. Structural MRI studies have reported OCD related volumetric increases of thalamic regions (Kim et al., 2001; Gilbert et al., 2000; Atmaca et al., 2007). Functional MRI studies have indicated changes for thalamic regions as well, although in contrast to our findings, these generally point to increased rather than decreased metabolism associated with the disorder (Mataix-Cols et al., 2004; Chen et al., 2004; Schienle et al., 2005). Results of PET/SPECT studies are inconclusive. Some perfusion studies showed increased thalamic regional cerebral blood flow (rCBF) (Alptekin et al., 2001; Lacerda et al., 2003; Saxena et al., 2001; Saxena et al., 2004), whereas others report thalamic rCBF decreases (Lucey et al., 1995; Busatto et al., 2001). One PET ligand study demonstrated reduced thalamic serotonin transporter (SERT) availability in OCD patients compared to healthy controls (Hesse et al., 2005). The pulvinar of the posterior thalamus is presumably involved in the integration of sensory information, visuo-spatial processing and visual

selective attention (Buchsbaum et al., 2006; Kastner and Pinsk, 2004; Michael and Buron, 2005; Laberge and Buchsbaum, 1990). Together with the dlPFC, the thalamus is implicated in the CSTC circuit. It is the key region in modulating subcortical input to frontal cortex, stimulates output of frontal brain regions, and plays a crucial role in the processing of sensory inputs thereby mediating both behaviors, emotion and cognition (Sherman and Guillery, 2002). Disturbances within this structure could therefore easily be coupled to the cognitive and behavioral deficits seen in OCD patients.

Finally, reduced brain activity related to OCS included premotor and inferior parietal regions. Similar to van den Heuvel et al. (2005a), activation changes in these brain regions were present exclusively in the 'task vs. baseline' contrast. Given that the 'task vs. baseline' contrast (as compared to 'task load') tests for all brain areas needed for correct planning, as well as the fact that premotor and parietal areas are involved in basic functions of motor response preparation (Mars et al., 2007; Hoshi and Tanji, 2000) and visuo-spatial processing (Cabeza and Nyberg, 2000), it is likely that these brain regions mainly support proper task execution rather than higher-order planning itself (Lazeron et al., 2000). For example, involvement of premotor regions might reflect differences in internal imagery of movement of the beads during planning (Rowe et al., 2001). Involvement of the parietal lobes during cognitive planning has been found previously (Lazeron et al., 2000; van den Heuvel et al., 2003), and parietal cortex abnormalities associated with OCD also have been reported. Anatomical studies indicated OCD-related parietal gray matter (Valente Jr. et al., 2005; Menzies et al., 2007) and white matter (Kitamura et al., 2006; Szeszko et al., 2005) reductions. Furthermore PET, SPECT as well as MEG studies reported decreased parietal activation in OCD patients compared to unaffected controls (Kwon et al., 2003; Lucey et al., 1995; Ciesielski et al., 2005). The dlPFC receives somatosensory and visuo-spatial information from the parietal lobes (Faw, 2003) and activation of the inferior parietal lobes has shown to be correlated with prefrontal activity (Baker et al., 1996; Dagher et al., 1999). Therefore reduced parietal cortex function may result in functional changes of the dlPFC which in turn could eventuate in OC symptoms. Parietal dysfunction may also relate to general problems in visuo-spatial ability and nonverbal memory which have been proposed as impaired cognitive domains in OCD patients (Lazeron et al., 2000; Savage et al., 1999b; Savage et al., 1999a).

Our results also indicated clusters of increased functional brain activation related to OCS. Regional fMRI signal increments in OCS high compared to low scoring twins were found in the right postcentral gyrus, left precentral gyrus, right supramarginal gyrus and left inferior temporal gyrus, and in the right medial frontal gyrus for the 'task load' contrast. Post/precentral, supramarginal and

medial frontal gyrus regions primarily relate to brain areas involved in sensory (Iwamura, 1998), and motor and premotor (Chouinard and Paus, 2006) processing, while the inferior temporal lobe has been implicated in the ventral visual stream associated with object and word recognition (Goodale and Milner, 1992; Nobre et al., 1994). It is therefore likely that these brain regions mainly relate to basic processing that supports proper planning execution, rather than higher-order cognitive planning. For the temporal lobes, functional activation changes in OCD patients have been reported earlier (van den Heuvel et al., 2005a; Mataix-Cols et al., 2004; Adler et al., 2000), although generally not in inferior temporal parts. Increased responsiveness of brain areas may be indicative of increased arousal or mechanisms that act to compensate for functional deficits elsewhere in the brain.

When contrasting the present findings with the ToL planning in OCD patients as reported by van den Heuvel et al. (2005a), an interesting difference is observed with respect to responsiveness of the caudate nuclei. Van den Heuvel et al. (2005a) found decreased activation of the caudate nuclei in OCD patients compared to controls, whereas a comparable difference was absent in our intrapair twin comparison. Functional changes of the caudate are in line with the general theory of a dysfunction of prefrontal-basal ganglia circuitry in OCD (Pauls et al., 1986; van den Heuvel et al., 2005a). The dissimilarity between our results and those of van den Heuvel et al. (2005a) may indicate a difference between neurobiological changes underlying OCS due to combined genetic and environmental influences and due to pure environmental influences. OCD patients represent a group in which OCD is caused by genetic, environmental, and combined influences. In discordant MZ twin pairs, neurobiological changes can only be due to environmental stressors. However, we cannot rule out alternative explanations, such as the limited sample size possibly obscuring between-group differences, and the possibility that basal ganglia abnormalities are more severe in clinically diagnosed OCD patients. In this respect, we should also note that post hoc analyses revealed a cluster of relatively reduced activation for the OCS high twins in the right caudate for the 'planning vs. baseline' contrast, similar to van den Heuvel al. (2005a), but only after lowering the statistical threshold to $p = 0.01$, uncorrected.

Finally, due to the limited sample size of this study, we were unable to analyse our data at a level of symptom dimensions. Previous studies have indicated washing behavior to be related to activation of caudate and ventral striatal regions, and checking to activation of dorsal regions (Mataix-Cols et al., 2004). Our whole group analyses did not reveal any of these patterns. Future studies, using a larger sample size, should address this issue.

In the present sample for MRI we did not find significant intrapair differences on life-events or data on health (including birth order and birth weight) between the high and low-scoring co-twins. However, our sample was drawn from a larger population of OCS high-low discordant twin pairs previously selected for behavioral characterization (Cath et al., 2008). Statistical analysis on survey data (self-reports including: life-events, life style factors and data on health, taken at 6 time points between 1991 and 2002) in that study indicated as risk factors: low educational level, sexual assault at a young age and low birth weight (low birth weight was significant only as a shared environment factor in the comparison with twin pairs concordant-high and low for OCS).

Taken together, our findings suggest that neurobiological changes underlying the environmentally mediated risk for OCS partly overlap with the neurobiological abnormalities reported in OCD patients where the disorder likely originates from a combination of adverse genetic and environmental influences. A possible difference between genetically and environmentally mediated backgrounds may relate to functional changes of the striatum, which appear to be less pronounced in environmentally mediated OCS. In future work, we will directly explore differences between the genetic and environmental neurobiology of OC behavior by comparing results from our intrapair OCS discordant twin comparisons with changes in fMRI brain scans during cognitive planning between MZ twin pairs concordant-high and MZ twin pairs concordant-low for OCS; a contrast particularly suited for identifying basic neural mechanisms behind OCS primarily due to genetic risks.

4 Brain activation during cognitive planning in twins discordant or concordant for obsessive-compulsive symptoms

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Abstract

Neuroimaging studies have indicated abnormalities in cortico-striato-thalamo-cortical circuits in obsessive-compulsive disorder patients compared with controls. However, there are inconsistencies between studies regarding the exact set of brain structures involved and the direction of anatomical and functional changes. These inconsistencies may reflect the differential impact of environmental and genetic risk factors for obsessive-compulsive disorder on different parts of the brain. To distinguish between functional brain changes underlying environmentally and genetically mediated obsessive-compulsive disorder, we compared task performance and brain activation during a Tower of London planning paradigm in monozygotic twins discordant (n = 38) or concordant (n = 100) for obsessive- compulsive symptoms. Twins who score high on obsessive-compulsive symptoms can be considered at high risk for obsessive-compulsive disorder. We found that subjects at high risk for obsessive-compulsive disorder did not differ from the low-risk subjects behaviorally, but we obtained evidence that the high-risk subjects differed from the low-risk subjects in the patterns of brain activation accompanying task execution. These regions can be separated into those that were mainly affected by environmental risk (dorsolateral prefrontal cortex and lingual cortex), genetic risk (frontopolar cortex, inferior frontal cortex, globus pallidus and caudate nucleus) and regions affected by both environmental and genetic risk factors (cingulate cortex, premotor cortex, and parts of the parietal cortex). Our results suggest that neurobiological changes related to obsessive-compulsive symptoms induced by environmental factors, involve primarily the dorsolateral prefrontal cortex, whereas neurobiological changes induced by genetic factors involve orbitofrontal-basal ganglia structures. Regions showing similar changes in high-risk twins from discordant and concordant pairs may be part of compensatory networks that keep planning performance intact, in spite of cortico-striato-thalamo-cortical deficits.

Introduction

Obsessive-compulsive symptoms are characterized by recurrent, persistent, and intrusive anxiety provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions) performed to reduce anxiety and/or distress caused by the obsessions (American Psychiatric Association, 1994). Common obsessions include fear of contamination, fixation on symmetry and orderliness and somatic and aggressive obsessions. Well-known compulsions are excessive hand washing, counting and detailed and rigid rituals or habits, such as excessive checking or specific morning or eating routines. When a person performs these obsessions and/or compulsions for more than one hour a day and these thoughts

and rituals significantly interfere with daily life routines, the person fulfils the criteria for obsessive-compulsive disorder. Obsessive-compulsive disorder is generally assessed by clinical interviews, e.g., Diagnostic and Statistical Manual of Mental Disorders [DSM-IV, fourth edn. (American Psychiatric Association, 1994)]. Questionnaires, such as the Padua Inventory (Sanavio, 1988) and quantitative versions of the Yale-Brown Obsessive-Compulsive Scale (Goodman et al., 1989a; Goodman et al., 1989b) can be utilized to explore obsessive-compulsive symptomatology on a more quantitative scale. While the estimates of the prevalence of life-time obsessive-compulsive disorder are found to be as high as 0.5-2% (American Psychiatric Association, 1994; Grabe et al., 2000), the prevalence of obsessive-compulsive symptoms in the general population is much higher, with estimates up to 72% as reported by Rachman and de Silva (1978).

Neuropsychological studies have shown that patients with obsessive-compulsive disorder suffer from deficits in executive functions, including cognitive planning, response inhibition, set-switching, working memory and sustained attention [for review see: (Chamberlain et al., 2005; Menzies et al., 2008a; Schultz et al., 1999)]. Recent neuroimaging studies have indicated several neurobiological changes associated with obsessive-compulsive disorder. Structural magnetic resonance imaging (MRI) has revealed brain volume changes in orbitofrontal cortex, dorsolateral prefrontal cortex, basal ganglia, anterior cingulate cortex, parietal cortex and thalamus (Menzies et al., 2007; Pujol et al., 2004; Radua and Mataix-Cols, 2009; Rotge et al., 2009; Valente Jr. et al., 2005; van den Heuvel et al., 2009), in line with the hypothesis of a disturbed cortico-striato-thalamo-cortical (CSTC) network. Functional neuroimaging studies also showed altered activation in abovementioned brain structures during performance of cognitive tasks and after symptom provocation (Breiter et al., 1996; Chamberlain and Menzies, 2009; Maltby et al., 2005; Menzies et al., 2008a; Rauch et al., 2007; Ursu et al., 2003). Although the overall picture points to a deficit in CSTC processing, there are considerable inconsistencies across studies regarding the brain areas involved and the direction of anatomical and functional changes. A possible explanation for this relates to the presence of methodological differences between studies such as heterogeneity of patient groups and differences in sample size, scanning modalities/parameters and analysis methods. However, there may also be 'true' variability in the underlying neurobiology of obsessive-compulsive disorder. That is, it may be that dysfunction of different brain regions leads to highly comparable changes at the behavioral level, because these regions are part of the same brain network involved in the regulation of anxiety and safety behaviors. Such heterogeneity in affected brain regions may, for instance, reflect the differential influence of environmental and genetic risk factors for obsessive-compulsive disorder that may impact on different parts of the brain.

Family studies (Hettema et al., 2001; Nestadt et al., 2000) and twin studies (Jonnal et al., 2000; van Grootheest et al., 2005) have indicated the importance of genetic as well as environmental risk factors with regard to the etiology of obsessive-compulsive disorder. Heritabilities for obsessive-compulsive disorder have been estimated between 27 – 47% in adults and 45 – 65% in children (Jonnal et al., 2000; van Grootheest et al., 2005) and linkage and association studies have mainly pointed towards functional deficits of genes involved in serotonergic, glutamatergic and dopaminergic neural signaling (Bengel et al., 1999; Billett et al., 1998; Enoch et al., 2001; Nicolini et al., 2009). Given these moderate heritabilities, as much as 35 – 73 % of the risk for obsessive-compulsive disorder should be accounted for by environmental stressors and/or adverse gene-environment interactions. Potential environmental risk factors for obsessive-compulsive disorder include traumatic life experiences, perinatal problems, streptococcal infection, psychosocial stress, aspects of parenting (e.g., parental overprotection), pregnancy, divorce and emotional neglect (Albert et al., 2000; Alonso et al., 2004; Cath et al., 2008; Geller et al., 2008; Lin et al., 2007; Wilcox et al., 2008).

Most brain imaging studies apply a group comparison of affected individuals with healthy controls. These standard case-control designs cannot disentangle differences in brain function that are due to environmental risk factors from those that are due to genetic risk factors. A design that makes a distinction between genetically and environmentally mediated neurobiological changes that underlie the development of behavioral traits such as obsessive-compulsive disorder, is the so-called discordant/concordant monozygotic twin design (de Geus et al., 2007; van 't Ent et al., 2009; Wolfensberger et al., 2008). As nearly all monozygotic twins begin life with identical genomes, discordance at the behavioral level is likely to arise from differential exposure to environmental influences. Consequently, differences in brain function between the high-risk twin and the low-risk co-twin from discordant pairs reflect environmental effects on the brain, rather than effects of genetic variation, although these environmental stressors may ultimately act through modification of gene expression (Heijmans et al., 2009).

In contrast, to maximize detection of the effects of genetic risk factors, neuroimaging results can be compared between monozygotic twins who both score high on obsessive-compulsive symptoms and monozygotic twins who both score very low on obsessive-compulsive symptoms. These monozygotic concordant-high and low-scoring twins are likely to come from families with either high or low vulnerability for obsessive-compulsive disorder. This familial vulnerability may consist of shared environmental or genetic vulnerability. However, since no influence of shared family environment on obsessive-compulsive behavior was found in any of the studies in adult twins (Clifford et al., 1984; Jonnal et al., 2000; van Grootheest et al., 2007), familial vulnerability for this

trait translates entirely to genetic vulnerability. Therefore, a comparison between monozygotic twins scoring both high (concordant-high) on obsessive-compulsive symptoms and monozygotic twins scoring both low (concordant-low) on obsessive-compulsive symptoms will reveal functional activation differences due to influences of genetic risk factors. Furthermore, comparing the regions affected in the high-risk discordant twins with those in high-risk concordant twins, allows for the identification of regions commonly affected in all high-risk subjects. These regions may be most closely correlated with the observed behavioral deficits of the disorder.

In the present study, the discordant/concordant monozygotic twin design was used to assess differences in functional brain activation during cognitive planning with the Tower of London paradigm (Shallice, 1982). The Tower of London paradigm has previously been found to activate the dorsolateral prefrontal cortex, anterior cingulate cortex, caudate nucleus, (pre)cuneus, supramarginal and angular gyrus of the parietal lobe and frontal opercular areas of the insula (Dagher et al., 1999; Lazeron et al., 2000; Newman et al., 2003; van den Heuvel et al., 2003). Several neuropsychological studies have used a computerized version of the Tower of London to assess problem solving and planning ability in patients with obsessive-compulsive disorder (Kuelz et al., 2004; Menzies et al., 2008a). Some studies revealed that deviant performance on the Tower of London was evident not so much as a deficit in planning accuracy, but rather that patients were slower to recover from an incorrect move (Veale et al., 1996) or had longer movement times (Purcell et al., 1998b; Purcell et al., 1998a) compared with healthy controls. Chamberlain and colleagues further revealed that patients with obsessive-compulsive disorder required more attempts to obtain a correct response on the Tower of London, but only for the highest difficulty levels (4-6 moves) (Chamberlain et al., 2007). Importantly, Delorme and colleagues (Delorme et al., 2007) found that unaffected relatives of patients with obsessive-compulsive disorder had significantly lower scores and increased response times on the Tower of London task compared with controls, which suggests genetic contribution to the behavioral planning deficits. A neuroimaging study further demonstrated that behavioral impairment on the Tower of London task in patients with obsessive-compulsive disorder was associated with decreased functional MRI activation in the dorsolateral prefrontal cortex and caudate nucleus as well as increased activation in the anterior cingulate cortex (van den Heuvel et al., 2005a). This differential brain activation does not only reflect a genetic etiology, since we replicated the reduced dorsolateral prefrontal cortex activation in 12 monozygotic twin pairs discordant for obsessive-compulsive symptoms (den Braber et al., 2008). No obsessive-compulsive symptom-related changes were found for the caudate nucleus or the anterior cingulate cortex which may be more specific to obsessive-compulsive symptoms caused by genetic factors.

Here we aimed to extend our previous findings, and to specifically examine whether different brain regions are affected in subjects at high risk for obsessive-compulsive disorder due to adverse environmental influences or to genetic influences. For this we compared performance and functional MRI data during the Tower of London task between twins scoring low and high on obsessive-compulsive symptoms from discordant monozygotic pairs and between concordant pairs where both twins scored low or both scored high on obsessive-compulsive symptoms. Furthermore, we explicitly tested for the presence of overlap in the regions that were affected by both environmental and genetic risk for obsessive-compulsive disorder.

Materials and methods

Subjects

The twin pairs in this study were recruited from the Netherlands Twin Register (Boomsma et al., 2006). In 2002, surveys were sent to twin families including the Padua Inventory Abbreviated. The Padua Inventory Abbreviated is derived from the Padua Inventory-Revised version, a widely used self-report inventory on obsessive-compulsive symptoms (Sanavio, 1988; van Oppen, 1992). The Padua Inventory-Revised measures obsessive-compulsive symptoms on a scale from 0 to 4, and contains five subcategories: washing, checking, rumination, precision and impulses (van Oppen et al., 1995). The Padua Inventory-Revised correlates moderately with the Yale-Brown Obsessive-Compulsive Scale symptom checklist, a clinician-derived inventory on obsessive-compulsive symptoms (Denys et al., 2004). Reduction of the Padua Inventory-Revised to 12 items was implemented by selecting two items of each of the five Padua Inventory-Revised subscales with highest factor loadings in a previous validation study (van Oppen et al., 1995), and adding another two items for each of the more equivocal obsession subscales: rumination and impulses.

Completed Padua Inventory Abbreviated questionnaires were returned by 815 monozygotic twin pairs (222 male; 593 female). From this sample we selected twin pairs in the age range between 18 and 60 years who scored discordant, concordant-high, or concordant-low for obsessive-compulsive symptoms. A twin pair was classified as discordant for obsessive-compulsive symptoms if one twin scored high (>16) and the co-twin scored low (≤7). A twin pair was classified as concordant-high for obsessive-compulsive symptoms if both twins scored ≥15, with at least one twin scoring ≥16. A twin pair was classified as concordant-low for obsessive-compulsive symptoms if both twins scored ≤7. These Padua Inventory Abbreviated cut-off scores were derived from sensitivity and specificity measurements in a sample of patients with obsessive-compulsive disorder when

compared with clinical controls [(n=120; mean scores 20.7, SD 8.1; sensitivity 0.74 and specificity 0.72 at the best cut-off point of 16) (Cath et al., 2008)]. This initial selection yielded 32 discordant monozygotic twin pairs, 40 concordant-high monozygotic twin pairs and 269 concordant-low monozygotic twin pairs for obsessive-compulsive symptoms. From the large sample of concordant-low twin pairs a selection was made to optimally match the concordant-high twin pairs by sex and age which resulted in a final concordant-low sample of 41 twin pairs. Two concordant-high twin pairs were omitted from the selection: in one pair, both twins were treated for severe anorexia, and had indicated that they were not willing to participate in research projects; in the other pair, the twins indicated that they were not willing to participate in research projects other than the filling out of questionnaires. The remaining 111 twin pairs were invited by letter. Exclusion criteria were neurological damage, colorblindness and contraindications for MRI (e.g., pregnancy, metal artifacts in the body, claustrophobia). From this group, 69 monozygotic twin pairs finally participated in our MRI study, including 19 discordant (7 pairs newly enrolled), 22 concordant-high and 28 concordant-low twin pairs (**table 4.1**). Of this final population, two twins with high obsessive-compulsive symptom scores from the discordant group and five twins with high obsessive-compulsive symptom scores from the concordant-high group met clinical diagnosis for obsessive-compulsive disorder. Furthermore, three twins with high obsessive-compulsive symptom scores and one twin with a low obsessive-compulsive symptom score from the discordant group and six twins from the concordant-high group used selective serotonin reuptake inhibitors.

The MRI protocol could not be completed by one of the twins from a concordant-low pair due to a metal artifact at the eyebrow level and by one of the twins from a concordant-high pair due to a panic attack.

Protocol

A self-report questionnaire, consisting of demographic questions, life events, comparative twin rating (Reynolds et al., 2005), the 13-item Beck Depression Inventory Short Form (Beck et al., 1961; Beck et al., 1974) and the 12-item Padua Inventory Abbreviated, was sent to the subjects at home to be filled in before the day of MRI scanning. On the day of MRI, the following diagnostic interviews and questionnaires were administered: (i) an adapted form of the Yale-Brown Obsessive-Compulsive Scale, to measure both life-time and current obsessive-compulsive symptoms and severity; (ii) the State Trait Anxiety Inventory; (iii) the State Trait Anger Scale (Spielberger et al., 1970; Spielberger et al., 1983); and (iv) the Mini International Neuropsychiatric Interview (Sheehan et al., 1998) to test for possible comorbidities. Comorbidities tested by the Mini International Neuro-psychiatric Interview include depression, panic disorder, agoraphobia, social phobia, post-traumatic stress disorder and generalized anxiety disorder.

Table 4.1. Twin sample demographics

	Twin pairs			
	Discordant		Concordant	
	high (13 female, 6 male)	low	high (17 female, 5 male)	low (20 female, 8 male)
Age at MRI scan (SD)	35.58 (8.92)		36.23 (10.87)	37.50 (8.87)
Obsessive-compulsive symptoms				
PI-R-ABBR 2002	19.55 (3.99)	4.53 (2.17)[a]	20.62 (4.56)	4.18 (2.19)[b]
PI-R-ABBR current	12.63 (7.34)	6.84 (4.15)[a]	15.27 (5.58)	4.43 (3.00)[b]
Y-BOCS severity lifetime	7.74 (5.85)	7.00 (8.29)	10.66 (7.21)	3.18 (4.54)[b]
Y-BOCS severity current	5.42 (5.78)	1.47 (2.25)[a]	7.64 (5.95)	0.95 (2.13)[b]
Y-BOCS symptom lifetime	22.11 (25.32)	7.11 (7.17)[a]	30.09 (27.34)	4.82 (6.15)[b]
Y-BOCS symptom current	24.32 (30.37)	7.26 (9.61)[a]	22.82 (20.64)	3.25 (5.11)[b]
Agressive \ checking lifetime	5.84 (7.34)	2.11 (2.73)[a]	9.43 (9.36)	1.79 (2.16)[b]
Agressive \ checking current	5.74 (7.82)	2.00 (3.59)[a]	6.89 (7.61)	1.05 (1.54)[b]
Hoarding \ saving lifetime	1.16 (1.38)	0.26 (0.56)[a]	1.48 (1.84)	0.36 (0.70)[b]
Hoarding \ saving current	1.21 (1.44)	0.37 (0.68)[a]	1.23 (1.64)	0.39 (0.78)[b]
Symmetry \ ordering lifetime	1.68 (3.48)	0.84 (1.64)	2.64 (3.44)	0.43 (1.29)[b]
Symmetry \ ordering current	1.58 (3.63)	0.68 (1.49)	2.02 (3.14)	0.23 (0.63)[b]
Washing \ cleaning lifetime	5.11 (8.09)	0.95 (2.39)[a]	4.84 (6.39)	0.77 (1.90)[b]
Washing \ cleaning current	5.21 (6.70)	1.32 (2.83)[a]	3.43 (4.74)	0.63 (1.74)[b]
Comorbidity				
Comorbidity lifetime (MINI)	1.58 (1.39)	0.74 (1.10)[a]	1.45 (1.42)	0.41 (0.78)[b]
Comorbidity current (MINI)	0.63 (1.71)	0.00 (0.00)[a]	0.27 (0.50)	0.02 (0.13)
Tic	0.37 (0.76)	0.16 (0.37)	0.27 (0.66)	0.05 (0.23)[b]
BDI	4.58 (6.61)	2.95 (2.84)	3.57 (3.22)	1.38 (2.18)[b]
STAI	13.95 (8.77)	12.53 (6.17)	13.64 (7.36)	8.55 (7.36)[b]
STAS	0.21 (0.71)	0.00 (0.00)	0.48 (2.14)	0.11 (0.49)

a Significant difference between discordant-high and discordant-low-scoring twins.

b Significant difference between concordant-high and concordant-low-scoring twins.

PI-R-ABBR 2002 = mean Padua Inventory Abbreviated scores (SD) assessed in 2002; PI-R-ABBR current = mean Padua Inventory abbreviated scores (SD) at time of MRI examination; Y-BOCS severity lifetime/current = mean Yale–Brown Obsessive–Compulsive Scale severity scores (SD) across whole life span and at the time of MRI; Y-BOCS symptom lifetime/current: mean compound Yale–Brown Obsessive–Compulsive Scale symptom scores (SD) across whole life span and at the time of MRI; aggressive/checking, hoarding/saving, symmetry/ordering and washing/cleaning lifetime/current = mean Yale–Brown Obsessive–Compulsive Scale subcategory scores (SD) across the life span or at time of MRI (assessed using the Yale–Brown Obsessive–Compulsive Scale symptoms list).

Comorbidity lifetime/current (MINI) = mean comorbidity scores (SD) across whole life span or at the time of MRI (measured using the Mini International Neuropsychiatric Interview); Tic = mean tic scores (SD) at time of MRI; BDI = mean Beck Depression Inventory scores (SD) at time of MRI; STAI = mean State Trait Anxiety inventory scores (SD) at time of MRI; STAS = mean State Trait Anger Scale scores (SD) at time of MRI.

In addition, subjects were screened for the eight most common tics (head shaking, eye blinking, other facial tics, shoulder raising, expressing swear words/foul language/dirty words, sound making, growling and throat clearing/coughing/sniffing), since high comorbidity rates have been found between obsessive-compulsive disorder and chronic tic disorders (Cath et al., 2001). The subjects were asked to indicate whether they were familiar with one of these tics by answering yes or no.

All subjects were asked to collect mucosal cell samples for DNA extraction to test zygosity. The ethical review board of the VU University medical centre approved the study and all subjects provided written informed consent.

Tower of London

Stimuli for the Tower of London task consisted of images of three colored beads (red, blue and yellow), placed on three vertical rods of decreasing height **(figure 4.1)**. On each trial a start configuration **(figure 4.1, bottom)** and final target configuration **(figure 4.1, top)** were simultaneously displayed. During planning trials **(figure 4.1A)**, subjects were requested to count the number of steps to get from the start to final target configuration, with the restrictions that only one bead could be moved at a time and that a bead could be moved only if there was no other bead on top. Five planning difficulty levels were included corresponding to the minimal number of moves (1 to 5) needed to achieve the target configuration. In addition, baseline stimuli were included **(figure 4.1B)** during which subjects only had to count the total number of yellow and blue beads. With each stimulus presentation, two possible answers (one correct and one incorrect) were presented at the bottom left and right of the screen. The correct answer had to be indicated by pressing a corresponding left or right hand button. No feedback regarding the correct answer was provided.

The stimuli were presented in an event-related design of 17 minutes with self-paced stimulus timing, i.e., a subsequent trial was presented on the screen immediately after the response on a previous trial, or directly after the maximum reaction time limit of 60 seconds. Presentation order of the stimuli was pseudo-random with distribution frequency of the six stimulus types similar to van den Heuvel et al. (2005a). The stimulus presentation order was the same for all subjects, however, the total number of trials completed by each subject depended on the subject's reaction times.

Stimuli were projected on a screen at the end of the MRI scanner table, viewed by the participant through a mirror. Two MRI compatible response boxes were used to record the subject's performance. Prior to performance of the Tower of London task within the scanner, subjects practiced the task on a personal computer

outside the scanner. Furthermore, subjects performed a number of practice trials while in the scanner, immediately before the actual task.

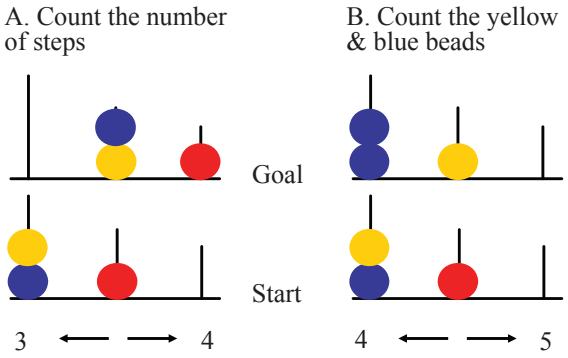

Figure 4.1. *Examples of Tower of London stimuli used in the present study. (A) Planning condition; (B) baseline condition [adapted from van den Heuvel et al. (2005a)].*

Image acquisition

The MRI session consisted of a structural part of ~ 6 minutes and a functional part of ~ 17 minutes. Subjects remained inside the scanner and were asked to minimize head movement during and between consecutive runs. To reduce motion artifacts, the subject's head was immobilized using foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3D gradient-echo T1-weighted sequence (flip angle 8°; Repetition Time, TR = 9.69 ms; Echo Time, TE = 4.60 ms, matrix, 256x256 pixels; voxel size, 1.00x1.00x1.20 mm). For functional MRI, an echo planar imaging sequence (flip angle 80°; TR = 2300 ms; TE = 30 ms, matrix, 96x96 pixels; field of view 220x220 mm) was used, covering the whole brain (40 axial slices; 2.29 mm x 2.29 mm in-plane resolution; 3.0 mm slice thickness). A total of 440 echo planar imaging volumes were collected per subject.

Data analysis

MRI data were analyzed using Statistical Parametric Mapping version 5 (SPM5) (Wellcome Department of Imaging Neuroscience, London, UK). Echo planar imaging scans were slice time corrected, realigned and normalized to the standard Montreal Neurological Institute (MNI) brain of SPM. Subsequently, data were resliced to 3 mm x 3 mm x 3 mm voxels and spatially smoothed using an 8 mm isotropic Gaussian kernel. After high-pass filtering (cut-off 128 seconds), functional scans were analyzed in the context of the general linear model using

delta functions convolved with a canonical haemodynamic response function. Event duration, computed as the time between stimulus and response onset, was included in the model to account for haemodynamic responses of varying lengths to each type of stimulus. Error trials and head-movement parameters were modeled as regressors of no interest. Post hoc analysis of subject motion during the scans, based on the functional scan realignment parameters, indicated that the twins with high obsessive-compulsive symptom scores did not exhibit significantly larger head-movement compared with those with low obsessive-compulsive symptom scores. For each individual, a 'planning versus baseline' main effect was computed in which brain activation during all planning trials was compared with brain activation during baseline trials. In addition, a main effect of 'task load' was computed using a linear contrast to identify brain regions that show magnetic resonance signal intensity variation correlated with task difficulty (van den Heuvel et al., 2005a).

Statistical tests

Differences in survey- and interview-based variables were tested using a mixed-model ANOVA [mixed models linear menu item in statistical package for the social sciences (SPSS; SPSS, Chicago, IL, USA)] with twin pair type (discordant versus concordant) and obsessive-compulsive symptom score (high versus low) as two fixed factors and family as a random factor to account for within-twin pair dependence. For the analysis of task performance data a similar mixed-model ANOVA was used, with task load (planning difficulty levels 1 to 5) as an additional repeated measures factor. Preplanned contrasts of significant 'task load' x 'obsessive-compulsive symptoms score' x 'twin pair type' interactions compared the discordant and concordant-high and low groups for each of the task load levels. Statistical results with regard to questionnaire and task performance data, were considered significant at $p < 0.05$, Bonferroni corrected.

First-level functional MRI contrast estimates for 'planning versus baseline' and 'task load' were entered into second-level analyses available in SPM5. Differences in contrast estimates between twins scoring high or low on obsessive-compulsive symptoms from discordant pairs were investigated by paired sample t-tests. Differences in contrast estimates between concordant twin pairs scoring high or low on obsessive-compulsive symptoms were assessed using an ANOVA group comparison. To account for within-twin pair correlations of functional MRI signals, first-level results of the twin and co-twin of each concordant pair were entered as repeated measures. For main task effects of selected contrasts we set an individual voxel threshold of $p < 0.05$, corrected for multiple comparisons (false discovery rate), with a minimal cluster extent of 10 voxels. Group differences, masked with the appropriate main task effect (mask thresholded at $p < 0.05$, uncorrected), are reported at an uncorrected individual voxel threshold of

$p < 0.005$, with a minimal cluster extent of five voxels.

Post hoc region of interest based comparison

After independent assessment of obsessive-compulsive symptom-related differences across the whole brain in discordant-high-low and concordant-high versus concordant-low twins, we performed an additional region of interest analysis to directly compare functional brain activation differences observed in both type of twin contrasts. That is, we tested for increased (or decreased) functional brain activation in concordant-high versus concordant-low twin pairs specifically in spherical regions of interest (radius 10 mm) centered on the coordinates where discordant-high twins showed maximally increased (or decreased) functional activation relative to discordant-low twins. Conversely, we tested for increased (or decreased) functional brain activation in discordant-high versus discordant-low twins in spherical regions of interest centered on the coordinates where concordant-high twins showed maximally increased (or decreased) functional activation relative to concordant-low twins. For these post hoc regions of interest analyses, we applied an individual voxel p-value threshold of $p < 0.05$, corrected for multiple comparisons (false discovery rate).

Results

Questionnaire and interview data

Demographics and data on obsessive-compulsive symptoms of the subjects are summarized in **table 4.1**. Significant main effects of 'obsessive-compulsive symptom score', were found for the Padua Inventory Abbreviated obtained in 2002 ($F(1, 120.66) = 579.32$, $p < 0.001$), Padua Inventory Abbreviated current scores ($F(1, 122.19) = 87.91$, $p < 0.001$), lifetime and current Yale-Brown Obsessive-Compulsive Scale symptom scores ($F(1, 124.23) = 34.26$, $p < 0.001$; $F(1, 122.31) = 34.95$, $p < 0.001$) as well as lifetime and current Yale-Brown Obsessive-Compulsive Scale severity scores ($F(1, 135.67) = 14.34$, $p < 0.001$; $F(1, 134.54) = 50.27$, $p < 0.001$). Furthermore, an interaction between 'obsessive-compulsive symptom score' and 'twin pair type' (discordant/concordant) was found for Padua Inventory Abbreviated current scores ($F(1, 122.19) = 8.12$, $p = 0.005$) and lifetime Yale-Brown Obsessive-Compulsive Scale severity scores ($F(1, 135.67) = 9.66$, $p = 0.002$). In both cases this was due to larger differences between high and low-scoring twins in concordant compared with discordant groups. There was no significant 'obsessive-compulsive symptom score' by 'twin pair type' interaction for the Yale-Brown Obsessive-Compulsive Scale subcategories aggressive\checking, hoarding\saving, symmetry\ordering and washing\cleaning, either across the whole life span (aggressive\checking: $F(1, 126.32) = 3.04$, $p = 0.084$, hoarding\saving: $F(1, 128.86) = 0.01$, $p = 0.929$, symmetry\ordering: $F(1, 126.35) =$

2.19, p = 0.141, washing\cleaning: F(1, 130.15) = 0.00, p = 0.962), or at the time of MRI (aggressive\checking: F(1, 126.49) = 1.13, p = 0.289, hoarding\saving: F(1, 115.37) = 0.00, p = 0.987, symmetry\ordering: F(1, 120.28) = 1.09, p = 0.299, washing\cleaning: F(1, 131.56) = 0.60, p = 0.439).

Table 4.1 also shows scores on questionnaires measuring comorbidities in the discordant and concordant twin pairs. Significant main effects of 'obsessive-compulsive symptom score', were found for lifetime and current comorbidity scores measured with the Mini International Neuropsychiatric Interview ($F(1, 132.70) = 21.60$, $p < 0.001$; $F(1, 116.75) = 11.48$, $p < 0.001$), tic scores ($F(1, 118.47) = 4.92$, $p = 0.028$), Beck Depression Inventory scores ($F(1, 136.69) = 8.67$, $p = 0.004$) and State Trait Anxiety scores ($F(1, 134.43) = 6.27$, $p = 0.013$). There was no significant main effect of 'obsessive-compulsive symptom score' with regard to State Trait Anger Scale scores ($F(1, 122.61) = 2.09$, $p = 0.150$). Significant 'obsessive-compulsive symptom score' by 'twin pair type' interactions were absent for all comorbidity measures.

Task performance

Figure 4.2 indicates Tower of London task response accuracy (top) and response latency (bottom) as a function of task load for twins scoring high and low on obsessive-compulsive symptoms in both the discordant **(figure 4.2A)** and concordant groups **(figure 4.2B)**. Significant main effects of variable 'task load' across groups indicated that reaction accuracy decreased and reaction times increased with increasing task difficulty (response accuracy: $F(1,221.14) = 89.37$, $p < 0.001$; response latency: $F(1,168) = 263.70$, $p < 0.001$). There was no significant main effect of 'obsessive-compulsive symptom score' for either the baseline condition (accuracy: $F(1,126.80) = 0.23$, $p = 0.632$; latency: $F(1,134.85) = 0.23$, $p = 0.629$) or during planning (accuracy: $F(1,181.76) = 0.51$, $p = 0.477$; latency: $F(1,285.81) = 0.94$, $p = 0.332$). In addition, there was no significant interaction between 'task load' and 'obsessive-compulsive symptom score' (accuracy: $F(1,221.14) = 0.94$, $p = 0.440$; latency: $F(1,168) = 1.09$, $p = 0.365$), or a significant 'task load' by 'obsessive-compulsive symptom score' by 'twin pair type' interaction (accuracy: $F(1,221.14) = 0.69$, $p = 0.600$; latency: $F(1,168) = 0.51$, $p = 0.728$). In short, high-scoring twins of either discordant or concordant pairs did not perform differently to the low-scoring twins.

Functional imaging

Main task effect

Activated brain regions for the 'planning versus baseline' and 'task load' contrasts are summarized in **figure 4.3** and **table 4.2**. In both the discordant and concordant groups, clusters of increased activation associated with Tower of London planning were noted, in parietal cortex (Brodmann areas (BA) 7 and 40), (pre)frontal cortex

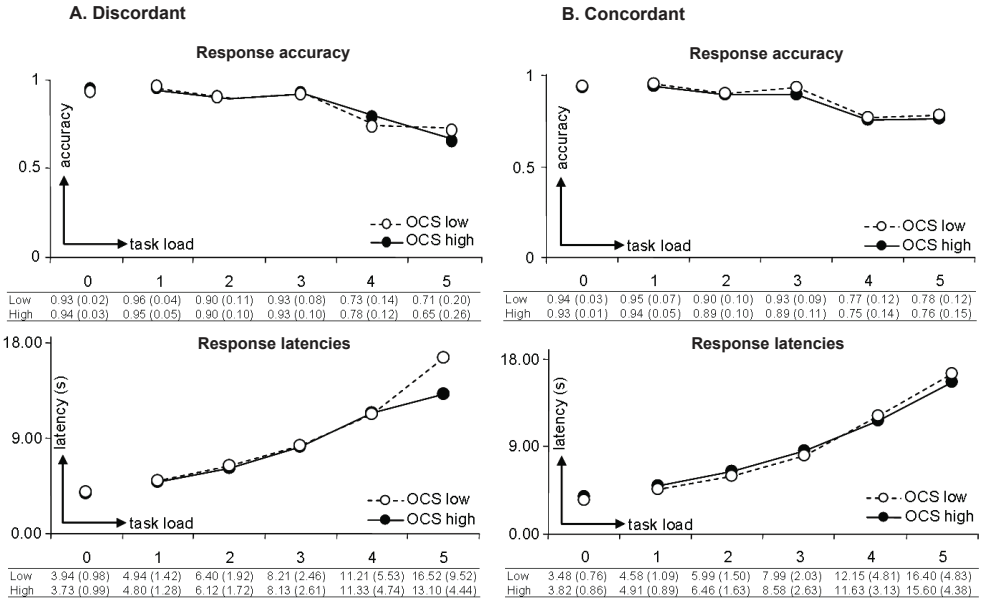

Figure 4.2. *Tower of London task performance. (Top): Response accuracy (between 0 and 1) as a function of task load levels 1, 2, 3, 4 and 5 (task load 0 = baseline condition) in the (A) discordant group, (B) concordant group. (Bottom): Mean latencies (s) of correct responses as a function of task load. Data for twins scoring high and low on obsessive-compulsive symptoms (OCS) are indicated by filled and open circles, respectively.*

(BA 6, 8, 9, 10 and 46), anterior cingulate (BA32), caudate nucleus and thalamus pulvinar. For the 'task load' contrast, relative to 'planning versus baseline', there was a tendency for more robust task-related activation in regions of the inferior frontal lobes (BA 44 & 47) as well as left and right frontopolar areas (compare the anatomical renderings in the top and bottom panels of **figure 4.3**).

Environmental risk: high- versus low-scoring twins from discordant pairs

Table 4.3, left, and **figure 4.4** show clusters of obsessive-compulsive symptoms-related differences in brain activation between the discordant-high and low twins. For the 'planning versus baseline' contrast **(figure 4.4A)**, twins scoring high on obsessive-compulsive symptoms compared with their low-scoring co-twins exhibited clusters of decreased brain activation in premotor cortex (clusters labeled A and B in **table 4.3, left** and **figure 4.4A**) and superior parietal cortex (clusters F-H), both bilaterally, and right medial frontal cortex (cluster C), right dorsolateral prefrontal cortex (cluster D) and left inferior parietal cortex (cluster E). Increased brain activation for twins scoring high on obsessive-compulsive symptoms was observed in the right middle temporal cortex (cluster I). For the 'task load' contrast **(figure 4.4B)**, clusters of decreased brain activation in twins scoring high on obsessive-compulsive symptoms relative to twins

Table 4.2. Brain activity for 'planning versus baseline' and 'task load' contrasts

Contrast	Anatomical location	Side	BA	Discordant (n = 38)				Concordant (n = 98)			
				MNI coordinates			Z score	MNI coordinates			Z score
				x	y	z		x	y	z	
'planning	parietal cortex	L	7	-6	-66	51	Inf	-9	-60	51	Inf
vs. baseline'		R	7	9	-69	57	7.30	3	-60	51	Inf
		L	40	-60	-36	36	5.36	-63	-33	36	4.72
		R	40	42	-42	42	6.54	45	-42	48	6.97
	frontal cortex	L	6	-30	0	51	7.10	-21	9	57	Inf
		R	6	27	9	57	7.11	21	12	54	7.34
		L	8	-30	15	48	5.40	-30	15	48	6.26
		R	8	33	12	51	5.80	21	12	54	7.34
		L	10	-42	48	-6	5.29				
		R	10	30	60	-3	4.60				
		L	9/46	-48	24	36	5.55	-48	33	27	5.00
		R	9/46	45	30	36	5.97	45	27	24	4.38
	occipital cortex	L	18					-33	-69	0	5.14
		R	18					21	-99	3	4.45
	anterior cingulate	L	32	-6	21	48	5.41	-9	21	45	3.95
		R	32	9	21	48	4.46				
	caudate nucleus	L	--	-12	15	-3	6.25	-12	15	-3	Inf
		R	--	12	9	0	5.81	15	18	-3	7.02
	thalamus pulvinar	L	--	-15	-30	12	2.72	-9	-30	6	3.03
		R	--	9	-27	12	4.07	3	-21	12	4.27
'task load'	parietal cortex	L	7	-3	-69	51	6.04	-9	-72	60	Inf
		R	7	6	-66	63	5.35	12	-66	66	Inf
		L	40	-45	-60	48	6.05	-42	-57	48	7.24
		R	40	57	-54	42	5.52	54	-54	45	7.60
	frontal cortex	L	6	-27	3	63	6.95	-27	12	60	Inf
		R	6	36	9	57	6.81	30	6	60	Inf
		L	8	-30	15	48	5.52	-3	27	45	Inf
		R	8	33	14	51	5.62	21	15	51	Inf
		L	9	-42	27	33	6.24	-42	30	33	Inf
		R	9	45	30	33	5.61	45	33	33	Inf
		L	10	-33	60	12	6.51	-36	51	9	7.08
		R	10	33	60	6	6.21	33	54	3	Inf
		L	44	-51	9	12	3.53				
		R	44					54	9	12	3.85
		L	47	-51	18	0	2.95	-48	15	0	3.94
		R	47	51	18	0	3.14	33	24	-6	3.70
	temporal cortex	L	37	-57	-48	-12	3.37				
	anterior cingulate	L	32	-6	24	36	5.90	-6	24	39	6.52
		R	32	9	33	30	5.30	9	24	36	4.32
	caudate nucleus	L	--	-15	12	12	5.65	-18	18	6	6.57
		R	--	18	21	6	4.87	18	18	6	6.71
	globus pallidus	L	--	-12	3	0	3.41	-15	0	-3	5.03
		R	--					12	3	-3	2.31
	thalamus pulvinar	L	--	-9	-24	12	2.62	-12	-27	15	2.66
		R	--	9	-27	12	4.14	9	-27	12	3.08

Brain regions showing significant functional MRI signal increase for the 'planning versus baseline' and 'task load' contrasts in the discordant and concordant twin groups. Anatomical location = activated brain region; L = left hemisphere; R = right hemisphere; BA = Brodmann area; MNI coordinates (mm) = location of voxel with largest effect size; Z score: z-value of voxel with largest effect size; Inf = infinite.

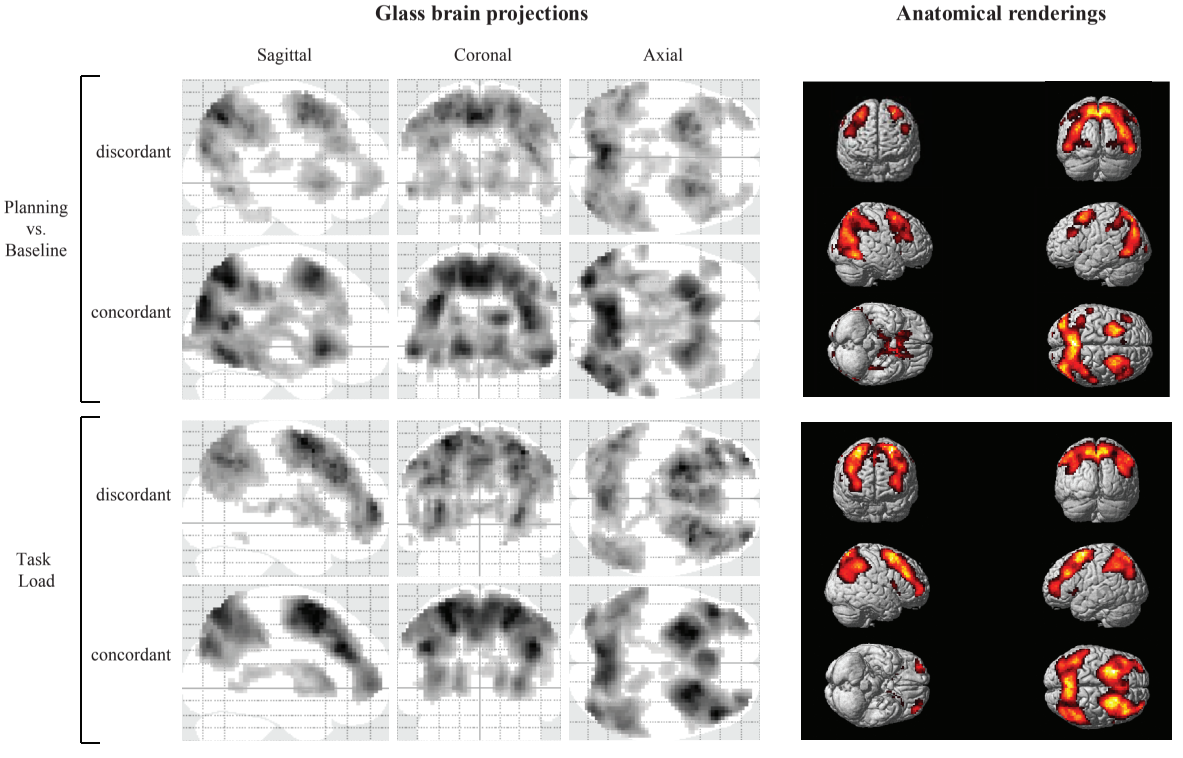

Figure 4.3. *Brain regions showing increased functional MRI signal during Tower of London cognitive planning. Glass brain overviews depict brain activity patterns for 'planning versus baseline' (top) and 'task load' (bottom) contrasts in discordant and concordant twins. Anatomical renderings on the right illustrate locations of functional brain activation for the 'planning versus baseline' (top) and 'task load' (bottom) contrasts, across all concordant twins.*

scoring low were noted in the left dorsolateral prefrontal cortex (cluster labeled J in **table 4.3, left** and **figure 4.4B**) and right lingual cortex (cluster K). Increased brain activation for the twins scoring high on obsessive-compulsive symptoms was observed bilaterally in the cingulate cortex (cluster L and M).

Genetic risk: concordant-high versus concordant-low-scoring twins

Table 4.4, left and **figure 4.5** show clusters of obsessive-compulsive symptom-related differences in brain activation between the concordant-high and low twin pairs. For the 'planning versus baseline' contrast **(figure 4.5A)**, concordant-high-scoring twins compared with concordant-low twins exhibited clusters of decreased brain activation, bilaterally, in temporal cortex (clusters labeled B, C and D in **table 4.4, left** and **figure 4.5A**), left globus pallidus (clusters labeled E) and left superior parietal cortex (cluster A). Clusters of increased brain activation for twins scoring high on obsessive-compulsive symptoms were noted in right parietal cortex (cluster F and G), and left cingulate cortex (cluster H). For the 'task load' contrast **(figure 4.5B)**, clusters of decreased brain activation in concordant-high twins were found in the left premotor cortex (clusters labeled K in **table 4.4, left** and **figure 4.5B**), right frontopolar cortex (clusters labeled L), left superior parietal cortex (cluster labeled I) and left caudate tail (cluster J). Increased brain activation for the concordant-high twins was observed in the left cingulate cortex (cluster M), and right inferior frontal cortex (cluster N).

Post hoc region of interest comparisons

Post hoc tests revealed no significant differences in brain activation for concordant-high versus concordant-low twin pairs in regions of interest centered around the clusters with functional activation differences in the whole brain discordant twin comparison (i.e., spherical regions of interest placed on each of the cluster peak coordinates from the discordant comparison listed in **table 4.3, left**). There were also no differences in brain activation in discordant-high versus discordant-low twin pairs in regions of interest centered around the clusters with functional activation differences in the whole brain concordant twin comparison (i.e., spherical regions of interest placed on each of the cluster peak coordinates from the concordant comparison listed in **table 4.4, left**).

Post hoc analyses using obsessive-compulsive symptoms scores at the time of scanning

This study had a prospective design in that selection of the twins preceded the actual MRI scans by 4-7 years. As a consequence many of the discordant pairs and some of the concordant pairs no longer met the criteria at the time of scanning. We therefore conducted new analyses on our data to test if a focus on the obsessive-compulsive symptom scores at the time of scanning would affect our results significantly. We re-run the analysis on a group of eight discordant pairs who still met the criteria at the time of MRI scanning, [high obsessive-compulsive

Table 4.3. Brain activation differences between twins scoring high and low on obsessive–compulsive symptoms from the discordant group

Test	Cluster label[a]	Anatomical location	BA	MNI coordinates (19 pairs)			Z score	*p*-value	No. of voxels	MNI coordinates (8 pairs)[b]			Z score	No. of voxels	Euclidean distance (mm)
				x	y	z				x	y	z			
'planning vs. baseline'															
high<low	A	Left premotor cortex	6	-27	3	54	3.41	<0.001	15	-30	0	48	3.84	9	7.3
	B	Right premotor cortex	6	12	6	69	3.33	<0.001	9	12	9	60	2.98	1	9.5
	C	Right medial frontal cortex	8	15	27	48	3.20	0.001	8	-	-	-	-	-	-
	D	Right dorsolateral prefrontal cortex	9	39	18	27	2.85	0.002	5	33	21	21	2.82	1	9
	E	Left inferior parietal cortex	40	-39	-54	51	4.33	<0.001	46	-39	-54	51	2.70	4	0
			40	-57	-36	45	3.18	0.001	9	-57	-42	45	2.66	1	6
	F	Left superior parietal cortex	7	-12	-54	54	3.40	<0.001	14	-	-	-	-	-	-
			7	-18	-69	42	2.87	0.002	6	0	-69	51	2.86	5	20.1
	G	Right superior parietal cortex	7	30	-72	48	2.93	0.002	5	-	-	-	-	-	-
	H	Right superior parietal cortex	19	30	-78	30	3.36	<0.001	9	39	-84	33	3.40	1	11.2
high>low	I	Right middle temporal cortex	39	54	-69	12	3.44	<0.001	12	57	-60	21	3.02	4	13.1
'task load'															
high<low	J	Left dorsolateral prefrontal cortex	9	-9	30	36	3.09	0.001	9	-21	15	48	2.61	1	22.6
	K	Right lingual cortex	30	21	-42	0	3.21	0.001	8	-	-	-	-	-	-
high>low	L	Right cingulate cortex	24	12	0	48	3.46	<0.001	10	12	0	48	2.76	1	0
	M	Left cingulate cortex	24	-9	0	42	3.01	0.001	9	-3	3	42	2.99	3	6.7

a Clusters with regional brain activation differences between discordant-high and discordant-low twins for the 'planning versus baseline' and 'task load' contrasts.
Test = test for significant decreases (high<low) or increases (high>low) in twins with high relative to low obsessive–compulsive symptoms scores; cluster label = alphabetical cluster label as displayed in anatomical overlays of figure 4.4A ('planning versus baseline' contrast) and B ('task load' contrast); BA = Brodmann area; MNI coordinates (19 pairs) (mm) = location of voxel with largest effect size for the 19-pair comparison; Z score = z-value of voxel with largest effect size; P-value = cluster P-value; no. of voxels = number of voxels in cluster.
b Montreal Neurological Institute coordinates (mm) of voxel with largest effect size for the post hoc within-pair comparison in the 8 pairs still extremely discordant at the time of scanning; Z score = z-value of voxel with largest effect size for the post hoc 8 pair comparison; no. of voxels = number of voxels in cluster for the post hoc 8 pair comparison; Euclidean distance (mm) = distance between the coordinates derived from the original 19 pair comparison and the coordinates derived from the additional 8 pair comparison.

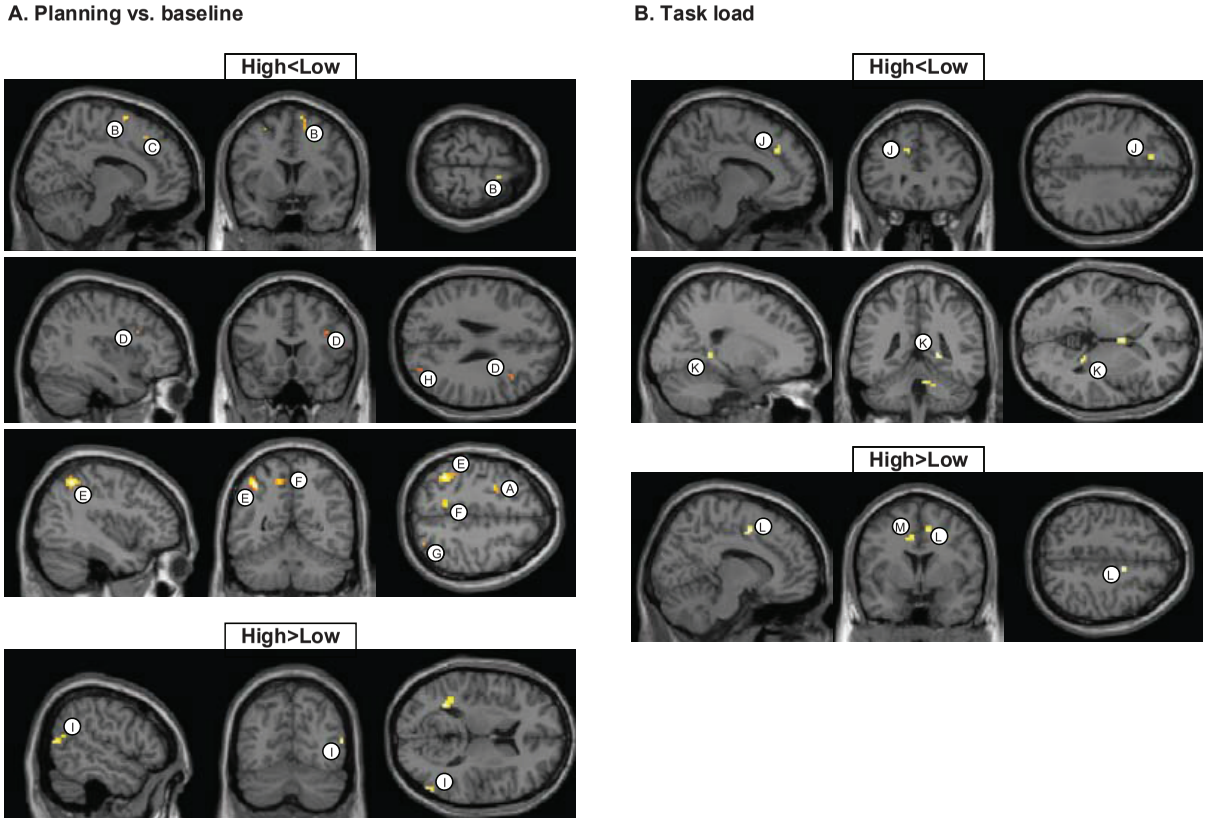

Figure 4.4. *Brain regions showing reduced (top: high<low) and increased (bottom: high>low) functional MRI signal in twins with high obsessive-compulsive symptoms scores versus twins with low scores from the discordant group. (A) 'Planning versus baseline' contrast; (B) 'task load' contrast.*

Table 4.4. Brain activation differences between twins scoring high and low on obsessive–compulsive symptoms from the concordant group

Test	Cluster label[a]	Anatomical location	BA	MNI coordinates (22/28pairs)			Z score	*p*-value	No. of voxels	MNI coordinates (10/23 pairs)[b]			Z score	No. of voxels	Euclidean distance (mm)
				x	y	z				x	y	z			
'planning vs.baseline'															
high<low	A	Left superior parietal cortex	19	-33	-78	39	3.34	<0.001	35	-36	-72	39	2.80	4	6.7
	B	Left superior temporal cortex	22	-54	-15	3	3.10	0.001	9	-57	-12	6	3.24	13	5.2
	C	Left inferior temporal cortex	37	-48	-51	-3	3.78	<0.001	29	-48	-51	-3	4.22	84	0
	D	Right middle temporal cortex	37	48	-63	0	3.27	0.001	23	-	-	-	-	-	-
	E	Left globus pallidus	-	-18	-3	-12	3.00	0.001	5	-	-	-	-	-	-
high>low	F	Right inferior parietal cortex	40	48	-51	21	3.28	0.001	15	63	-54	21	2.66	1	15.3
	G	Right superior parietal cortex	7	24	-57	54	3.14	0.001	15	27	-54	57	3.75	47	5.2
	H	Left cingulate cortex	31	-6	-45	48	3.08	0.001	12	-	-	-	-	-	-
'task load'															
high<low	I	Left superior parietal cortex	19	-33	-75	39	3.54	<0.001	39	-33	-72	39	3.15	10	3
	J	Left caudate tail	-	-15	-30	18	3.65	<0.001	14	-24	-27	18	3.05	20	9.5
	K	Left premotor cortex	6	-33	-6	66	3.19	0.001	11	-	-	-	-	-	-
	L	Right frontopolar cortex	10	24	48	3	3.11	0.001	7	24	48	0	3.00	15	3
high>low	M	Left cingulate cortex	32	-9	18	39	3.81	<0.001	26	-12	18	39	3.29	10	3
	N	Right inferior frontal cortex	47	51	18	0	3.50	<0.001	7	51	18	0	2.65	5	0

a Clusters with regional brain activation differences between concordant-high and concordant-low twins for the 'planning versus baseline' and 'task load' contrasts.
Test = test for significant decreases (high<low) or increases (high>low) in twins with high relative to low obsessive–compulsive symptoms scores; Cluster label = alphabetical cluster label as displayed in anatomical overlays of figure 4.5A ('planning versus baseline' contrast) and B ('task load' contrast); BA = Brodmann area; MNI coordinates (22/28 pairs) (mm) = location of voxel with largest effect size for the 22-high to 28-low twin pair comparison; Z score = z-value of voxel with largest effect size; P-value = cluster P-value; no. of voxels = number of voxels in cluster. b Montreal Neurological Institute coordinates (mm) of voxel with largest effect size for the post hoc comparison between the 10 twin pairs that scored high at the time of scanning and the 23 twin pairs that scored low at the time of scanning; Z score = z-value of voxel with largest effect size for the post hoc 10-high to 23-low-scoring twin pair comparison; no. of voxels = number of voxels in cluster for the post hoc 10-high to 23-low-scoring twin pair comparison; Euclidean distance (mm) = distance between the coordinates derived from the original 22-high to 28-low-twin pair comparison and the coordinates derived from our additional 10-high to 23-low-scoring twin pair comparison.

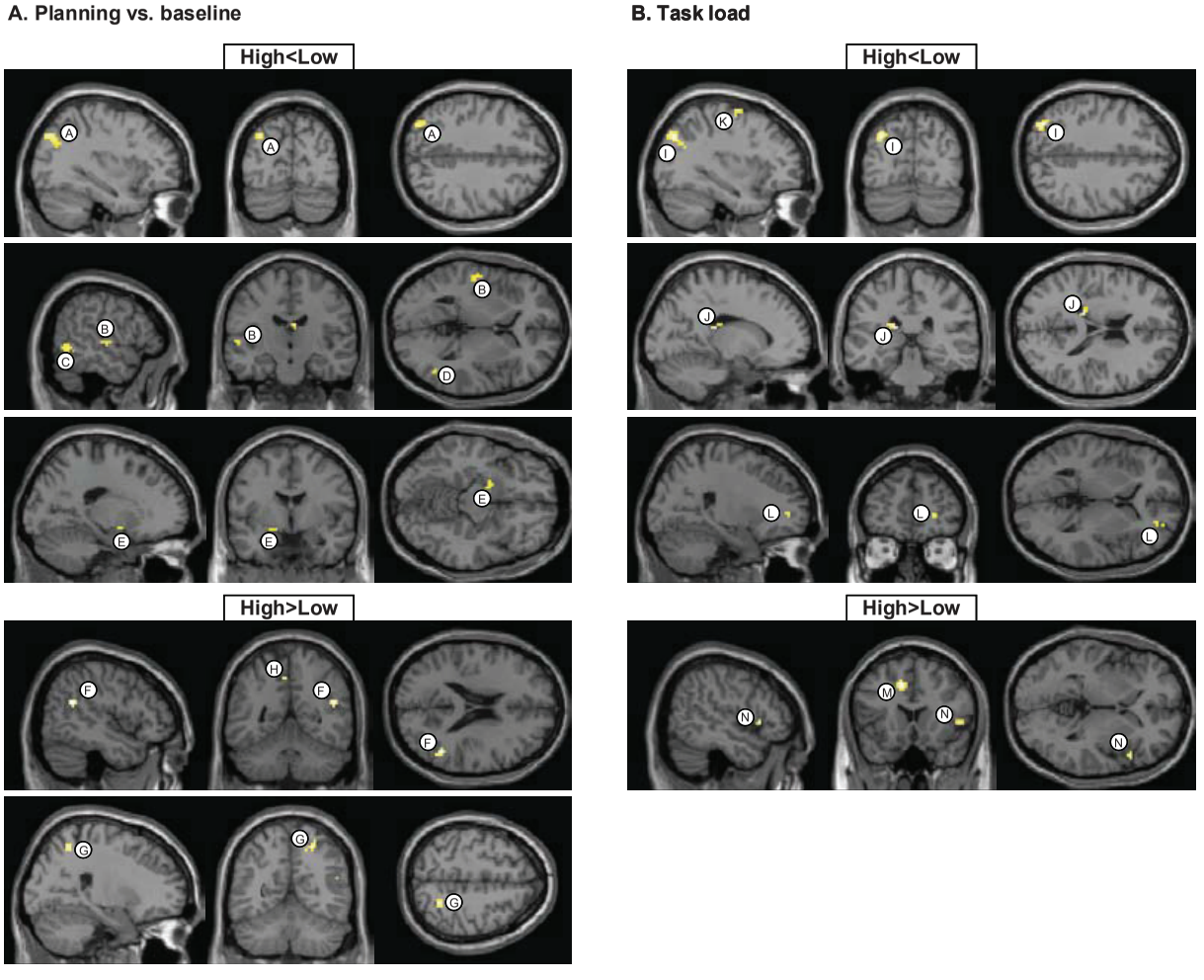

Figure 4.5. *Brain regions showing reduced (top: high<low) and increased (bottom: high>low) functional MRI signal in concordant-high versus concordant-low twins. (A) 'Planning versus baseline' contrast; (B) 'task load' contrast.*

symptom score: mean(SD) = 17.75(7.6); low obsessive-compulsive symptom score: mean(SD) = 4.75(3.1)] and on those concordant pairs with a mean obsessive-compulsive symptom score meeting the cut-off criteria at the time of scanning [10 concordant- high twin pairs with mean(SD) = 19.30(5.1) and 23 concordant-low twin pairs with mean(SD) = 3.76(2.2)]. To directly compare functional brain activation differences observed from the original analysis in 19 discordant pairs with those obtained from the analysis in the selected 8 pairs, we tested for increased (or decreased) functional brain activation ($p<0.005$, uncorrected) in our 8 pair comparison specifically at the coordinates where the analysis on 19 pairs showed maximally increased (or decreased) functional activation. If no significant cluster was found at the exact coordinate derived from our 19 pair comparison we searched for the nearest local maxima within that anatomical location. Results are reported in **table 4.3, right**. The same analysis was performed for the concordant group, in which we tested for increased (or decreased) functional brain activation ($p<0.005$, uncorrected) in our 10 concordant-high to 23 concordant-low pair comparison specifically at the coordinates where the analysis on the original 22 concordant-high to 28 concordant-low pair comparison showed maximally increased (or decreased) functional activation. Results are reported in **table 4.4, right**. Post hoc analyses in both the discordant and concordant groups revealed highly similar results compared with those obtained from the original analyses, although a few areas were lost due to reduced statistical power.

Discussion

In the present study, task performance and brain activation during a Tower of London cognitive planning paradigm were compared within monozygotic twin pairs discordant for obsessive-compulsive symptoms and between monozygotic twin pairs who scored concordant-low or concordant-high for obsessive-compulsive symptoms. No differences were found in response accuracy or latency measures between discordant twins, which implies that the environmentally mediated risk for obsessive-compulsive disorder did not influence behavioral task performance. Likewise, concordant-high-twins did not perform worse than concordant-low-scoring twins suggesting that the genetically mediated risk for obsessive-compulsive disorder did not interfere with actual task performance. These results partly disagree with studies comparing Tower of London performance in patients with obsessive-compulsive disorder versus controls. Purcell and colleagues (1998a) found no significant differences in response accuracy in Tower of London task performance between patients with obsessive-compulsive disorder and controls, but the patients with obsessive-compulsive disorder reacted significantly slower. In addition, van den Heuvel and colleagues (2005) found patients with obsessive-compulsive disorder to be significantly less accurate

and slower. It is unclear whether the absence of performance deficits in our study reflects the lower severity of obsessive-compulsive symptoms in this largely non-clinical sample, the fact that only few of our subjects had a history of anti-depressant medication (in contrast to the studies with patient groups), or a combination.

Although their performance remained intact, there was evidence that the high-risk subjects in our study deviated from the low-risk subjects in the patterns of brain activation accompanying execution of the Tower of London task. The brain regions in which subjects with high obsessive-compulsive symptoms scores differed from subjects with low obsessive-compulsive symptoms scores can be separated into regions that were mainly affected by environmental risk (dorsolateral prefrontal cortex (BA9), and lingual cortex (BA30)), regions mainly affected by genetic risk (frontopolar cortex (BA10), inferior frontal cortex (BA47), globus pallidus and caudate nucleus), and regions affected by both environmental and genetic risk factors (cingulate cortex (BA24, 31, 32), premotor cortex (BA6) and parts of the parietal cortex (BA7, 19, 40)). We discuss these findings in more detail below.

Regions affected by environmental risk

Brain regions showing different activation patterns in twins with high obsessive-compulsive symptoms scores compared with those with low obsessive-compulsive symptoms scores that were present in only the discordant group and, therefore, are probably related to environmental risk factors for obsessive-compulsive disorder, include the dorsolateral prefrontal cortex (BA9) ('planning versus baseline' and 'task load'), and right lingual cortex (BA30) ('task load'). Our findings of decreased 'planning versus baseline' and 'task load' associated dorsolateral prefrontal cortex activity in the twins with high obsessive-compulsive symptoms scores compared with those with low obsessive-compulsive symptoms scores, replicates our previous findings in a subsample of the present discordant twin population (den Braber et al., 2008). In addition, these results are in line with the findings of a study in patients with obsessive-compulsive disorder (van den Heuvel et al., 2005a). The dorsolateral prefrontal cortex has been related to executive processing, including attention, response inhibition, cognitive planning and decision making (Faw, 2003; Newman et al., 2003; Ridderinkhof et al., 2004). In addition, neuropsychological studies have typically associated dysfunction of this brain structure with perseverative, disinhibited behaviors, which patients with obsessive-compulsive disorder particularly show during the completion of their compulsions (Friedlander and Desrocher, 2006). Reduced activity in the dorsolateral prefrontal cortex also agrees with the commonly accepted neurobiological model of CSTC abnormalities in obsessive-compulsive disorder (Graybiel and Rauch, 2000; Insel and Winslow, 1992; Menzies et al., 2008a).

In line with our results, a decrease in lingual cortex activity ('task load') in patients with obsessive-compulsive disorder compared with unaffected controls has been found in a symptom provocation study by Mataix-Cols and colleagues (2004). In their study patients with obsessive-compulsive disorder and controls were presented with emotional (e.g., washing-related, checking-related) pictures during functional MRI scanning. The observed decrease in lingual activity was specifically associated with the checking symptom dimension. The lingual cortex is part of the occipital cortex, which is involved in visual processing. The authors suggested that the patients with obsessive-compulsive disorder directed their attention more to the emotional salience of the pictures rather than focusing on the visual details, which would explain the decrease in activation of the occipital cortex.

Regions affected by genetic risk

Brain regions showing different activation patterns in twins with high obsessive-compulsive symptoms scores compared with those with low obsessive-compulsive symptoms scores that were present in only the concordant group and therefore are suggested to be related to genetic risk factors for obsessive-compulsive disorder, include the right frontopolar cortex (BA10) ('task load'), the right inferior frontal cortex (BA47) ('task load'), the left caudate nucleus ('task load') and the left globus pallidus ('planning versus baseline'). The 'task load'-related decrease in frontopolar activity (BA10) in twins with high obsessive-compulsive symptoms scores is in agreement with lower activity in this area in patients with obsessive-compulsive disorder after performing a set switching paradigm (Gu et al., 2008). Although its specific role in cognitive functioning is not yet clearly understood, the frontopolar region appears to be engaged in a wide variety of higher-order cognitive functions, such as learning and exploration, memory retrieval, relational reasoning, multitasking behavior and 'the human ability to hold in mind goals while exploring and processing secondary goals' (Burgess et al., 2007; Koechlin and Hyafil, 2007; Ramnani and Owen, 2004). This region is connected to areas in the CSTC network, including the prefrontal cortex and cingulate cortex (Koechlin and Hyafil, 2007; Ramnani and Owen, 2004) and may influence obsessive-compulsive disorder through these connections.

Our finding of increased 'task load'-related activity in the inferior frontal cortex is in line with findings in patients with obsessive-compulsive disorder (van den Heuvel et al., 2005a). The inferior frontal cortex has been implicated in a wide range of cognitive processes, including task switching, reversal learning and cognitive and emotional inhibition (Dillon and Pizzagalli, 2007; Ramnani and Owen, 2004). Furthermore, this region is involved in regulating socially appropriate behaviors and when impaired a patient may show tactless, impulsive and disinhibited behavior (Friedlander and Desrocher, 2006).

Our findings of decreased caudate nucleus ('task load') and globus pallidus ('planning versus baseline') activity are consistent with several neuroimaging studies (Giedd et al., 2000; Mataix-Cols and van den Heuvel, 2006; Szeszko et al., 2004; van den Heuvel et al., 2005a). Reduced activity patterns in these basal ganglia structures agrees with the general theory of a dysfunction in the CSTC circuitry in obsessive-compulsive disorder (Graybiel and Rauch, 2000; Menzies et al., 2008a). The basal ganglia have strong connections with associative, orbitofrontal and sensorimotor cortices and participate in many neuronal pathways implicated in motor, emotional, motivational, associative and cognitive functions (Herrero et al., 2002). In addition, the basal ganglia play a role in reinforcing wanted behaviors and suppressing unwanted behavior (Schultz et al., 1997). A dysfunction in globus pallidus and/or caudate nucleus might therefore result in the behavioral deficits seen in obsessive-compulsive disorder, which is supported by the fact that focal lesions in the caudate nucleus or globus pallidus produce striking obsessive-compulsive disorder like behavior (Laplane et al., 1989).

Taken together, our findings of altered prefrontal and striatal activity in twins with high obsessive-compulsive symptoms scores compared with those with low scores fit very well with a model of neurobiological changes due to the genetic risk for obsessive-compulsive disorder. Since family and twin studies have shown that obsessive-compulsive disorder is heritable (van Grootheest et al., 2005), several studies have tried to identify genetic variants involved in obsessive-compulsive disorder etiology (Nicolini et al., 2009). Glutamine and serotonin system genes are among the candidate genes for which replication has most often been reported (Nicolini et al., 2009). In prefrontal regions and their projection areas in the striatum both glutamergic and serotonergic neurotransmission is highly abundant (Carlsson, 2001; Fineberg et al., 2010). Interestingly, pharmacological studies have indicated glutamate/serotonin interactions in these particular regions, which are further supported by positron emission tomography and magnetic resonance spectroscopy studies (Carlsson, 2001).

Regions affected by environmental and genetic risk

The additional regions of interest analysis employed in this study, testing the presence of overlap in brain activation changes observed in our discordant and concordant twins did not reveal any significant results, after correction for multiple testing. Nonetheless, there was an implication that some areas in the uncorrected whole-brain analyses were affected by both environmental and genetic risk factors for obsessive-compulsive disorder. These regions included the cingulate, premotor and parietal cortices.

In agreement with our findings, increased activity in the cingulate cortex ('task load') was also found in patients with obsessive-compulsive disorder

(van den Heuvel et al., 2005a). A priori, we hypothesized that regions affected by both environmental and genetic risk factors for obsessive-compulsive disorder should be closest related to the behavioral abnormalities characteristic of the disorder. At first sight, this appears to make sense for the cingulate cortex, since this brain region, through its connections with other regions of the limbic system, is implicated in the assessment of emotional information and the regulation of emotional responses, and thereby might mediate the anxiety provoking thoughts and subsequent repetitive behaviors seen in obsessive-compulsive disorder (Aouizerate et al., 2004).

However, in view of the full pattern of our results, we a posteriori favor the alternative explanation that the regions found to be affected by both environmental and genetic risk factors for obsessive-compulsive disorder, including the cingulate cortex, act to compensate for the disturbances in CSTC circuits rather than playing a central role in obsessive-compulsive symptomatology. The cingulate cortex is related to performance monitoring (MacDonald III, et al., 2000) and error signaling (Magno et al., 2006), and the high obsessive-compulsive symptom group may feel a strong need to perform well and avoid errors, as perfectionism is highly associated with obsessive-compulsive disorder (Frost and Steketee, 1997). This is in line with our finding that subjects with high obsessive-compulsive symptoms scores in both discordant and concordant groups kept their performance intact.

Decreases in brain activity in the high-scoring compared with low-scoring twins from both groups were found in the premotor cortex (BA6) and regions of the parietal cortex (BA7, BA19, BA40). Activation decreases in these regions, almost exclusively present in the 'planning versus baseline' contrast, are in line with our previous findings (den Braber et al., 2008) and those from van den Heuvel and colleagues (2005a). Since these areas are involved in basic functions of motion processing (Rowe et al., 2001), motor preparation (Hoshi and Tanji, 2000; Mars et al., 2007), and visuospatial processing (Cabeza and Nyberg, 2000) they may mainly support proper task execution (e.g., analysis of the planning stimulus, imaginary of movement of the beads, executing a response) rather than higher order planning.

Obsessive-compulsive disorder-related abnormalities in superior and inferior parietal regions have been found by others as well (Ciesielski et al., 2005; Kitamura et al., 2006; Kwon et al., 2003; Lucey et al., 1995; Menzies et al., 2007; Menzies et al., 2008a; Szeszko et al., 2005; Valente Jr. et al., 2005). While the decrease in brain activation in the parietal cortex in the high obsessive-compulsive symptoms group might indicate a deficit in visual processing, there could also be another explanation. The superior and inferior parietal cortex are connected with each other, and results from animal studies have shown that these structures are

strongly interconnected with the prefrontal cortex, dorsal premotor area, supplementary motor area and anterior cingulate cortex (Diwadkar et al., 2000; Faw, 2003; Goldman-Rakic, 1988; Petrides and Pandya, 1984). The superior parietal cortex also has major subcortical connections with the claustrum, caudate nucleus and putamen (Leichnetz, 2001; Yeterian and Pandya, 1993). These considerations indicate that the parietal cortex and DLPFC (or caudate nucleus), do not act independently, but influence each other. Therefore, the decrease in parietal activity found in our study might be directly related to the decreased activity observed in the dorsolateral prefrontal cortex and caudate nucleus. This is in line with recent evidence that the underlying pathology of obsessive-compulsive disorder is not limited to orbitofrontal-striatal regions and associated limbic structures, but also involves parietal lobe abnormalities (Menzies et al., 2008a).

This study had a prospective design in that selection of the twins preceded the actual scans by 4-7 years. As a consequence, some of the discordant and concordant pairs no longer matched the stringent selection criteria at the time of MRI scanning, which could have influenced our results adversely. Nevertheless, the within-pair difference in the discordant group and the between-pair difference in the concordant-high-low group were still significant at the time of scanning and the post hoc analysis; comparing only those twins that matched selection criteria at the time of scanning revealed highly comparable results. These results indicate that environmentally or genetically mediated functional brain alterations in obsessive-compulsive symptoms remain unchanged regardless of having present obsessive-compulsive symptoms, suggesting that these brain alterations are trait-like in nature. This is consistent with conclusions drawn by others (Bannon et al., 2006; Rao et al., 2008) that used neuropsychological tests rather than functional MRI.

To summarize, the present results suggest that brain regions affected by the environmental risk for obsessive-compulsive disorder are partly distinct from brain regions affected by the genetic risk for obsessive-compulsive disorder. Regions with neurobiological changes induced by environmental risk factors include the dorsolateral prefrontal cortex and lingual cortex, which are part of the dorsolateral prefrontal-subcortical loop (Cummings, 1995) of the CSTC network in which several imaging studies have reported abnormalities (Menzies et al., 2008a). Disturbances in the dorsolateral prefrontal-subcortical loop may result in perseveration, reduced mental control and impaired response inhibition, as seen in obsessive-compulsive disorder. Regions with neurobiological changes induced by genetic factors include orbitofrontal-basal ganglia structures that are part of the orbitofrontal-basal ganglia loop of the CSTC network (Menzies et al., 2008a). Disturbances in the orbitofrontal-basal ganglia loop may result in the tactless, impulsive and disinhibited behavior seen in obsessive-compulsive

disorder (Graybiel and Rauch, 2000). Regions that show similar decreases in activity in discordant and concordant groups, such as superior and inferior parietal regions may indirectly reflect the deficits in dorsolateral prefrontal and orbitofrontal-striatal networks, to which they are highly connected. Regions that show similar increases in activity in discordant and concordant groups, such as the cingulate cortex may be part of compensatory networks that keep planning performance intact, at least during a relatively unchallenging task like the Tower of London.

5 Brain activation during response interference in twins discordant or concordant for obsessive-compulsive symptoms

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Abstract

One of the core behavioral features associated with obsessive-compulsive symptomatology is the inability to inhibit thoughts and/or behaviors. Neuroimaging studies have indicated abnormalities in frontostriatal and dorsolateral prefrontal – anterior cingulate circuits during inhibitory control in patients with obsessive-compulsive disorder compared with controls. In the present study, task performance and brain activation during Stroop color-word and Flanker interference were compared within monozygotic twin pairs discordant for obsessive-compulsive symptoms and between groups of pairs scoring very low or very high on obsessive-compulsive symptoms, in order to examine the differential impact of non-shared environmental versus genetic risk factors for obsessive-compulsive symptomatology on inhibitory control related functional brain activation. Although performance was intact, brain activation during inhibition of distracting information differed between obsessive-compulsive symptom high compared to low-scoring subjects. Regions affected in the discordant group (e.g., temporal and anterior cingulate gyrus) were partly different from those observed to be affected in the concordant groups (e.g., parietal gyrus and thalamus). A robust increase in dorsolateral prefrontal activity during response interference was observed in both the high-scoring twins of the discordant sample and the high-scoring twins of the concordant sample, marking this structure as a possible key region for disturbances in inhibitory control in obsessive-compulsive disorder.

Introduction

Obsessive-compulsive symptoms (OCS) are characterized by recurrent, persistent, and intrusive anxiety provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions) performed to reduce anxiety and/or distress caused by the obsessions. When a person has these obsessions and/or performs compulsions for more than one hour a day and these thoughts and rituals significantly interfere with his/her daily life routines, the person fulfills the criteria for obsessive-compulsive disorder (OCD). The life-time prevalence of OCD is 0.5-2% (American Psychiatric Association, 1994; Grabe et al., 2000), but obsessions are much more prevalent in the general population – as high as 72% (Rachman and de Silva, 1978; Salkovskis and Harrison, 1984) and the prevalence of OC symptoms reaches up to 20% (Fullana et al., 2009).

Numerous twin (Jonnal et al., 2000; van Grootheest et al., 2005) and family studies (Hettema et al., 2001; Nestadt et al., 2000) have indicated the importance of genetic as well as environmental risk factors with regard to the etiology of OCD.

Heritabilities for OCD have been estimated to be between 27 – 47% in adults and 45 – 65% in children (Jonnal et al., 2000; van Grootheest et al., 2005) and linkage and association studies have mainly pointed towards functional deficits of genes involved in serotonergic, glutamatergic and dopaminergic neural signaling (for review see Nicolini et al., 2009). Potential environmental risk factors for OCD include traumatic early life experiences, perinatal problems, streptococcal infection, psychosocial stress, aspects of parenting (e.g., parental overprotection), pregnancy, divorce and emotional neglect (Albert et al., 2000; Alonso et al., 2004; Cath et al., 2008; Geller et al., 2008; Grisham et al., 2008; Lin et al., 2007; Wilcox et al., 2008).

Over the last two decades, neuroimaging studies have indicated several neurobiological changes underlying the psychological and behavioral dysfunction of OCD. Results from structural and functional magnetic resonance imaging (sMRI/fMRI) studies mainly point to volume differences and altered regional brain activation in the ventral prefrontal cortex (PFC), dorsolateral prefrontal cortex (DLPFC), basal ganglia, anterior cingulate cortex (ACC) and thalamus (Menzies et al., 2008a; Radua and Mataix-Cols, 2009; Radua et al., 2010; Rotge et al., 2009). These findings have contributed to the widely accepted neuroanatomical model of OCD involving the direct and indirect cortico-striato-thalamo-cortical (CSTC) loops (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). The direct loop functions as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors. The indirect loop functions as a negative feedback loop important for inhibiting and switching between behaviors. It has been hypothesized that an imbalance between these loops, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate OC symptomatology (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). Although a disturbance in these CSTC loops may represent the main neurological basis for OCD, several imaging studies also suggest the involvement of other brain regions, such as amygdala, hippocampus and parietal areas in OCD (Menzies et al., 2008a; Pujol et al., 2004; Valente Jr. et al., 2005). Therefore, Menzies and colleagues (2008) proposed an extended model that includes these brain areas that are functionally connected to the ventral and dorsal frontal-striatal circuits.

One of the core behavioral features associated with OC symptomatology is the inability to inhibit thoughts and/or behaviors. The process of inhibitory control has been linked to frontal-striatal networks, but the ACC and its interactions with the DLPFC may also play a crucial role since this circuitry has been repeatedly linked to conflict monitoring and adjustments in control (Kerns et al., 2004; Melcher et al., 2008). Numerous imaging studies in OCD specifically focused on the neurobiological processes underlying inhibitory control by exposing both OCD

patients and controls to cognitive tasks that are developed to measure inhibitory control, such as response interference in the Stroop color-word and Eriksen Flanker task (Melcher et al., 2008). Regarding task performance, in which prolonged reaction times and error rates are generally considered a direct indicator for cognitive conflict or interference, OCD patients have been repeatedly described to be in the normal range (Fitzgerald et al., 2005; Maltby et al., 2005; Nakao et al., 2005; Page et al., 2009; Viard et al., 2005; Yucel et al., 2007). However, some studies showed prolonged reaction times during high-conflict trials, suggestive for impaired inhibitory control in OCD patients (Menzies et al., 2007; van den Heuvel et al., 2005b). Furthermore, of interest for the present study, Menzies et al. (2007) found delayed response inhibition on the stop-signal task in OCD patients as well as in unaffected first degree relatives of OCD patients, suggesting familial vulnerability of this aspect of OCD. Even if performance was intact, there is evidence that OCD patients show a different pattern of brain activation during the execution of tasks measuring inhibitory control. Most neuroimaging studies that investigated inhibitory control showed a higher response-conflict related increase in ACC activity in OCD patients than in controls (Fitzgerald et al., 2005; Maltby et al., 2005; Page et al., 2009; Ursu et al., 2003). Increases in regional activity during high-conflict trials has also been reported for frontal-striatal regions (orbitofrontal cortex, caudate and thalamus), as well as the DLPFC, cerebellum, temporal and parietal regions (Maltby et al., 2005; Nakao et al., 2005; Nakao et al., 2009; Page et al., 2009; van den Heuvel et al., 2005b). However, the findings regarding the direction of activation changes are not consistent, since hypoactivation of ACC, caudate, temporal and parietal regions during response interference has also been reported (Nakao et al., 2005; Nakao et al., 2009; Page et al., 2009; Yucel et al., 2007). These inconsistencies may be explained by methodological differences between studies such as the paradigm used to measure response interference, heterogeneity of patient groups and differences in sample size, scanning modalities/parameters and analysis methods. However, there may also be 'true' variability in the underlying neurobiology of (response interference in) OCD. That is, it may be that dysfunction of different brain regions leads to highly comparable changes at the behavioral level, because these regions are part of the same brain network involved in controlling behaviors. Such heterogeneity in affected brain regions could reflect a differential influence of environmental and genetic risk factors for OCD impacting on different parts of the brain.

Most brain imaging studies compare groups of affected individuals with healthy controls. These standard case-control designs cannot disentangle differences in brain function that are due to environmental risk factors from those that are due to genetic risk factors. A distinction between genetically and environmentally mediated neurobiological changes that underlie the development of OCD can

be accomplished by using a discordant/concordant monozygotic (MZ) twin design (de Geus et al., 2007; den Braber et al., 2010; den Braber et al., 2011; van 't Ent et al., 2009; Wolfensberger et al., 2008). Excluding post-twinning de novo mutations, all MZ twins begin life with identical genomes, so behavioral discordances are likely to arise from differential exposure to environmental influences. Consequently, differences in brain function between the high-risk twin and the low-risk co-twin from MZ discordant pairs reflect environmental effects on the brain, rather than effects of genetic variation. In contrast, to maximize detection of the effects of genetic risk factors for OCD, neuroimaging results can be compared between MZ twins who both score high on OC symptoms and MZ twins who both score very low on OC symptoms. These MZ concordant-high and low-scoring twins likely represent either high or low familial vulnerability for OCD. This familial vulnerability could be due to shared environmental or genetic vulnerability. However, shared family environment has not been found to contribute to OC behavior in adults (Clifford et al., 1984; Jonnal et al., 2000; van Grootheest et al., 2007). Therefore, a comparison between MZ concordant-high and MZ concordant-low pairs on OC symptoms is likely to reveal functional activation differences due to influences of genetic risk factors.

In previous studies by our group we applied the discordant/concordant twin design to investigate both white matter volume differences and planning-related activation changes in the brains of subjects with an environmental etiology or genetic predisposition for OC symptoms (den Braber et al., 2010; den Braber et al., 2011). The results from these studies suggest that brain regions affected in environmentally mediated OC symptoms are distinct from those affected in genetically mediated OC symptoms. Interestingly, observed white matter changes and planning related changes in brain activity converge on the CSTC loops. Neurobiological changes in OC symptoms induced by environmental risk factors involve the dorsal frontal CSTC loop (dorsolateral prefrontal region), whereas neurobiological changes in OC symptoms induced by genetic risk factors seem to involve regions implicated in the ventral frontal CSTC loop (inferior frontal region).

The present study aimed to examine the differential impact of non-shared environmental versus genetic influences for OC symptomatology on inhibitory control related functional brain activation. For this end we compared performance and fMRI data during the Stroop color-word and the Eriksen Flanker task, between twins scoring low and twins scoring high on OC symptoms from discordant MZ pairs and between concordant pairs where both twins scored either low or high on OC symptoms.

Methods

Participants

The twin pairs included in this study were recruited from the Netherlands Twin Register (NTR) (Boomsma et al., 2006). Surveys were sent to twin families including the Padua Inventory Abbreviated (PI-R-ABBR) (Cath et al., 2008; van Oppen et al., 1995). Completed PI-R-ABBR questionnaires were returned by 815 MZ twin pairs (222 male; 593 female). From this sample we selected twin pairs in the age range between 18 and 60 years who scored discordant, concordant-high or concordant-low for OCS. A twin pair was classified as discordant for OC symptoms if one twin scored OCS high (>16) and the co-twin scored OCS low (≤7). A twin pair was classified as concordant-high for OC symptoms if both twins scored ≥15, with at least one twin scoring ≥16. A twin pair was classified as concordant-low for OC symptoms if both twins scored ≤7. These PI-R-ABBR cut-off scores were derived from sensitivity and specificity measurements in an independent sample of OCD patients when compared to clinical controls (n=120; mean scores 20.7, SD 8.1; sensitivity 0.74 and specificity 0.72 at the best cut-off point of 16 (Cath et al., 2008)). For more details on sample selection we refer to den Braber et al. (2010). A final sample of 71 MZ twin pairs participated in this MRI study, including 20 discordant, 23 concordant-high and 28 concordant-low twin pairs **(table 5.1)**. The MRI protocol could not be completed by two subjects (metal artifact, panic attack). In the final sample (n=140), 2 twins with high OCS scores from the discordant group and 5 twins with high OCS scores from the concordant-high group met clinical diagnosis for OCD. Furthermore, 3 twins with high OCS scores and 1 twin with a low OCS score from the discordant group and 6 twins from the concordant-high group used selective serotonin reuptake inhibitors.

Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, comorbidity, type and severity of OC symptoms, tics, state-anger and state anxiety (for a detailed description of the administered diagnostic interviews and questionnaires, please refer to den Braber et al. (2010)). All twins were asked to collect mucosal cell samples for DNA extraction to test zygosity. The ethical review board of the VU University medical centre approved the study. All participants provided written informed consent.

Task paradigms

Stroop

During the Stroop color-word task, subjects had to report the ink color of written color-words. Dutch translations of the words “red”, “yellow”, “blue” and “green” were used that were written in any of these four colors. Word meaning and ink color could be either congruent (e.g., the word “green” written in green)

Table 5.1. MZ twin sample demographics

		Twin pairs							
		Discordant (environmental risk contrast)				Concordant (genetic risk contrast)			
		high (n=20 subjects)	low (n=20 subjects)			high (n=46 subjects)	low (n=56 subjects)		
Demographics	Female	14 pairs				17 pairs	20 pairs		
	Male	6 pairs				6 pairs	8 pairs		
	Age (years (SD))	35.60 (8.68)				36.00 (10.55)	37.50 (8.79)		
		mean (SD)	mean (SD)	t-value	*p*	mean (SD)	mean (SD)	t-value	*p*
Obsessive-compulsive symptoms	PI-R-ABBR (0-48)	20.07 (5.03)	4.73 (1.84)	14.51	<0.001	20.42 (4.56)	4.18 (2.19)	22.31	<0.001
	Y-BOCS severity (0-40)	5.45 (5.62)	1.45 (2.19)	3.64	0.001	7.54 (5.83)	0.95 (2.13)	6.95	<0.001
Comorbidity	MINI :								
	Depression (n)	2	0			0	0		
	Panic disorder (n)	1	0			0	0		
	Agoraphobia (n)	2	0			0	0		
	Social disorder (n)	1	0			2	0		
	Post-traumatic stress disorder (n)	1	0			0	0		
	Generalized anxiety disorder (n)	3	0			7	0		
	Tic (0-8)	0.40 (0.75)	0.20 (0.41)	1.25	0.214	0.30 (0.66)	0.09 (0.29)	2.03	0.046
	BDI (0-39)	4.65 (7.50)	3.05 (2.80)	1.73	0.089	3.50 (3.17)	1.38 (2.18)	2.47	0.016
	STAI (0-60)	13.85 (8.54)	12.25 (6.13)	0.83	0.409	13.37 (7.39)	8.55 (7.36)	2.91	0.005
	STAS (0-30)	0.20 (0.70)	0.00 (0.00)	0.91	0.365	0.46 (2.09)	0.11 (0.49)	1.09	0.282

Twin pairs: number of female and male twin pairs; Age: age at time of MRI examination (in years). PI-R-ABBR: mean Padua Inventory Abbreviated scores (SD); Y-BOCS severity: mean Yale–Brown Obsessive–Compulsive Scale severity scores (SD). MINI (depression, panic disorder, agoraphobia, social disorder, post-traumatic stress disorder, generalized anxiety disorder): number of subjects with current comorbid disorder (measured using the Mini International Neuropsychiatric Interview); Tic: mean tic scores (SD) at time of MRI; BDI: mean Beck Depression Inventory scores (SD) at time of MRI; STAI: mean State Trait Anxiety Inventory scores (SD) at time of MRI; STAS: mean State Trait Anger Scale scores (SD) at time of MRI.

or incongruent (e.g., the word "red" written in blue). The correct answer had to be indicated by pressing buttons: left middle finger for ink color yellow, left forefinger for green, right forefinger for red and right middle finger for blue. The task was administered in 18 blocks of similar stimulus types. Of these, 3 blocks contained congruent and 3 blocks contained incongruent color-word stimuli. In each individual block 16 words were presented for 2 seconds separated by small intervals of 200 milliseconds. The other 12 blocks consisted of words with an emotional content, which were not used here (for a full description of the task we refer to van den Heuvel et al. (2005b). The subjects were asked to respond to the stimuli as fast and accurate as possible. The onset of each individual stimulus together with the subject's response was recorded, such that the data could be analyzed in an event-related manner. Total task duration was ±10 minutes.

Flanker

In the flanker task subjects had to indicate, as quickly as possible, the direction of a central target arrow (i.e., "<" left hand press; ">" right hand press) which was surrounded by four task irrelevant flankers of the same size and shape. The direction of the flanker arrows could be either congruent ('< < < < <' or '> > > > >') or incongruent ('< < > < <' or '> > < > >') to the direction of the central target arrow. Flankers and targets were displayed simultaneously. The task was administered in an event-related design. During the task 120 congruent and 120 incongruent trials were presented in random order. Stimuli were shown for 200 ms and the interstimulus interval consisted of a period of gray screen after each stimulus (randomized between 600 and 1600 ms) and a subsequent fixation cross for 1000 ms before the next stimulus. Total task duration was ±10 minutes.

For both the Stroop and Flanker task, stimuli were projected on a screen at the end of the MRI scanner table, viewed by the participants through a mirror. Two magnet compatible response boxes were used to record the subject's performance. Before the experiment, the subjects practiced a number of trials on a computer outside the scanner and again inside the scanner, prior to the actual start of the session.

Image acquisition

The MRI session consisted of a structural part of about 6 minutes and a functional part of approximately 20 minutes (Stroop ± 10 minutes, Flanker ± 10 minutes). The twins remained inside the scanner and were asked to minimize head movement during and between consecutive runs. To reduce motion artifacts, the participant's head was immobilized using foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. The anatomical scan consisted of

182 coronal slices with a 3D gradient-echo T1-weighted sequence (flip angle 8°; Repetition Time, TR = 9.69 ms; Echo Time, TE = 4.60 ms, matrix, 256x256 pixels; voxel size, 1.00x1.00x1.20 mm). For fMRI, an echo planar imaging (EPI) sequence (flip angle 80°; TR = 2300 ms; TE = 30 ms, matrix, 96x96 pixels; field of view 220x220 mm) was used, covering the whole brain (40 axial slices; 2.29 mm x 2.29 mm in-plane resolution; 3.0 mm slice thickness). For the Stroop task a total of 260 and for the Flanker a total of 250 EPI volumes were scanned per subject.

Data analysis

MRI data were analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK). EPI scans were slice time corrected, realigned and normalized to the standard MNI (Montreal Neurological Institute) brain of SPM. Subsequently, data were resliced to 3 mm x 3 mm x 3 mm voxels and spatially smoothed using an 8 mm isotropic Gaussian kernel. After high-pass filtering (cut-off 128 seconds), functional scans were analyzed in the context of the general linear model using delta functions convolved with a canonical hemodynamic response function. Error trials and head-movement parameters were modeled as regressors of no interest. Post hoc analysis of subject motion during the scans, based on the functional scan realignment parameters, indicated that the twins with high OC symptom scores did not exhibit significantly larger head-movements compared to the twins with low OC symptom scores. For each subject and task, contrast images were computed for simple main effects of congruent and incongruent trials, as well as the effect of response interference (incongruent minus congruent trials). For all contrasts, only trials with correct reactions were included.

Statistical tests

Survey- and interview-based variables and task performance data were investigated using a mixed-model analysis of variance (ANOVA; Mixed Models Linear menu item in SPSS; SPSS, Chicago, Illinois) with twin pair type (discordant versus concordant) and OC symptom score (high versus low) as two fixed factors and family as a random factor to account for within-twin pair dependence. Statistical results with regard to questionnaire and task performance data were considered significant at $p < 0.05$, Bonferroni corrected.

First-level functional MRI contrast estimates for 'Stroop interference' and 'Flanker interference' were entered into second-level analyses available in SPM5. Differences in contrast estimates between OCS high and OCS low-scoring twins from discordant pairs were investigated by paired sample t-tests. Differences in contrast estimates between concordant OCS high and concordant OCS low twin pairs were assessed using an ANOVA group comparison. To account for within-twin pair correlations of fMRI signals, first-level results of the twin and

Table 5.2. Response interference effects on task performance

Measure	Sample	Stroop incongruent-congruent				Flanker incongruent-congruent			
		OCS High	OCS Low	F	*p*	OCS High	OCS Low	F	*p*
Response latencies	Discordant	179.29 (110.31)	145.60 (117.80)	1.69	0.197	65.17 (23.59)	68.00 (28.15)	0.35	0.559
	Concordant	170.28 (108.61)	156.56 (122.47)	0.32	0.570	63.06 (21.10)	67.14 (18.79)	0.61	0.438
Response accuracy	Discordant	-0.14 (0.08)	-0.13 (0.09)	0.40	0.525	-0.04 (0.05)	-0.07 (0.07)	4.80	0.032*
	Concordant	-0.18 (0.17)	-0.13 (0.12)	3.03	0.086	-0.06 (0.04)	-0.05 (0.05)	0.35	0.556

Effects of information conflict on Stroop task (left) and Flanker task (right) behavior, measured by computing differences in response latencies (top rows) and response accuracy (bottom rows) on incongruent relative to congruent stimulus trials. Columns "F" and "p" indicate results from statistical tests on OCS-related differences between selected discordant and concordant twin samples. Indicates statistically significant difference.*

co-twin of each concordant pair were entered as repeated measures. For main task effects of selected contrasts we set an individual voxel threshold of $p < 0.05$, corrected for multiple comparisons (false discovery rate: FDR), with a minimal cluster extent of 100 voxels. Group differences, masked with the appropriate main task effect (mask thresholded at $p < 0.05$, uncorrected), are reported at an uncorrected individual voxel threshold of $p < 0.005$, with a minimal cluster extent of 10 voxels.

Results

Questionnaire and interview data

As expected, OCS high compared to low-scoring twins in both the discordant and concordant groups showed significant higher scores on the PI-R-ABBR, and Y-BOCS severity scale (see **table 5.1**). In addition, high-scoring twins were more often diagnosed with current co-morbid disorders, which were absent in the low-scoring twins.

Task performance

Across all individuals, reaction times for both the Stroop and the Flanker task were significantly delayed after incongruent compared to congruent stimuli [Stroop: incongruent 988.92 ± 167.44 ms vs. congruent 826.27 ± 148.84 ms, $F(1,139) = 279.82$, $p<0.001$; Flanker: incongruent 590.44 ± 191.19 ms vs. congruent 524.77 ± 192.82 ms, $F(1,139) = 1294.77$, $p<0.001$]. In addition, for both the Stroop and Flanker task, percentages of trials with correct reactions were significantly reduced after incongruent stimuli [Stroop: incongruent 80.4 ± 15.3 vs. congruent 95.0 ± 6.4, $F(1,139) = 171.02$, $p<0.001$; Flanker: incongruent 92.5 ± 6.9 vs. congruent 97.9 ± 3.8, $F(1,139) = 173.98$, $p<0.001$].

In **table 5.2** response interference effects, quantified by computing differences in response latency and response accuracy between incongruent and congruent stimulus trials, are displayed separately for the discordant and concordant twin sample. Response latencies did not differ significantly between OCS high and low-scoring twins from both the discordant and concordant sample. For response accuracy, a smaller effect of response interference was found in the discordant-high-risk relative to their discordant-low-risk co-twins during the Flanker task.

Functional imaging

Main effects

Figure 5.1 and **table 5.3** show brain areas with significant fMRI-BOLD activations, across all subjects, during Stroop interference **(figure 5.1, top; table 5.3, left)** and Flanker interference **(figure 5.1, bottom; table 5.3, right)**. In both the Stroop and

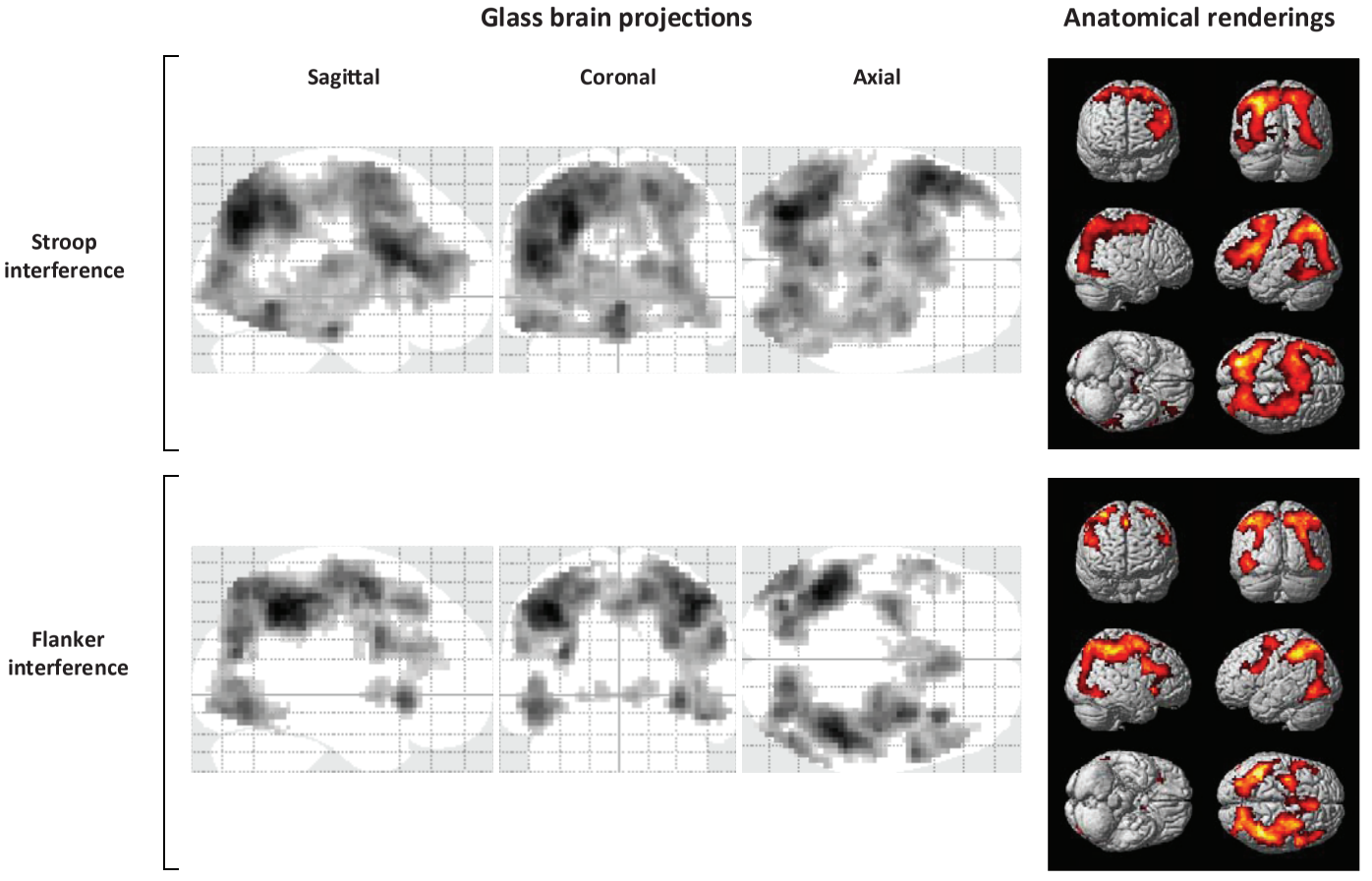

Figure 5.1. *Main effects of fMRI-BOLD activation, across all participating twins. Glass brain overviews depict brain activity patterns for 'Stroop interference' (top) and 'Flanker interference' (bottom). Anatomical renderings on the right illustrate locations of functional brain activation for 'Stroop interference' (top) and 'Flanker interference' (bottom), across all participating twins.*

Table 5.3. Brain activity for Stroop and Flanker interference contrasts across both the discordant and concordant sample

Anatomical location	Side	BA	Stroop interference (incongruent-congruent)				Flanker interference (incongruent-congruent)			
			MNI coordinates			T value	MNI coordinates			T value
			x	y	z		x	y	z	
Frontal Cortex	Left	4	-21	-24	66	2.70	-36	-24	60	3.29
	Right	4	36	-21	57	5.25	42	-15	60	6.14
	Left	6	-33	0	63	6.94	-30	-6	63	5.96
	Right	6	36	0	48	6.13	33	-3	60	6.95
	Left	8	-6	15	48	6.47	-6	18	51	3.26
	Right	8	6	18	51	3.95	6	18	51	5.61
	Left	9	-42	9	27	8.46	-54	15	27	4.83
	Right	9	--	--	--	--	54	6	30	6.15
	Left	10	-39	45	18	6.44	--	--	--	--
	Left	45	-48	18	21	9.13	-48	24	24	2.62
	Right	45	--	--	--	--	48	24	24	4.86
	Left	46	-45	33	15	7.65	-45	36	21	2.67
	Right	46	--	--	--	--	48	36	27	4.20
	Left	47	-30	30	-3	5.13	--	--	--	--
	Right	47	--	--	--	--	36	21	-3	6.46
Parietal Cortex	Left	3	--	--	--	--	-57	-24	42	3.33
	Right	3	--	--	--	--	57	-24	42	2.74
	Left	7	-27	-69	42	10.13	-36	-60	54	6.40
	Right	7	18	-69	60	7.32	15	-72	54	6.33
	Left	40	-36	-51	51	8.59	-39	-48	45	8.47
	Right	40	36	-51	51	3.41	42	-42	48	7.99
Occipital Cortex	Left	19	-48	-57	-12	6.30	-48	-72	6	4.93
	Right	19	33	-84	12	6.49	45	-81	-9	4.85
Temporal Cortex	Left	37	-54	-54	-15	6.29	-48	-45	-18	3.03
	Right	37	51	-54	-12	4.46	60	-54	-12	4.08
ACC	Left	32	-9	24	36	3.52	-3	24	42	3.10
	Right	32	12	24	36	4.35	3	24	39	2.62
Thalamus	Left	--	-18	-21	18	5.46	--	--	--	--
	Right	--	18	-21	18	5.75	--	--	--	--
Caudate	Left	--	-15	0	21	2.90	-6	6	0	3.42
	Right	--	15	0	21	2.29	9	6	0	4.40
Putamen	Left	--	-18	0	9	5.05	-21	3	-3	3.48
	Right	--	--	--	--	--	18	9	3	3.67

Anatomical locations of significant clusters for main effects of fMRI-BOLD activation during Stroop interference (incongruent-congruent trials) (left) and Flanker interference (incongruent-congruent trials) (right). MNI coordinates and T-values are listed for the voxels with the largest effect size. ACC = anterior cingulate cortex.

Table 5.4. Environmental risk: Brain activation differences between OCS high and low MZ twins of the discordant sample

	Stroop interference (incongruent - congruent)					Flanker interference (incongruent - congruent)				
Test	Anatomical location	MNI coordinates			Z score	Anatomical location	MNI coordinates			Z score
		x	y	z			x	y	z	
high>low	Right dorsolateral prefrontal gyrus	51	27	27	2.96	Left middle temporal gyrus	-48	6	-27	3.38
						Left cingulate gyrus	-9	-3	48	3.09
						Right cerebellum	15	-48	-27	3.18
high<low	Left precentral gyrus	-27	-15	54	3.53	*no significant clusters*				

Differences in brain activation to trials with information conflict in discordant-high compared to low-risk twins. Test: test for significant increases (high>low) or decreases (high<low) in OCS high relative to OCS low-scoring twins. MNI coordinates (mm): location of voxel with largest effect size; Z score: z-value of voxel with largest effect size.

Table 5.5. Genetic risk: Brain activation differences between OCS high and low MZ twin pairs of the concordant sample

	Stroop interference (incongruent - congruent)					Flanker interference (incongruent - congruent)				
Test	Anatomical location	MNI coordinates			Z score	Anatomical location	MNI coordinates			Z score
		x	y	z			x	y	z	
high>low	Left dorsolateral prefrontal gyrus	-36	30	15	3.73	Right thalamus	9	-21	-3	3.14
	Left middle frontal gyrus	-33	0	45	3.68					
	Left precuneus	-33	-66	36	3.39					
	Right angular gyrus	30	-66	36	2.86					
	Left inferior parietal gyrus	-45	-36	42	3.32					
	Right inferior parietal gyrus	39	-39	57	3.15					
high<low	*no significant clusters*					Left inferior parietal gyrus	-54	-45	51	3.30

Differences in brain activation to trials with information conflict in concordant-high compared to low-risk twins.

Flanker task response interference was associated with enhanced activation of bilateral occipital, parietal, temporal, caudate/putamen as well as several prefrontal lobe regions including ACC, DLPFC, premotor and inferior frontal cortices. For the Stroop task, increased activation was also noted in left and right thalamus, whereas for the Flanker task an additional cluster was observed in left and right postcentral gyrus.

Environmental risk: OCS high versus low-scoring twins from discordant pairs

Paired comparisons between the high-risk twin and the low-risk co-twin from discordant pairs revealed significant clusters of increased activation to response interference, but located in different brain regions for the Stroop and Flanker task. For the Stroop task, a single cluster of increased activation was found in the right DLPFC (**table 5.4** and **figure 5.2**, label A). For the Flanker task increased activation was found in left middle temporal gyrus, left cingulate gyrus and right cerebellum (**table 5.4** and **figure 5.2**, labels B, C and D, respectively).

High-risk discordant twins also showed an area of reduced activation during response interference, exclusively in the Stroop task, in the left precentral gyrus (**table 5.4** and **figure 5.2**, label E).

Genetic risk: concordant-high versus concordant-low-scoring twin pairs

Table 5.5 and **figure 5.2 (right)** show clusters of OCS-related differences for brain activation to response interference between the concordant-high and concordant-low twin pairs. Concordant-high compared to concordant-low twin pairs showed several significant clusters of increased activation to response interference, again in different brain regions for the Stroop and Flanker task. For the Stroop task, high-risk twins from concordant pairs showed relatively increased activity in regions of the left DLPFC, left middle frontal gyrus, left precuneus, right angular gyrus and bilateral inferior parietal gyrus (**figure 5.2**, labels F, G, H, I, J and K, respectively). For the Flanker task, there was a single cluster of increased activation in high-risk twins in the right thalamus (**figure 5.2**, label L).

High-risk concordant twins also showed an area of reduced activation during response interference, exclusively in the Flanker task, in the left inferior parietal gyrus (**figure 5.2**, label M).

There were 10 subjects (3 discordant-high, 7 concordant-high) with current comorbid disorders. Removing these subjects from the analyses did not affect the pattern of results.

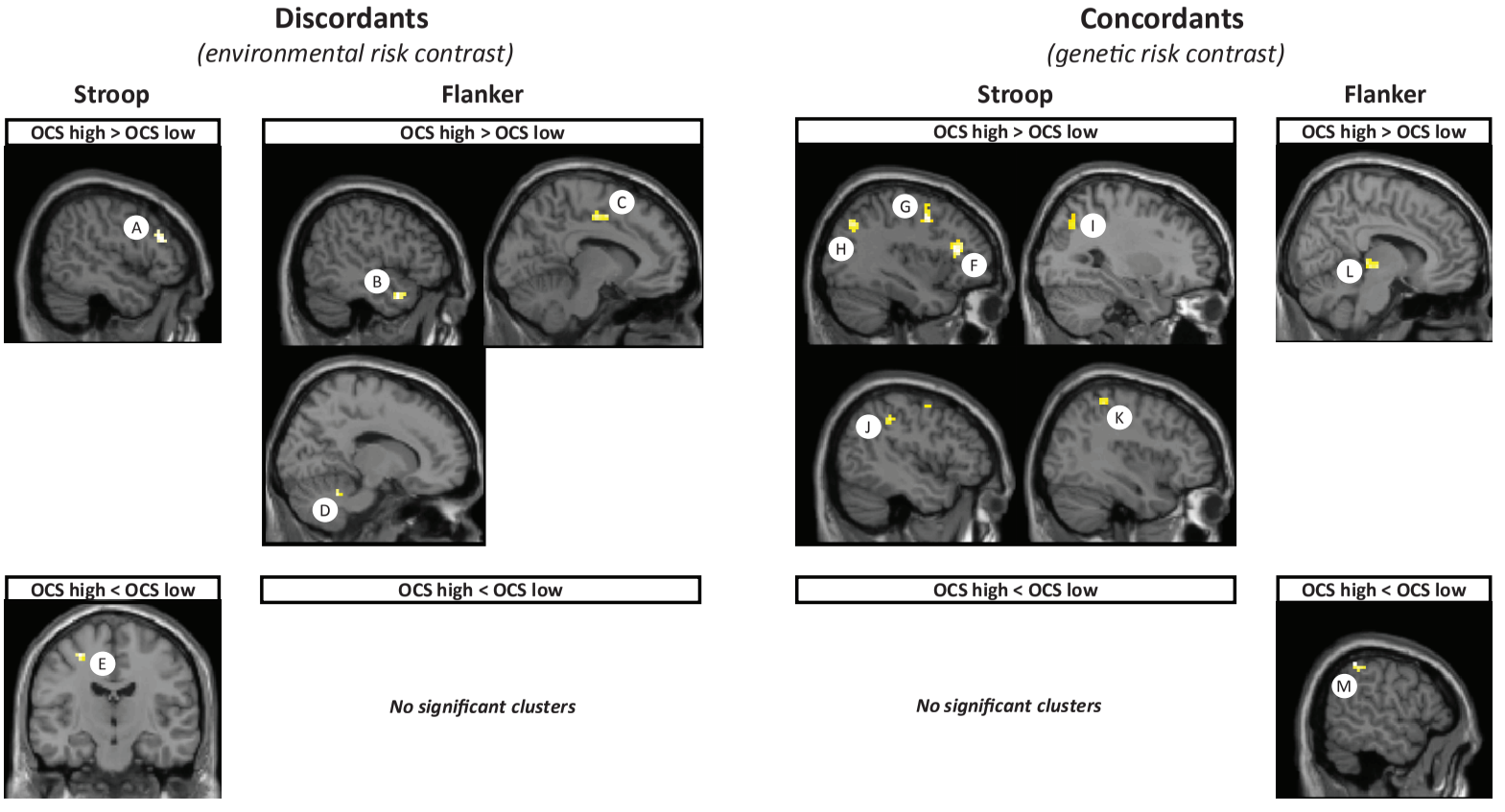

Figure 5.2. *Most significant clusters, overlaid on MR sections, from statistical evaluations of OCS-related differences in brain activation to Stroop and Flanker trials with response interference. Left panels: hyperactivations (top row) and hypoactivations (bottom row) for discordant-high compared to low-risk twins (environmental contrast). Right panels: hyperactivations (top row) and hypoactivations (bottom row) for concordant-high compared to low-risk twins (genetic contrast). A = Right dorsolateral prefrontal gyrus; B = Left middle temporal gyrus; C = Left cingulate gyrus; D = Right cerebellum; E = Left precentral gyrus; F = Left dorsolateral prefrontal gyrus; G = Left middle frontal gyrus; H = Left precuneus; I = Right angular gyrus; J = Left inferior parietal gyrus; K = Right inferior parietal gyrus; L = Right thalamus; M = Left inferior parietal gyrus.*

Discussion

Task performance and brain activation during Stroop color-word and Flanker interference were compared within MZ twin pairs discordant for OC symptoms and between groups of pairs scoring very low or very high on OC symptoms, in order to examine the differential impact of non-shared environmental versus genetic and shared environmental risk factors for OC symptomatology on inhibitory control related functional brain activation. Shared family environment has never been reported to influence OC behavior in adult twin studies (Clifford et al., 1984; Jonnal et al., 2000; van Grootheest et al., 2007). Therefore, familial risk factors for this trait were taken to translate mainly to genetic risk.

Analysis of task-related behavior indicated classical effects of response interference on response latencies and response accuracy for both the Stroop and Flanker task. In line with previous studies, task performances of twins high and low on OC symptoms were comparable, with the exception of marginally reduced interference in high compared to low-scoring twins of the discordant sample during the Flanker task, which was mainly due to the fact that high-scoring twins made less errors during incongruent trials than their low-scoring co-twins.

The fMRI main effects of our study indicated that highly similar brain processes were active during Stroop and Flanker task performance. Brain areas involved include bilateral occipital, parietal, temporal, caudate/putamen as well as several frontal lobe regions including ACC, DLPFC, premotor and inferior frontal cortices. These results highly overlap with findings from previous studies that investigated response interference using these paradigms (Kerns et al., 2004; van 't Ent et al., 2009; van den Heuvel et al., 2005b). OC symptom status clearly affected this pattern of brain activation, with increased conflict related DLPFC activity in high-risk compared to low-risk twins from both the discordant and concordant sample during the Stroop task. It has been hypothesized that evaluative and control functions are represented by a dorsolateral prefrontal- anterior cingulate cortical circuit, where the ACC is involved in detecting the occurrence of conflict and the DLPFC in performance adjustments (Melcher et al., 2008). A study that examined predictions of this conflict hypothesis, indeed showed more ACC activity in high-conflict correct trials and error trials, which was associated with adjustments in behavior on the next trial that reflect improved control (Kerns et al., 2004). In addition, this study showed that trials exhibiting the largest adjustments in behavior following conflict were associated with increased activity in DLPFC. Furthermore, previous studies provided evidence for hyperactivity in this dorsolateral prefrontal – anterior cingulate circuit during cognitive control in OCD patients compared to healthy controls (Maltby et al., 2005; Schlosser et al., 2010) and showed that OCD patients exhibited enhanced dorsal ACC to DLPFC

connectivity, in agreement with the hypothesis that OCD is related to an overactive control system (Schlosser et al., 2010). During Stroop interference there was no evidence for increased conflict related activity in the ACC in our study, but increased conflict related activity in the ACC was observed in OCS high compared to low-scoring twins from the discordant sample during Flanker interference. In addition, this increase in ACC activity during response interference was accompanied by a better performance of the OC symptom high-scoring twins compared to their low-scoring co-twins.

While the ACC was not significantly more activated in high versus low-scoring twins during Stroop interference, the DLPFC, involved in performance adjustments, showed an increased conflict related activity in the OCS high-scoring twins from discordant OCS pairs as well as twins from concordant OCS pairs. This finding suggests that although the degree of color-word conflict detection was the same, OCS high-scoring subjects have a higher propensity to adjust their behavior following conflict compared to low-scoring subjects. This supports the assumption of an overactive control network in OC symptomatology. As the DLPFC was affected in both the high-scoring twins from discordant and concordant pairs, it seems to act as a final common pathway for both genetic and environmental risk factors for OC symptomatology. Deviant DLPFC activity during inhibitory control may be closely correlated with the actual behavioral deficits of the disorder.

Response conflict related brain alterations in OCS high compared to low-scoring twins were also observed in several other regions of the brain. Regions affected in the discordant group were different from those observed in the concordant group. Brain regions showing different activation patterns in twins with high OC symptoms scores compared with those with low OC symptoms scores that were present in only the discordant group and, therefore, are likely related to environmental risk factors for OC symptomatology include the precentral gyrus (Stroop), middle temporal gyrus and cerebellum (Flanker). Brain regions showing different activation patterns in twins with high OC symptoms scores compared with those with low OC symptoms scores that were present in only the concordant group and, therefore, are likely related to genetic risk factors for OC symptomatology, include the middle frontal gyrus, precuneus, angular gyrus, parietal gyrus (Stroop) and thalamus (Flanker).

An environmentally mediated increase in temporal activity and genetically mediated increase in parietal activity has also been observed in a previous study by our group that used the discordant/concordant twin design to study OC symptom related brain alterations during a cognitive planning paradigm (den Braber et al., 2010). OCD related alterations in temporal and parietal cortices have been found by others as well, and have therefore been included in an

extended model for OCD (Menzies et al., 2008a). An altered function of these regions might, through their functional connections with the ventral and dorsal PFC, lead to an imbalance of the direct and indirect pathway of the CSTC networks, which could subsequently induce OC behavior. Abnormalities in thalamic volume and function in OCD have been extensively reported (Menzies et al., 2008a). The thalamus is implicated in the CSTC model of OCD. It is the key region in modulating subcortical input to the frontal cortex, stimulates output of frontal brain regions, and plays a crucial role in the processing of sensory inputs thereby mediating both behaviors, emotion and cognition (Sherman and Guillery, 2002). Therefore, disturbances within this structure are likely to be coupled to the cognitive and behavioral deficits seen in OCD patients. Further research into the association between these structures and the control network is warranted.

A total of ten subjects included in this study had comorbid diagnoses, which could have confounded our result. Although excluding these subjects did not change the patterns of results, comorbid traits that do not meet threshold for clinical diagnoses in the remaining subjects with high levels of OC symptoms may have influenced the results. This is a limitation of the design used, as the selection for high levels of OC symptoms will by necessity lead to co-selection for comorbid traits.

In summary, the present study demonstrates decreases as well as increases in brain activation during the inhibition of distracting information in OCS high compared to low-scoring subjects. A robust increase in DLPFC activity during response interference was observed in both the high-scoring twins of the discordant sample as well as the high-scoring twins of the concordant sample, marking this structure as a possible key region for disturbances in inhibitory control in OCD.

6 White matter differences in monozygotic twins discordant or concordant for obsessive-compulsive symptoms: A combined Diffusion Tensor Imaging/Voxel-Based Morphometry study

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Abstract

Background: Neuroimaging studies of obsessive-compulsive disorder (OCD) patients point to deficits in cortico-striato-thalamo-cortical circuits that might include changes in white matter. The contribution of environmental and genetic factors to the various OCD-related changes in brain structures remains to be established.
Methods: White matter structures were analyzed in 140 subjects with both diffusion tensor imaging and voxel-based morphometry. We studied 20 monozygotic twin pairs discordant for obsessive-compulsive symptoms (OCS) to detect the effects of environmental risk factors for obsessive-compulsive (OC) symptomatology. Furthermore, we compared 28 monozygotic twin pairs concordant for low OCS scores with 23 twin pairs concordant for high OCS scores to detect the effects of genetic risk factors for OC symptomatology.
Results: Discordant pair analysis showed that the environmental risk was associated with an increase in dorsolateral-prefrontal white matter. Analysis of concordant pairs showed that the genetic risk was associated with a decrease in inferior frontal white matter. Various white matter tracts showed opposite effects of environmental and genetic risk factors (e.g., right medial frontal, left parietal and right middle temporal) illustrating the need for designs that separate these classes of risk factors.
Conclusions: Different white matter regions were affected by environmental and genetic risk factors for OC symptomatology, but both classes of risk factors might, in aggregate, create an imbalance between the indirect loop of the cortico-striato-thalamo-cortical network (to the dorsolateral-prefrontal region) –important for inhibition and switching between behaviors– and the direct loop (to the inferior frontal region), that contributes to the initiation and continuation of behaviors.

Introduction

Obsessive-compulsive symptoms (OCS) have been defined as recurrent, persistent, and intrusive anxiety provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions) performed to reduce anxiety and/or distress caused by the obsessions (American Psychiatric Association, 1994). When a person has these obsessions and/or performs compulsions for more than one hour a day and these thoughts and rituals significantly interfere with his/her daily life routines, the person fulfills the criteria for obsessive-compulsive disorder (OCD). OCD is generally assessed with structured clinical interviews (e.g., the Structured Clinical Interview for DSM Disorders [SCID]) (American Psychiatric Association, 1994). Additionally, questionnaires such as the Padua Inventory (Sanavio, 1988) and quantitative versions of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)

(Goodman et al., 1989a; Goodman et al., 1989b) might be used to rate OCS severity. The life-time prevalence of OCD is 0.5-2% (American Psychiatric Association, 1994; Grabe et al., 2000), but obsessions are much more prevalent in the general population – as high as 72% (Rachman and de Silva, 1978), and the prevalence of OCS reaches 20% (Fullana et al., 2009).

Over the last two decades, neuroimaging studies have indicated several neurobiological changes underlying the psychological and behavioral deficits of OCD. Structural magnetic resonance imaging (MRI) has revealed regional volume differences in the ventral prefrontal cortex, dorsolateral prefrontal cortex (DLPFC), basal ganglia, anterior cingulate cortex, parietal cortex and thalamus (Menzies et al., 2007; Pujol et al., 2004; Radua and Mataix-Cols, 2009; Radua et al., 2010; Rotge et al., 2009; Valente Jr. et al., 2005; van den Heuvel et al., 2009). Findings from functional neuroimaging studies are largely consistent with structural MRI findings and show altered regional activation in the aforementioned brain structures during performance of cognitive tasks and after symptom provocation (for a review, see Menzies et al. (2008a)). Together, these findings contributed to the widely accepted neuroanatomical model of OCD involving the direct and indirect cortico-striato-thalamo-cortical (CSTC) loops (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). The direct loop functions as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors. The indirect loop functions as a negative feedback loop important for inhibiting and switching between behaviors (Mataix-Cols and van den Heuvel, 2006). It has been hypothesized that an imbalance between these loops, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate obsessive-compulsive (OC) symptomatology (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). Although a disturbance in these CSTC loops seems to be the neurological basis for OCD, several imaging studies suggest the involvement of other brain regions in OCD as well. For example, Menzies et al. (2008) recently proposed an extended model that includes various brain areas (e.g., anterior cingulate cortex, amygdala, parietal areas) that are functionally connected to the ventral and dorsal CSTC loops.

So far, anatomical models of OCD have been mainly based on structural and functional MRI analyses that focused on gray matter differences in OCD patients compared with control subjects. More recent studies suggest an additional role for white matter abnormalities in the etiology of OCD (Cannistraro et al., 2006; Garibotto et al., 2010; Ha et al., 2009; Menzies et al., 2008b; Nakamae et al., 2008; Saito et al., 2008; Szeszko et al., 2005; Yoo et al., 2007; Nakamae et al., 2011), possibly related to variation in genes involved in oligodendrocyte development (Stewart et al., 2007b). Diffusion tensor imaging (DTI) can be used to study white matter integrity, for instance in tracts that interconnect the brain regions of

the CSTC loops. DTI provides a measure of diffusion of water molecules within tissues, permitting the investigation of brain tissue microstructure. In structures with a highly coherent directional organization, (e.g., white matter tracts in the brain), the dominant direction of diffusion is parallel to the fiber direction, so that diffusion becomes more anisotropic (Basser and Pierpaoli, 1996; Beaulieu, 2002; Le Bihan et al., 2001; Mori and Zhang, 2006). Fractional anisotropy (FA), a value that can be derived from diffusion tensor images, describes the degree of anisotropy within a voxel. Reduced FA might be interpreted as a reduced density of fibers, less directional coherence of fibers, or a reduced degree of myelination of fibers, all suggesting damaged or disorganized or under-developed white matter (Basser and Pierpaoli, 1996; Beaulieu, 2002; Le Bihan et al., 2001; Mori and Zhang, 2006). To further investigate the nature of white matter alterations, T1-weighted scans can be analyzed with voxel-based morphometry (VBM). VBM, performed on white matter segmentations provides information on regional white matter volume differences (Ashburner and Friston, 2000; Ashburner and Friston, 2001). If an increase in FA is accompanied by an increase in white matter volume, the higher white matter integrity might indicate fibers that are more dense or more myelinated.

A few studies that used DTI to measure white matter abnormalities in OCD patients compared with healthy control subjects (Cannistraro et al., 2006; Garibotto et al., 2010; Ha et al., 2009; Menzies et al., 2008b; Nakamae et al., 2008; Saito et al., 2008; Szeszko et al., 2005; Yoo et al., 2007; Nakamae et al., 2011) have reported white matter differences near the caudate nucleus and thalamus (Cannistraro et al., 2006; Yoo et al., 2007), whereas others found differences predominantly in medial frontal and parietal regions (Ha et al., 2009; Menzies et al., 2008b; Szeszko et al., 2005). In addition, directly conflicting results were found, with reports of lower (Garibotto et al., 2010; Ha et al., 2009; Szeszko et al., 2005) and higher (Cannistraro et al., 2006) FA in the left cingulate, or no differences between patients and control subjects in this region at all (Menzies et al., 2008b). Such inconsistencies are usually explained as being due to methodological differences between studies, such as heterogeneity of patient groups and differences in sample size, scanning modalities/parameters, and analysis methods. However, there might also be 'true' variability in the underlying neurobiology of OCD. That is, different white matter tract abnormalities might lead to comparable behavioral changes, because they occur in parts of the same brain network that regulates anxiety and safety behaviors. Such heterogeneity in affected white matter fibers might, in turn, reflect the differential influence of environmental and genetic risk factors for OC symptomatology that might impact different parts of the brain (den Braber et al., 2010).

Most clinical DTI studies employ standard case-control designs, comparing healthy control subjects with a group of affected individuals. These studies, however, cannot disentangle whether differences in brain white matter integrity are due to environmental versus genetic risk factors. One design that makes a distinction between environmentally and genetically mediated neurobiological differences that underlie the development of behavioral traits such as OCD is the discordant/concordant monozygotic (MZ) twin design (de Geus et al., 2007; den Braber et al., 2010; van 't Ent et al., 2009; Wolfensberger et al., 2008). Nearly all MZ twins begin life with identical genomes, so behavioral discordances are likely to arise from differential exposure to environmental influences that can differentially modify gene expression in subjects with identical genotypes (Heijmans et al., 2009). Consequently, differences in central nervous system white matter between the high-risk twin and the low-risk co-twin reflect environmental effects on the brain, rather than effects of genetic variation. To detect the effects of genetic risk factors, neuroimaging results can be compared between MZ twins who both score high for measures of OCS and MZ twins who both score very low for OCS. These MZ concordant-high- and low-scoring twins are likely to come from families with either high or low vulnerability for OCD. Genetic epidemiological studies that compare MZ and dizygotic twin resemblance in OCS have already shown that the shared family environment does not influence these symptoms and that the familial vulnerability for this trait translates entirely to genetic vulnerability (Clifford et al., 1984; Jonnal et al., 2000; van Grootheest et al., 2007). Therefore, a comparison between MZ twins who both score high (concordant-high) for measures of OCS and MZ twins who both score low (concordant-low) for OCS can reveal white matter differences due to influences of genetic risk factors.

Our study aimed to examine the differential impact of non-shared environmental versus genetic influences on white matter regions in subjects at high risk for OCD. We compared DTI-derived FA maps between twins scoring low and twins scoring high on OCS from discordant MZ pairs and between concordant pairs where both twins scored either low or high on OCS. To confirm that an increase (decrease) in FA was accompanied by an increase (decrease) in white matter volume, we additionally examined white matter volumes in these specific regions with VBM.

Methods and materials

Participants

The twin pairs included in this study were recruited from the Netherlands Twin Register (Boomsma et al., 2006). Surveys were sent to twin families including the Padua Inventory Abbreviated (PI-R-ABBR) (Cath et al., 2008; van Oppen et al., 1995). Completed PI-R-ABBR questionnaires were returned by 815 MZ twin pairs

Table 6.1. Twin sample demographics

		Twin pairs							
		Discordant (environmental risk measure)				Concordant (genetic risk measure)			
		high (n=20)	low (n=20)			high (n=46)	low (n=56)		
Demographic data	Female	14				17	20		
	Male	6				6	8		
	Age (years (SD))	35.60 (8.68)				36.00 (10.55)	37.50 (8.79)		
		mean (±SD)	mean (±SD)	t-value	*p*	mean (±SD)	mean (±SD)	t-value	*p*
Obsessive-compulsive symptoms	PI-R-ABBR (0-48)	20.07 (5.03)	4.73 (1.84)	14.51	<0.001	20.42 (4.56)	4.18 (2.19)	22.31	<0.001
	Y-BOCS severity lifetime (0-40)	7.70 (5.69)	6.70 (8.18)	0.64	.527	10.41 (7.15)	3.18 (4.54)	5.13	<0.001
	Y-BOCS severity current (0-40)	5.45 (5.62)	1.45 (2.19)	3.64	0.001	7.54 (5.83)	0.95 (2.13)	6.95	<0.001
Comorbidity	MINI :								
	Depression (n)	2	0			0	0		
	Panic disorder (n)	1	0			0	0		
	Agoraphobia (n)	2	0			0	0		
	Social disorder (n)	1	0			2	0		
	Post-traumatic stress disorder (n)	1	0			0	0		
	Generalized anxiety disorder (n)	3	0			7	0		
	Tic (0-8)	0.40 (0.75)	0.20 (0.41)	1.25	0.214	0.30 (0.66)	0.09 (0.29)	2.03	0.046
	BDI (0-39)	4.65 (7.50)	3.05 (2.80)	1.73	0.089	3.50 (3.17)	1.38 (2.18)	2.47	0.016
	STAI (0-60)	13.85 (8.54)	12.25 (6.13)	0.83	0.409	13.37 (7.39)	8.55 (7.36)	2.91	0.005
	STAS (0-30)	0.20 (0.70)	0.00 (0.00)	0.91	0.365	0.46 (2.09)	0.11 (0.49)	1.09	0.282

Values are given as (n), unless otherwise indicated. Twin pairs: number of female and male twin pairs; age: age at time of magnetic resonance imaging (MRI) examination (in years). PI-R-ABBR: mean Padua Inventory Abbreviated Scores (SD); Y–BOCS severity lifetime/current: mean Yale–Brown Obsessive–Compulsive Scale severity scores (SD) across whole life span and at the time of MRI; MINI (depression, panic disorder, agoraphobia, social disorder, posttraumatic stress disorder, generalized anxiety disorder): number of subjects with current comorbid disorder (measured with the Mini International Neuropsychiatric Interview); Tic: mean Tic scores (SD) at time of MRI; BDI: mean Beck Depression Inventory scores (SD) at time of MRI; STAI: mean State Trait Anxiety Inventory scores (SD) at time of MRI; STAS: mean State Trait Anger Scale scores (SD) at time of MRI.

(222 male; 593 female). From this sample we selected twin pairs in the age range between 18 and 60 years that scored discordant, concordant-high or concordant-low for OCS. A twin pair was classified as discordant for OCS if one twin scored OCS high (>16) and the co-twin scored OCS low (≤7). A twin pair was classified as concordant-high for OCS if both twins scored ≥15, with at least one twin scoring ≥16. A twin pair was classified as concordant-low for OCS if both twins scored ≤7. These PI-R-ABBR cut-off scores were derived from sensitivity and specificity measurements in a sample of OCD patients when compared with clinical control subjects (n=120; mean scores 20.7, SD 8.1; sensitivity 0.74 and specificity 0.72 at the best cut-off point of 16 (Cath et al., 2008)). For more details on sample selection we refer to den Braber et al. (2010). A final sample of 71 MZ twin pairs participated in this MRI study, including 20 discordant, 23 concordant-high and 28 concordant-low twin pairs **(table 6.1)**. The MRI protocol could not be completed by two subjects (metal artifact, panic attack). In the final sample (n=140), 2 twins with high OCS scores from the discordant group and 5 twins with high OCS scores from the concordant-high group met clinical diagnosis for OCD. Furthermore, 3 twins with high OCS scores and 1 twin with a low OCS score from the discordant group and 6 twins from the concordant-high group used selective serotonin reuptake inhibitors (SSRIs).

Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, comorbidity, type and severity of OCS, tics, state-anger, and state anxiety (for a detailed description of the administered diagnostic interviews and questionnaires, please refer to den Braber et al. (2010)). All twins were asked to collect mucosal cell samples for DNA extraction to test zygosity. The ethical review board of the VU University medical centre approved the study. All participants provided written informed consent.

Image acquisition

The MRI session consisted of an anatomical scan of approximately 6 minutes and a DTI scan of approximately 3 minutes. During the scan sessions, the twins remained inside the scanner and were asked to minimize head movement during and between consecutive runs. To reduce motion artifacts, the head of each participant was immobilized with foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best, The Netherlands) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3-dimensional T1-weighted gradient-echo sequence (flip angle 8°; Repetition Time = 9.69 ms; Echo Time = 4.60 ms; matrix, 256x256 pixels; voxel size, 1.00x1.00x1.20 mm). Diffusion tensor images were obtained in 32 directions by using single-shot echoplanar acquisition (flip angle

90º; Repetition Time = 4834 ms; Echo Time = 94 ms; matrix, 112x110 pixels; voxel size, 2.00x2.00x3.00 mm; b-value 1000 s/mm2, 38 axial slices).

Data analysis

FA maps were calculated from raw DTI scans with the Medical Image Navigation and Research Tool by INRIA (MEDINRIA, Asclepios Research Project - INRIA Sophia Antipolis, France). MRI data were further analyzed with SPM8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom). T1-weighted magnetic resonance images were segmented into gray matter, white matter and cerebrospinal fluid, and normalized to a group template (i.e., a specific template for the discordant and concordant twins) with the Diffeomorphic Anatomical Registration Through Exponential Lie algebra (DARTEL) algorithm, and subsequently warped from DARTEL space to the standard Montreal Neurological Institute (MNI) brain. FA maps were first co-registered with T1-weighted magnetic resonance images and normalized with each subject's T1 to DARTEL to MNI warp parameters. Subsequently, data were spatially smoothed with an 8 mm isotropic Gaussian kernel.

Statistical tests

Differences in survey- and interview-based variables were tested with a mixed-model analysis of variance (Mixed Models Linear menu item in SPSS; SPSS, Chicago, Illinois) with twin pair type (discordant vs. concordant) and OCS score level (high vs. low) as two fixed factors and family as a random factor to account for within-twin pair dependence. Statistical results were considered significant at $p < 0.05$, Bonferroni corrected.

Differences in FA maps between OCS high- and OCS low-scoring twins from discordant pairs were investigated with a paired sample t-test. Differences in FA maps between concordant OCS high and concordant OCS low twin pairs were assessed with an analysis of variance group comparison. To account for within-twin pair correlations, FA maps of the twin and co-twin of each concordant pair were entered as repeated measures. Group differences are reported at an uncorrected individual voxel threshold of $p < 0.005$, with a minimal cluster extent of 50 voxels, which is slightly more conservative as used in previous DTI studies of OCD (Cannistraro et al., 2006; Garibotto et al., 2010; Szeszko et al., 2005).

To test whether an increase (decrease) in FA is accompanied by an increase (decrease) in white matter volume, an additional region of interest (ROI) analysis was performed on the structural VBM data. That is, we tested for increased (decreased) white matter volumes in the discordant and concordant structural data specifically in spherical ROIs (radius 10 mm) centered on the coordinates where discordant and concordant pairs showed maximally increased (decreased)

FA. For these post hoc ROI analyses, an individual voxel p-value threshold of $p<0.05$ was applied, corrected for multiple comparisons (Family Wise Error).

Results

Questionnaire and interview data

As expected, OCS high- compared with low-scoring twins in both the discordant and concordant groups showed significant higher scores on the PI-R-ABBR, and current Y-BOCS severity scale **(table 6.1)**. In addition, high-scoring twins were more often diagnosed with current comorbid disorders, which were absent in the low-scoring twins.

Environmental risk: OCS high- versus low-scoring twins from discordant pairs

Diffusion tensor imaging – fractional anisotropy

Table 6.2A and **figure 6.1** summarize the FA results of the OCS high versus low within twin pair comparison of the discordant pairs. Relative to their low-scoring co-twins, OCS high-scoring twins exhibited clusters of increased FA in right orbitofrontal (cluster label A in **table 6.2A** and **figure 6.1**), left dorsolateral prefrontal (cluster label B), left precentral (cluster label C), left corpus callosum (cluster label D), left cingulate (cluster label E), left insula (cluster label F), right superior parietal (cluster label G), and right temporal (cluster label H) regions and bilaterally in cerebellar regions (cluster labels I and J). Clusters of decreased FA in OCS high compared with their low-scoring co-twins were observed bilaterally in medial frontal and temporal regions (cluster labels K, L, N, P and Q in **table 6.2A** and **figure 6.1**), right insula (cluster label M), left parietal (cluster label O), and right occipital (cluster label R) regions and left brainstem/pons (cluster label S).

Structural VBM data – region of interest analysis

ROI analysis of the structural VBM data at coordinates that showed maximal within pair differences in FA in the discordant sample revealed a significant volume increase in left dorsolateral prefrontal white matter. Furthermore, a trend towards decreased white matter volumes in the high- compared with low-scoring twins was found in right medial frontal and left parietal regions (**table 6.2B** and indicated z-scores in **figure 6.1**).

Genetic risk: concordant-high- versus concordant-low-scoring twins

Diffusion tensor imaging – fractional anisotropy

Table 6.3A and **figure 6.2** show clusters of OCS related FA differences between the concordant-high and low twin pairs. Concordant-high-scoring twins compared with OCS low-scoring twins exhibited clusters of increased FA in right medial frontal (cluster label T in **table 6.3A** and **figure 6.2**) and right temporal (cluster

Table 6.2. Environmental Risk: Regional White Matter Differences Between OCS High and Low Twins of the Discordant Sample

Regional White Matter FA Within-Pair Differences[a]									Regional White Matter Volume Within-Pair Differences[b]					
Test	MNI coordinates			Z score	p-value	voxels (*n*)	Anatomical location	Cluster label	MNI coordinates (ROI VBM)			T-value	p-value	voxels (*n*)
	x	y	z						x	y	z			
high>low	18	39	-23	3.6	<0.001	98	right orbitofrontal	A	–	–	–	–	–	–
	-34	15	31	3.19	0.001	57	left dorsolateral prefrontal	B	-25	14	31	4.94	0.008[c]	194
	-25	-27	66	3.46	<0.001	65	left precentral	C	–	–	–	–	–	–
	-13	-10	27	3.63	<0.001	189	left corpus callosum	D	–	–	–	–	–	–
	-13	-7	63	3.31	<0.001	81	left cingulate	E	–	–	–	–	–	–
	-41	-30	21	3.37	<0.001	101	left insula	F	–	–	–	–	–	–
	29	-51	57	3.16	0.001	69	right superior parietal	G	–	–	–	–	–	–
	48	-16	-26	3.08	0.001	50	right temporal	H	–	–	–	–	–	–
	-17	-73	-39	3.34	<0.001	295	left cerebellum, pyramis/tonsil	I	–	–	–	–	–	–
	20	-70	-35	3.67	<0.001	488	right cerebellum, uvula/piramis	J	–	–	–	–	–	–
high<low	11	-15	63	4.23	<0.001	167	right medial frontal	K	11	-18	68	3.63	0.075[d]	88
	27	-15	36	3.6	<0.001	551	right medial frontal	L	–	–	–	–	–	–
	32	2	15	3.36	<0.001		right insula	M	–	–	–	–	–	–
	-25	15	24	3.12	0.001	128	left medial frontal	N	–	–	–	–	–	–
	-33	-67	32	3.35	<0.001	50	left parietal	O	-28	-61	26	3.59	0.081[d]	33
	-28	-67	12	3.24	0.001	53	left temporal	P	–	–	–	–	–	–
	35	-6	-30	4.08	<0.001	128	right middle temporal	Q	–	–	–	–	–	–
	15	-91	17	3.92	<0.001	91	right occipital	R	–	–	–	–	–	–
	-9	-12	-27	3.27	0.001	64	left brainstem, pons	S	–	–	–	–	–	–

MNI, Montreal Neurological Institute; VBM, voxel-based morphometry; other abbreviations as in table 6.1.

***a** Clusters with regional fractional anisotropy (FA) differences between OCS high and low twins in the discordant sample. Test: test for significant FA increases (high > low) or decreases (high < low) in OCS high- relative to OCS low-scoring twins; MNI coordinates (mm): location of voxel with largest effect size; Z score: z value of voxel with largest effect size; p-value: cluster p-value; Voxels (n): number of voxels in cluster; cluster label: alphabetical cluster label as displayed in anatomical overlays of figure 6.1.* **b** *Clusters with regional white matter volume differences between OCS high and low twins in the discordant sample in spherical region of interest (ROIs) (radius 10mm) centered on the coordinates where discordant pairs showed maximally increased (decreased) FA. MNI coordinates (mm): location of voxel with largest effect size; p-value: cluster p-value family-wise error (FWE)-corrected; voxels (n): number of voxels in cluster.* **c** *$p < .05$, FWE-corrected.* **d** *Trend toward $p < .05$, FWE-corrected.*

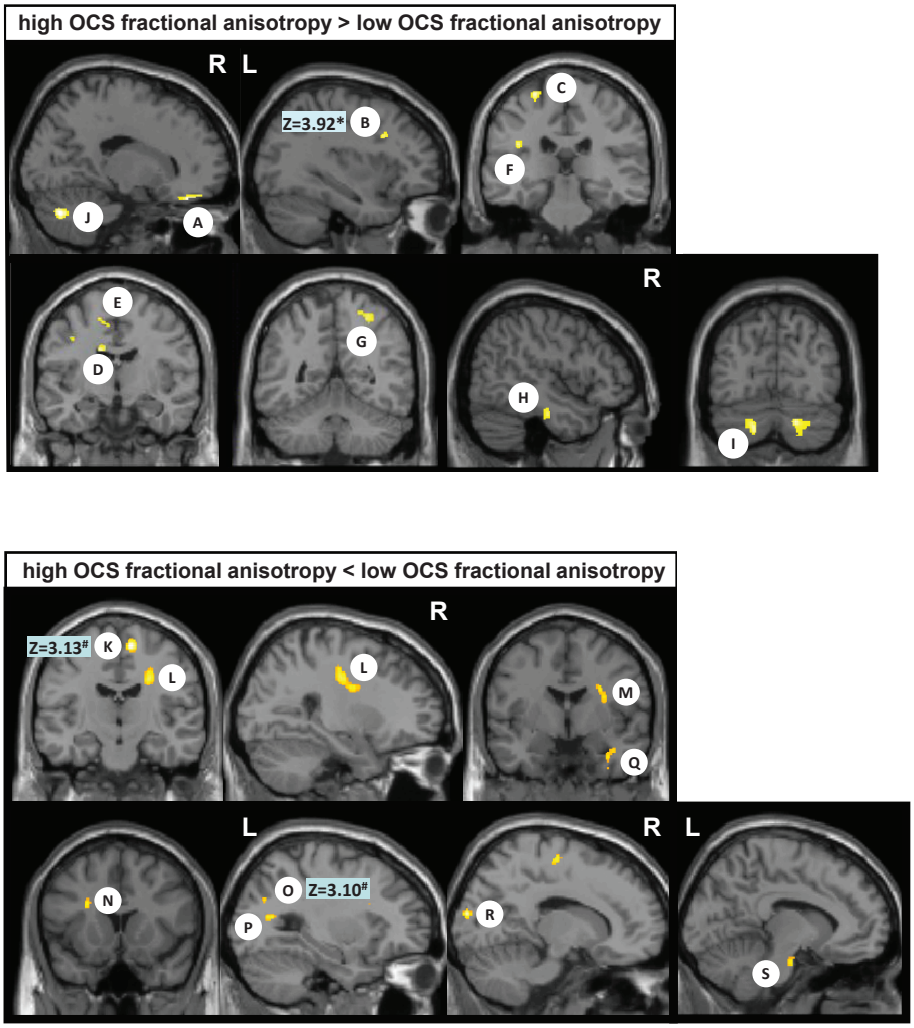


Figure 6.1. *Environmental risk: fractional anisotropy (FA) in high obsessive-compulsive symptoms (OCS) relative to low OCS discordant twins. Brain regions showing increased (top panels: high > low) and reduced (bottom panels: high < low) FA in OCS high vs. low twins of the discordant sample. Z: z value of voxel with largest effect size derived from additional voxel-based morphometry–region-of-interest analysis, *p < .05, family-wise error (FWE)-corrected; #trend toward p < .05, FWE-corrected.*

label W) regions and bilaterally in parietal regions (cluster label U and V). A cluster of decreased FA in OCS high- compared with low-scoring twins was observed in the left inferior frontal lobe (cluster label X in **table 6.3A** and **figure 6.2**).

Structural VBM data - region of interest analysis

ROI analysis of the structural VBM data at coordinates that showed maximal between-group differences in FA in the concordant sample revealed a significant decrease in white matter volume in the high compared with low concordant twins

in the left inferior frontal lobe. No clusters of increased white matter volumes were found (**table 6.3B** and indicated z-scores in **figure 6.2**).

Because this study included subjects with SSRI medication we conducted additional analyses to test whether removing these subjects from the analyses would affect the results. We re-ran the analysis on 17 discordant pairs and 20 concordant-high versus 28 concordant-low pairs not taking SSRIs. These analyses did not affect the pattern of results.

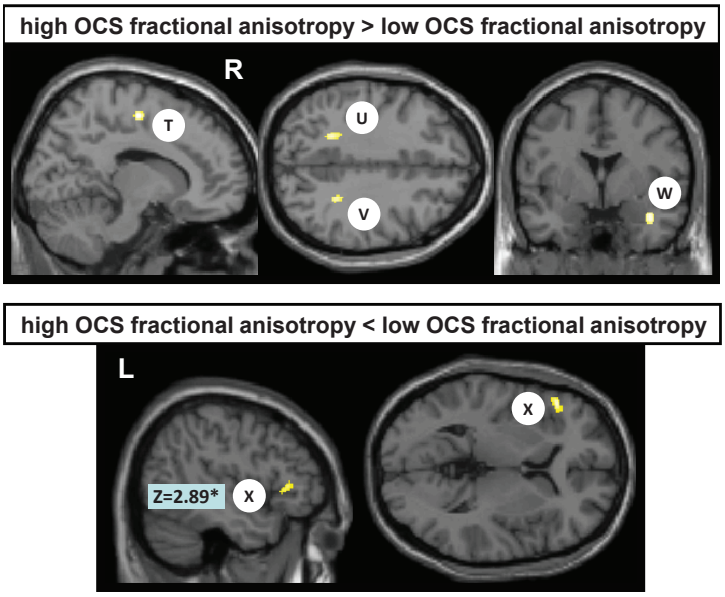


Figure 6.2. *Genetic risk: FA in high OCS relative to low OCS concordant twins. Brain regions showing increased (top panels: high > low) and reduced (bottom panels: high < low) FA in concordant-high vs. concordant-low twins. Z: z value of voxel with largest effect size derived from additional voxel-based morphometry–region-of-interest analysis, *p < .05, FWE-corrected. Abbreviations as in figure 6.1.*

Table 6.3. Genetic Risk: Regional White Matter Differences Between OCS High and Low Twins of the Concordant Sample

Regional White Matter FA Between-Group Differences[a]									Regional White Matter Volume Between-Group Differences[b]					
Test	MNI coordinates			Z score	p-value	voxels (*n*)	Anatomical location	Cluster label	MNI coordinates (ROI VBM)			T-value	p-value	voxels (*n*)
	x	y	z						x	y	z			
high>low	14	-13	56	3.41	<0.001	78	right medial frontal	T	–	–	–	–	–	–
	-21	-48	38	3.22	0.001	142	left parietal	U	–	–	–	–	–	–
	29	-43	36	3.08	0.001	51	right parietal	V	–	–	–	–	–	–
	39	0	-24	3.40	<0.001	86	right middle temporal	W	–	–	–	–	–	–
high<low	-51	27	3	3.99	<0.001	166	left inferior frontal	X	-46	27	1	3.03	0.047[c]	41

a *Cluster with regional FA differences between concordant-high and low twins. Test: test for significant FA increases (high>low) or decreases (high<low) in OCS high- relative to OCS low-scoring twins; MNI coordinates (mm): location of voxel with largest effect size; Z score: z value of voxel with largest effect size; p-value: cluster p-value; voxels (n): number of voxels in cluster; cluster label: alphabetical cluster label as displayed in anatomical overlays of figure 6.2.*

b *Clusters with regional white matter volume differences between OCS high and low twins in the concordant sample in spherical ROIs (radius 10 mm) centered on the coordinates where concordant pairs showed maximally increased (decreased) FA. MNI coordinates (mm): location of voxel with largest effect size; p-value: cluster p-value FWE-corrected; voxels (n): number of voxels in cluster. Abbreviations as in table 6.2.*

c *$p < .05$, FWE-corrected.*

Discussion

White matter structures were compared with a combined DTI-VBM analysis within MZ twin pairs discordant for OCS and between MZ twin pairs concordant-low or concordant-high for OCS. Discordant pair analysis indicated that environmental risk factors for OC symptomatology were associated with increases in dorsolateral prefrontal white matter. Concordant pairs analysis suggested that genetic risk factors for OC symptomatology were associated with decreases in inferior frontal white matter. Remarkably, DTI analysis indicated that some white matter tracts show FA alterations that are in the opposite direction in subjects at high environmental risk compared with subjects at high genetic risk (e.g., right medial frontal, left parietal and right middle temporal). Results are discussed in more detail in the following text, in which we focus on the areas that were detected by DTI and confirmed by the VBM analysis. VBM not only shows that the white matter differences identified by DTI indicate a change in the number of fibers or higher degree of myelination of fibers, it also provides a within-study replication of white matter abnormalities by a different method.

Environmentally mediated white matter alterations

The dorsolateral prefrontal region showed increased white matter integrity (FA), accompanied by increased white matter volume (VBM), in OCS high- compared with OCS low-scoring twins only in the discordant sample. Thus, these white matter differences are likely related to environmental risk factors for OC symptomatology. An increased FA in the dorsolateral prefrontal region, as found in subjects at high environmental risk for OCD, was also found by Ha et al. (2009) and is in line with literature in OCD (Menzies et al., 2008a). The DLPFC has been related to executive processing, including attention, response inhibition, cognitive planning, and decision making (Faw, 2003; Newman et al., 2003; Ridderinkhof et al., 2004). Neuropsychological studies have typically associated dysfunction of the DLPFC with perseverative, disinhibited behaviors, which OCD patients particularly show during the completion of their compulsions (Friedlander and Desrocher, 2006). The finding of systematic differences in white matter integrity in the DLPFC is consistent with the commonly accepted neurobiological model of CSTC abnormalities in OCD (Graybiel and Rauch, 2000; Insel and Winslow, 1992). In addition, this region was also implicated in OCD by a previous functional MRI study by our group that showed an environmentally mediated decrease in DLPFC activity during the performance of a Tower of London planning paradigm (den Braber et al., 2010).

Some white matter regions showed altered FA in high compared with low discordant twins that were not corroborated by our VBM-ROI analysis, but replicate previous DTI findings in OCD patients. These include the corpus callosum and

the cingulate bundle (Cannistraro et al., 2006; Garibotto et al., 2010; Yoo et al., 2007). An environmentally mediated increase in FA was found in the body of the corpus callosum, which interconnects motor and posterior parietal regions (Saito et al., 2008). This is in line with results from a morphological study that found significantly larger corpus callosal areas in pediatric OCD patients compared with control subjects (Rosenberg et al., 1997). The cingulate bundle is one of the main white matter tracts that connects the gray matter nodes of the neural circuitry implicated in OCD (Locke et al., 1961; Yakovlev and Locke, 1961). We found FA was higher in the cingulate bundle in the discordant OCS high twins, which replicates the finding by Cannistraro et al. (2006) in patients with OCD compared with healthy control subjects. The cingulate effect might be specific to environmental risk factors, because Menzies et al. (2008b), who performed a ROI analysis in OCD patients and their first degree relatives, did not find FA alterations in the cingulate bundle in either patients or their first-degree relatives.

Genetically mediated white matter alterations

The inferior frontal region showed lower white matter integrity (FA), accompanied by decreased white matter volume (VBM) in OCS high- compared with OCS low-scoring twins only in the concordant sample. These white matter deficits are likely related to genetic risk factors for OC symptomatology. The inferior frontal region is also implicated in the widely accepted neuroanatomical CSTC model for OCD and is involved in a wide range of cognitive processes, including task switching, reversal learning, and cognitive and emotional inhibition (Dillon and Pizzagalli, 2007; Ramnani and Owen, 2004). Furthermore, this region is involved in regulating socially appropriate behaviors, and when impaired a person might show impulsive and disinhibited behavior (Friedlander and Desrocher, 2006). In addition, this region was also implicated in a previous functional study by our group that showed a genetically mediated increase in inferior frontal activity during the performance of a Tower of London planning paradigm (den Braber et al., 2010).

White matter changes related to both environmental and genetic risk factors

Regions that showed FA alterations in both the discordant and concordant sample include medial frontal, parietal, and temporal regions. Interestingly, if we examine our results in more detail, roughly the same white matter regions show FA alterations, but these are in the opposite direction (increased vs. decreased) in subjects at high environmental risk compared with subjects at high genetic risk (e.g., right medial frontal: reduced FA in discordants, increased FA in concordants; left parietal: reduced FA in discordants, increased FA in concordants; right temporal: reduced FA in discordants, increased FA in concordants). This pattern of opposite DTI findings for the same anatomical region echoes a similar disparity in the extant literature. For instance, some studies found decreased FA in

the corpus callosum in patients with OCD compared with control subjects (Garibotto et al., 2010; Saito et al., 2008), whereas others reported increased FA in OCD patients for this region (Yoo et al., 2007). This pattern of findings makes most sense when we allow environmental and genetic risk factors to affect the brain in different ways. This is, in fact, a major rationale to apply the discordant/concordant twin design.

Patient samples represent an unknown mixture of individuals who developed OCD due to a genetic predisposition and/or environmental triggers. Our results illustrate that, if the study sample predominantly consists of patients with a familial predisposition, findings might differ from those of a study of patients who might have developed the disorder due to a negative environmental experience (e.g., divorce, sexual assault). A previous study attempting to identify genetic markers for OCD by comparing FA alterations in OCD patients, their first-degree relatives, and control subjects found an FA increase in right medial frontal white matter (Menzies et al., 2008b). This result is in line with our finding of a genetically mediated FA increase in the same region, which might indicate that this alteration is specific to individuals at increased genetic risk for OCD. However, this finding could be easily missed if a sample represents a mixture of subjects at increased genetic risk and subjects with environmentally mediated OCD. This might be true of the various studies that failed to find FA alterations in this region (Cannistraro et al., 2006; Garibotto et al., 2010; Yoo et al., 2007). Medial frontal, parietal, and temporal regions have been implicated in the neuroanatomical model for OCD, predominantly through their functional connections with the ventral prefrontal cortex and DLPFC. An alteration in these functional connections may lead to an imbalance between the direct and indirect pathways of the ventral and dorsal frontal-sriatal loops, which subsequently may induce OC-like behavior.

To summarize, inconsistencies between results of previously performed imaging studies might be related, at least in part, to 'true' variability in the underlying neurobiology of OCD. The present results suggest that the effects on central nervous system white matter regions of environmental risk factors for OC symptomatology are distinct from those of the genetic risk for OC symptomatology. These findings are in line with results from a previous functional imaging study by our group (den Braber et al., 2010) in which these same regions were found to be differentially affected by environmental and genetic risk factors for OC symptomatology. Interestingly, when the DTI-VBM and functional MRI results are taken together, they point toward an inverse relation between task-related activity and white matter integrity in OCS (e.g., environmentally mediated decrease in DLPFC activity coupled with higher dorsolateral prefrontal white matter integrity, and genetically mediated increase in inferior frontal activity together with lower inferior frontal white matter integrity). This inverse relation

might be the result of an increase or decrease, respectively, in inhibitory signaling in these specific regions that depends on white matter integrity.

Although different regions seemed to be affected by environmental and genetic risk factors for OC symptomatology, both classes of risk factors strikingly converge on the CSTC loops. Neurobiological changes in OCS induced by environmental risk factors involve the indirect loop of the dorsal CSTC circuit (dorsolateral prefrontal region), which functions as a negative feedback loop important for the inhibition of and switching between behaviors. By contrast, neurobiological changes in OCS induced by genetic risk factors, involve the direct loop of the ventral CSTC circuit (inferior frontal region), which functions as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors **(figure 6.3)**.

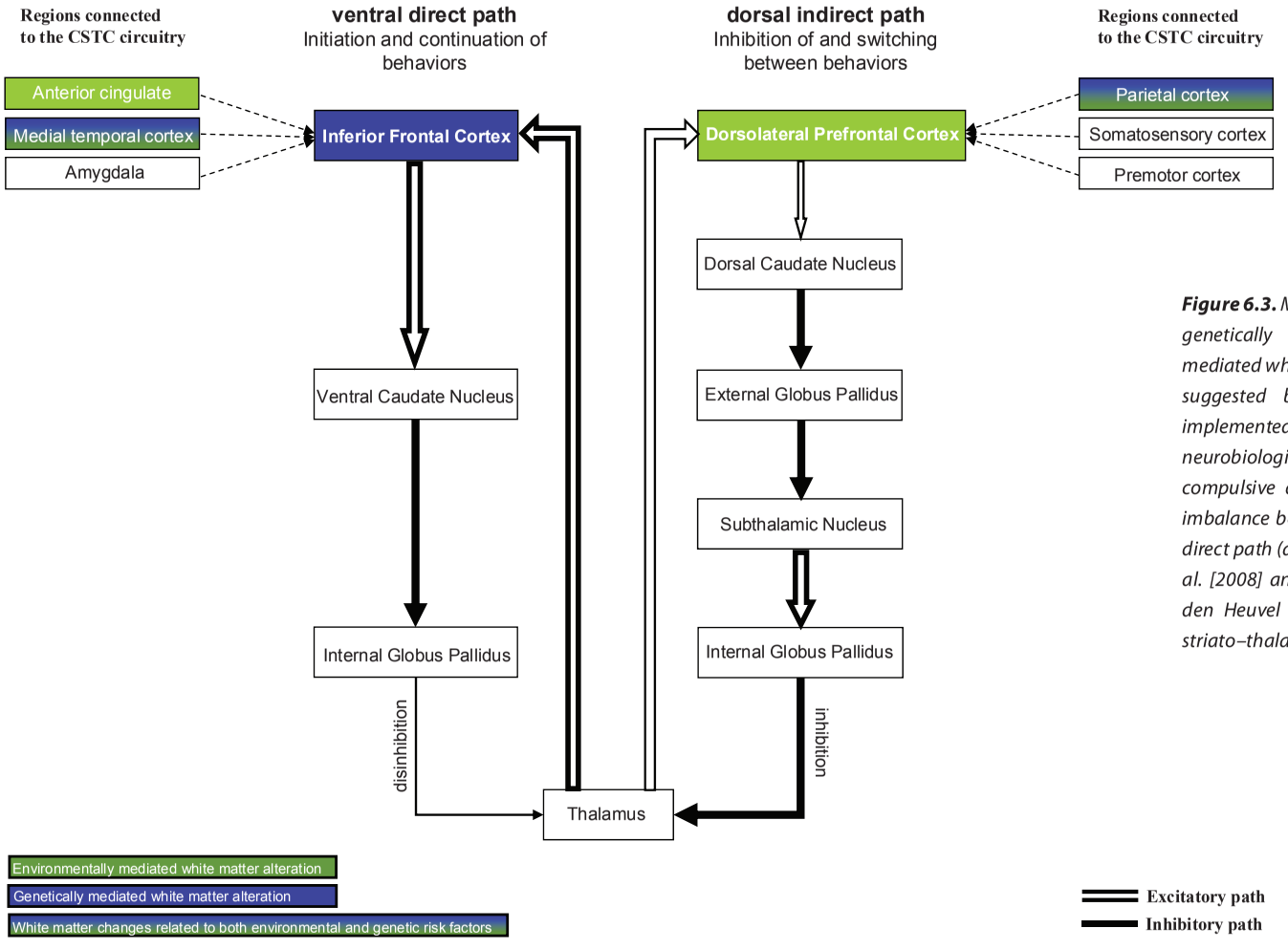


Figure 6.3. *Model illustrating distinct genetically and environmentally mediated white matter alterations as suggested by the present study implemented in the widely accepted neurobiological model for obsessive-compulsive disorder, suggesting an imbalance between the indirect and direct path (adapted from Menzies et al. [2008] and Mataix-Cols and van den Heuvel [2006]). CSTC, cortico–striato–thalamo–cortical.*

7

The use of fMRI to detect neural responses to cognitive tasks: is there confounding by task related changes in heart rate?

This chapter is submitted as:
D. van 't Ent, A. den Braber, E. Rotgans, E.J.C. de Geus, and J.C. de Munck. The use of fMRI to detect neural responses to cognitive tasks: is there confounding by task related changes in heart rate? (in revision, Psychophysiology).

Abstract

In agreement with the fact that functional Magnetic Resonance Imaging (fMRI) signals represent local Blood Oxygenation Level Dependent (BOLD) changes and variations in blood flow and blood volume, it has recently been demonstrated that fMRI signals during rest are strongly correlated with heart rate variations. Since heart rate/fMRI correlations show up in every part of the brain they may form an important confound in brain activation studies, particularly if heart rate is affected by the task. To assess the impact of task-related heart rate variation, we co-registered the electrocardiogram with fMRI in 91 subjects during a color-word Stroop task, administered using a block design, and a Tower of London (ToL) cognitive planning task, administered using an event-related design. We found that both Stroop interference and ToL planning were associated with significantly increased heart rate and confirmed significant main effects of heart rate regressors on the fMRI signals during both tasks. Nevertheless, statistical results from General Linear Model contrasts that test for increased fMRI signal during Stroop color-word interference and ToL planning were not significantly influenced by inclusion of heart rate regressors as nuisance variables. We conclude therefore that fMRI signal changes associated with fluctuations in heart rate do not impact strongly on higher-order fMRI task effects.

Introduction

The applicability of functional Magnetic Resonance Imaging (fMRI) as a neuroimaging tool rests on the assumption that fMRI signal changes include a Blood Oxygenation Level Dependent (BOLD) mechanism (Ogawa et al., 1992). This implies that regional brain activations result in local excess of oxy-hemoglobin supply, which leads to an increase in the homogeneity of magnetic susceptibility, a decrease in T2*, and hence increased fMRI signal (Buxton, 2009). Obviously, fMRI is only a very indirect measure of brain activity and, apart from the BOLD-effect, T2* is also influenced by other physiological factors such as respiratory and cardiac cycles which modulate blood oxygen levels and microvessel diameters (Birn et al., 2006; Glover et al., 2000; van Houdt et al., 2010; Windischberger et al., 2002; Bhattacharyya and Lowe, 2004; Katura et al., 2006; Tong et al., 2011). Indeed, it has recently been demonstrated for recordings during resting state conditions that fMRI signals over large parts of the brain are correlated with changes in heart rate (Chang et al., 2009; de Munck et al., 2008; Shmueli et al., 2007).

If there are no systematic differences in cardiac activity between different conditions of a task, one could argue that the effects of this physiological noise can always be compensated by recording a sufficient number of trials. However,

in paradigms where heart rate is modulated by the task, e.g., when there are different levels of task difficulty or emotional valence, fMRI signal changes correlated with cardiac activity pose a serious threat to the interpretation of the statistical parametric maps (SPMs). Statistically significant differences between task conditions may then not be caused by the BOLD-effect alone, but also by non-neuronal responses of the vascular bed to heart rate variations.

The goal of our study is to explore the extent to which fMRI signal changes between cognitive task conditions are influenced by between-condition differences in heart rate. To this end, we performed simultaneous electrocardiogram and BOLD fMRI recordings in a group of subjects during a color-word Stroop task and a Tower of London (ToL) cognitive planning task. The two tasks covered the two basic experimental setups for BOLD fMRI measurements; the Stroop task was administered in a blocked paradigm and the ToL in an event-related paradigm. The influence of non-neural contributions on fMRI task effects due to heart rate modulation was assessed by computing task related fMRI changes using a general linear model (GLM) that accounts for heart rate effects by adding heart rate regressors as confounders, and comparing the results with the fMRI signal changes from a GLM without inclusion of heart rate information, as is standard practice in BOLD fMRI research.

Methods

Subjects

From the Netherlands Twin Registry (Boomsma et al., 2002) we recruited a 'Test sample' of 46 subjects. All subjects (13M/33F: mean age 36.9 ± 8.9 yrs) were twins from monozygotic pairs, but by selecting only one of the members from each pair, shared family backgrounds were avoided. To investigate the stability of our findings, we repeated our analyses in the set of co-twins of the subjects in the test sample, which we will refer to as the 'Repetition sample'. However, for one of the co-twins (F: 35 yrs) no MRI data was available, leaving 45 subjects in the repetition set. None of the twins in both samples had a history of neurological illness as assessed from self-report surveys, and all twins provided written informed consent. The study was approved by the VU University medical centre Amsterdam ethical review board.

Tasks

The Stroop task of this study consisted of standard color-word stimuli as well as words with emotional content. For our purpose we investigated only the results pertaining to color-word interference, which had the largest effect on task performance and heart rate. During the Stroop color-word task, subjects had

to report the ink color of written color-words. Dutch translations of the words "red", "yellow", "blue" and "green" were used that could be written in any of these four colors. Word meaning and ink color could be either congruent (e.g., the word "green" written in green) or incongruent (e.g., the word "red" written in blue). The correct answer had to be indicated by pressing buttons: left middle finger for ink color yellow, left forefinger for green, right forefinger for red and right middle finger for blue. The task was administered in a block design with 18 blocks in total. Of these, 3 blocks contained congruent and 3 blocks contained incongruent color-word stimuli. The remaining blocks were filled with words that convey general emotional content (3 blocks, e.g., cancer, suffocate, etc), words with content related to obsessions/compulsions (3 blocks, e.g., guilty, dirty, etc), and neutral words with similar linguistic parameters (e.g., word length and frequency of occurrence) as the words with emotional content (3 blocks) and neutral words with similar linguistics as the words conveying obsession/compulsion related content (3 blocks). In each individual block of 35 seconds, 16 words were presented for 2 seconds and separated by small intervals of 200 milliseconds. The subjects were asked to respond to the stimuli as quickly and accurately as possible. Total task duration was 10.5 minutes.

Stimuli for the ToL task consisted of images of colored beads (red, blue, yellow), placed on three vertical rods of decreasing height **(figure 7.1)**. On each trial a start configuration and final target configuration were simultaneously depicted at the bottom and top of the screen, respectively. Subjects were requested to count the number of steps from the starting configuration to reach the target configuration. Five planning difficulty levels were included that corresponded with the minimal number of moves (1–5) actually needed to achieve the target. As a baseline condition, similar stimuli were presented but this time the subject only had to count the number of beads with specified colors. Each time, two possible answers (one correct and one incorrect) were presented at the bottom left and right of the screen, from which the correct one had to be indicated by pressing a corresponding left or right hand button. No feedback was provided during the task. The stimuli were presented in an event-related design with self-paced stimulus timing, i.e., a subsequent trial was presented on the screen with a delay of 32 ms after the response on a previous trial. For all subjects the stimulus presentation order was the same, but the total number of trials depended on the subject's reaction times. Total task duration was 17 minutes. Here we will focus on the comparison of 4-steps planning versus baseline, because it showed the largest modulation of heart rate. The heart rate effect for 5-steps planning was less pronounced and not statistically significant (Test sample: $p = 0.148$: Repetition sample: $p = 0.859$). This is likely because several of our subjects experienced this condition as very difficult and reported that they had given up on a number of trials. On average subjects completed 16 ± 3 trials

with 4-steps planning stimuli (~9% of the total number of trials) versus 62 ± 15 trials with baseline stimuli (~36%).

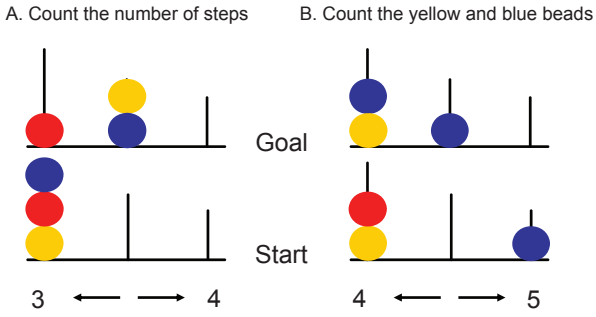


Figure 7.1. *Examples of Tower of London stimuli. (A) 4-steps planning condition; (B) baseline condition (adapted from van den Heuvel et al. (2005a)).*

For both the Stroop and ToL, stimuli were projected on a screen at the end of the MRI scanner table, viewed by the participants through a mirror. Two magnetic compatible response boxes were used to record the subject's performance. Before the experiment, the subjects practiced a number of trials on a computer outside the scanner and again inside the scanner, prior to the actual start of the session.

MRI and ECG

MR scans were made on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with an 8-channel standard SENSE receiver head coil. Of each subject a three-dimensional T1-weighted gradient-echo sequence anatomical scan was made consisting of 182 coronal slices of 256×256 pixels; voxel size was 1.0×1.0×1.2 mm^3. For fMRI, an echo planar imaging (EPI) sequence (flip angle 80°; repetition time = 2300 ms; echo time = 30 ms, matrix, 96 × 96 pixels; field of view 220 × 220 mm) was used, covering the whole brain (40 axial slices; 2.29 mm × 2.29 mm in-plane resolution; 3.0 mm slice thickness; no gap between slices). For the Stroop task a total of 260 and for the ToL a total of 440 EPI volumes were scanned per subject in one single run. During fMRI scanning a four-lead electrocardiogram (ECG) was recorded using the ECG system provided with the MR scanner, and sampled at 500 Hz. The ECG was stored in an ASCII log file and time aligned to the fMRI scanning using information from the additionally stored MRI field gradient onsets.

Inter heart Beat Interval (IBI) regressors

Inter heart Beat Interval (IBI) regressors were constructed similarly as described by de Munck et al. (2008). In brief, first the R-peaks in the ECG were detected automatically and large changes (> 30%) in consecutive RR intervals were flagged

and if necessary manually corrected after visual inspection. Subsequently, the ECG time series were subdivided into epochs corresponding to each of the fMRI scans (a single volume was acquired in 2.3 s). Since the RR interval times are irregularly sampled over time they cannot be directly used as IBI regressor. To compute an IBI value per fMRI epoch, all RR intervals were averaged having at least one point of overlap with that epoch. Since the effect of time varying heart beats on the fMRI signal does not follow the standard hemodynamic response function used for neuroimaging, IBI-regressors were shifted in time over multiple time steps of the MRI volume repetition time (TR), to account for possible delayed responses of the fMRI-signal to heart beat variations. In this way, the effect of heart beat on fMRI is described with a general impulse response model, where the optimal response shape is a priori unknown and is extracted from the data. In this study we used 7 IBI-regressors corresponding to time shifts of [-2, -1, 0, 1, 2, 3, 4]*TR. The IBI time series were either considered as effects of interest, or as 7 additional nuisance regressors in the GLM for statistical testing, or ignored.

We also used the ECG data to test if heart rate changed with task difficulty. To compute a mean IBI value per stimulus type for the Stroop task in each individual we took the mean of all RR interval times that overlapped each individual stimulus block and then averaged these mean RR times separately across the 3 blocks with congruent and incongruent color-word stimuli, respectively. For the ToL we first computed, for each stimulus, the mean of all RR times that overlapped the interval between stimulus onset and the subject's response and then averaged the mean RR times per stimulus type. Similar to the computation of IBI values per fMRI epoch, all RR times were included having at least one point of overlap with the relevant epoch (i.e., stimulus block for the Stroop task and interval between stimulus onset and the subject's response for the ToL task).

Statistical analyses

MRI data were analyzed using Statistical Parametric Mapping version 5 (SPM5: Wellcome Department of Imaging Neuroscience, London, UK). Echo planar imaging scans were slice time corrected, realigned and normalized to the standard Montreal Neurological Institute (MNI) brain of SPM. Subsequently, data were re-sliced to 3 mm × 3 mm × 3 mm voxels and spatially smoothed using an 8 mm isotropic Gaussian kernel. After high-pass filtering with cut-off at 128 s (0.0078125 Hz), functional scans were analyzed in the context of the general linear model using delta functions convolved with a canonical hemodynamic response function. For the ToL task, event duration, computed as the time between stimulus and response onset, was included in the GLM to account for hemodynamic responses of varying lengths to each type of stimulus. Error trials and head-movement parameters were modeled as regressors of no interest. For the Stroop task, a 'color-word interference' main effect was computed in which brain

Table 7.1. Task performance and IBI changes

		Test sample			Repetition sample		
Task	Condition	Reaction time (ms)	Accuracy (%)	IBI (ms)	Reaction time (ms)	Accuracy (%)	IBI (ms)
Stroop	congruent	808.7 ± 145.5	95.2 ± 6.0	836.3 ± 137.1	792.5 ± 129.8	96.3 ± 5.0	820.2 ± 129.4
	incongruent	956.9 ± 168.4	82.6 ± 12.9	827.7 ± 132.9	967.7 ± 157.9	83.4 ± 10.9	815.3 ± 121.0
ToL	baseline	3566.1 ± 874.8	93.2 ± 4.3	825.1 ± 125.8	3636.6 ± 995.6	94.2 ± 2.6	814.5 ± 121.6
	planning	11580.0 ± 5229.7	75.1 ± 13.1	817.3 ± 124.8	11830.8 ± 4716.1	77.9 ± 13.5	809.2 ± 120.1

Mean reaction times (ms ± sd), mean reaction accuracies (% ± sd) and mean Inter heart Beat Intervals (IBIs: ms ± sd) on color-word congruent and incongruent trials for the Stroop task and on baseline and 4-steps planning trials for the ToL task. Results for the twins in the Test sample are listed in the columns on the left; results for the twins in the Repetition sample are listed in the columns on the right.

activation during trials with color-word incongruent stimuli was compared with brain activation during trials with color-word congruent stimuli. For the ToL task, we applied a 'planning versus baseline' contrast to compare brain activation during 4-steps planning with brain activation during baseline. The 1st-level results for each individual were computed twice; once with and once without taking into account all 7 IBI-regressors. The influence of including IBI-regressors in the GLM was assessed statistically by means of a paired t-test that compared the 1st level results of each subject with and without inclusion of the 7 IBI-regressors. Task main effects across subjects are reported after correction for multiple comparisons using a false discovery rate of 5% and a minimal cluster size of 10 voxels.

Results

Table 7.1 shows mean reaction times, mean reaction accuracies and mean Inter heart Beat Intervals (IBIs) on color-word congruent and incongruent trials for the Stroop task and on baseline and 4-steps planning trials for the ToL task in the Test sample (left columns) and Repetition sample (right). Differences in task performance and IBIs between the two trial types of both tasks are highlighted in **figure 7.2**. Both Stroop color-word interference **(figure 7.2: left)** and ToL planning **(figure 7.2: right)** were associated with significantly reduced reaction accuracies and significantly increased reaction times. Furthermore, reduced performance in both tasks was accompanied by shorter IBIs (= increased heart rate) which were all significant, except for Stroop performance in the Repetition sample. These task related IBI changes create the potential for a confounding effect on the computed fMRI responses.

Figures 7.3 and **7.4** show fMRI main effects of the 7 individual IBI regressors included in the GLM when interpreted as effects of interest. Results are shown separately for tests of positive statistical contrast (T-test contrast: +1) and negative statistical contrast (T-test contrast: -1). Since decreased IBIs indicate increased heart rate and vice versa, the positive contrast tests for an inverse relation between heart rate and fMRI, whereas the negative contrast tests for covariation between heart rate and fMRI. The correlation patterns across the 7 regressors are highly equivalent in the Test sample (top) and Repetition sample (bottom) and also appear similar for the Stroop task **(figure 7.3)** and ToL task **(figure 7.4)**, although the patterns are most robust for recordings during the ToL. For the unshifted regressor (0*TR) and the regressor with a positive time shift of 1*TR, co-variations between heart rate and fMRI (suprathreshold voxels for negative statistical contrast) are evident across the whole brain, with posterior dominance. In contrast for larger positive shifts of 2, 3 and 4*TR, heart rate and fMRI are inversely related (suprathreshold voxels for positive statistical contrast).

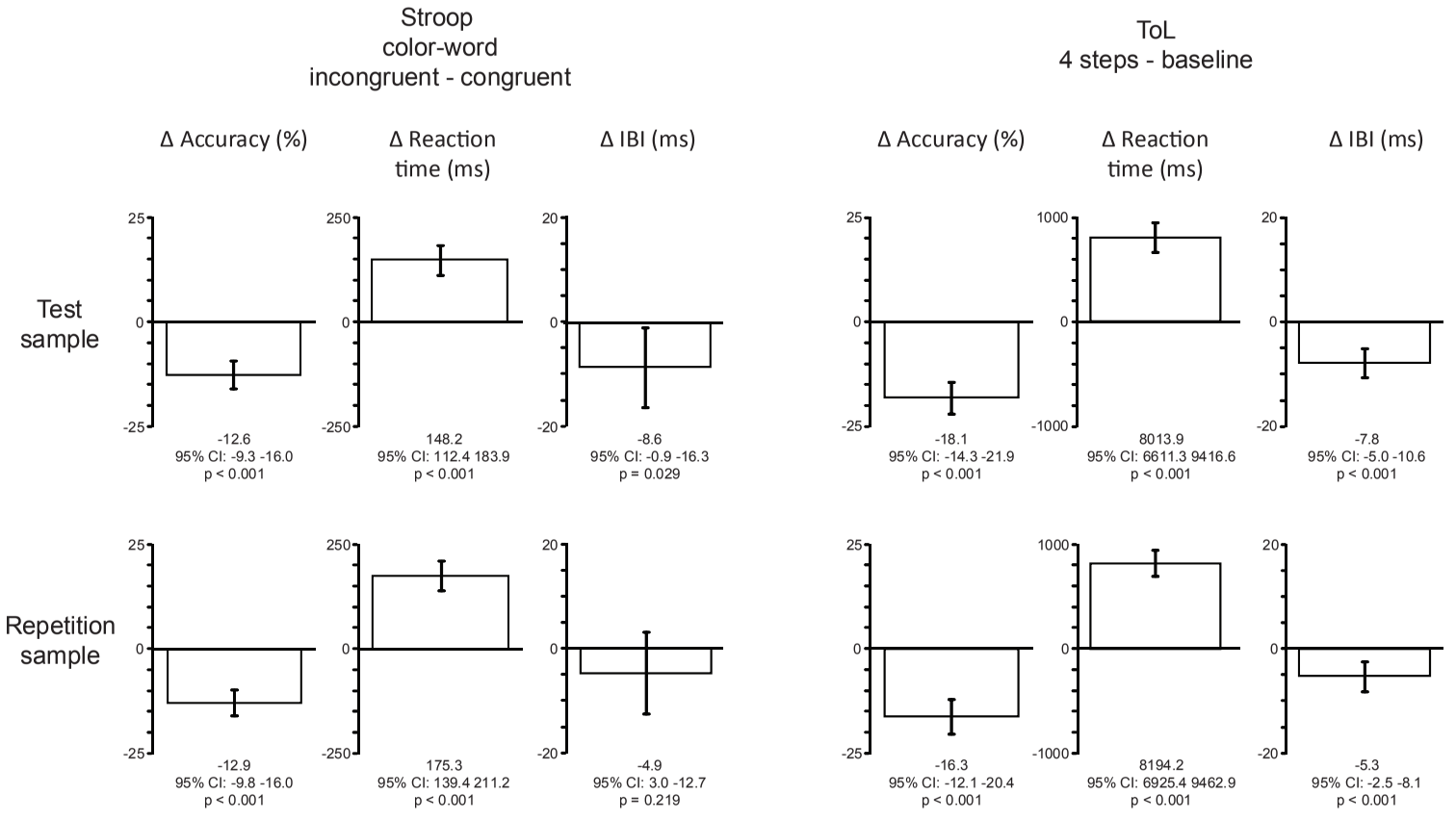


Figure 7.2. *Means of within subject differences in task performance (accuracy and reaction time) and Inter heart Beat Intervals (IBIs) on color-word incongruent compared to congruent trials for the Stroop task (left) and on 4-steps planning compared to baseline trials for the ToL task (right). Results of between condition paired t-test comparisons, for the Test sample (top) and Repetition sample (bottom), are indicated by 95% confidence intervals of the differences (CI: Error bars) and p-values.*

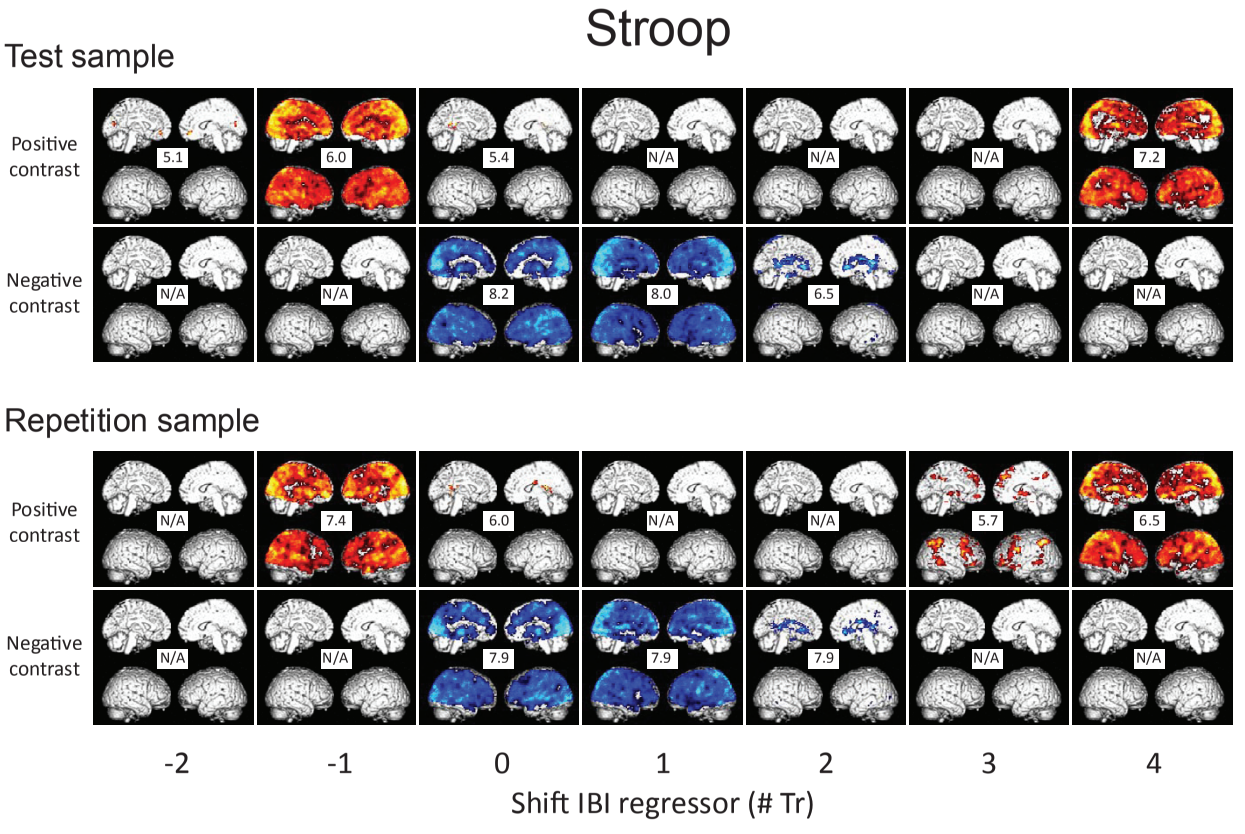


Figure 7.3. *Stroop task main effects of the 7 individual IBI regressors included in the GLM, corresponding to timeshifts relative to the recorded fMRI signals of [-2, -1, 0, 1, 2, 3, 4]*Repetition time (TR=2.3s), for the Test sample (top) and Repetition sample (bottom).*

Since decreased IBIs indicate increased heart rate and vice versa, the tests for positive contrast between IBI and fMRI changes (top rows) indicate voxels where heart rate and fMRI are inversely related, whereas the test for negative contrast (bottom rows) indicate voxels with covariation between heart rate and fMRI modulations. Numbers in each plot indicate statistical T-values for the voxel with highest test significance.

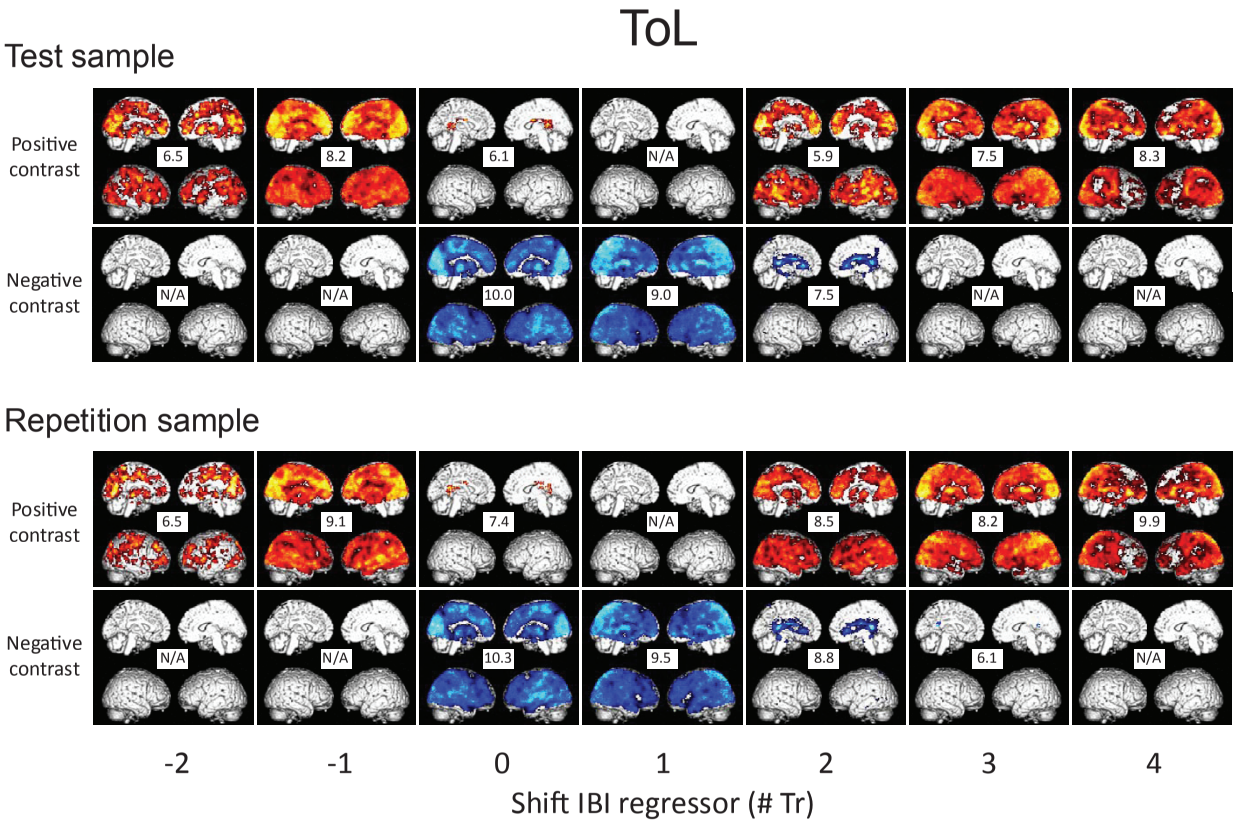


Figure 7.4. *ToL task main effects of the 7 individual IBI regressors included in the GLM, corresponding to timeshifts relative to the recorded fMRI signals of [-2, -1, 0, 1, 2, 3, 4]*Repetition time (TR=2.3s), for the Test sample (top) and Repetition sample (bottom). Numbers in each plot indicate statistical T-values for the voxel with highest test significance.*

Similar inverse heart rate versus fMRI associations are observed for shifts of the IBI regressor back in time, in particular for a shift of -1*TR.

Next, we examined the extent to which the higher heart rates (decreased IBIs) after Stroop color-word incongruent stimuli and after ToL planning stimuli confounded the fMRI main effects obtained using the color-word incongruent versus congruent contrast for the Stroop task and 4-steps planning versus baseline contrast for the ToL task. **Figure 7.5** shows glass brain projections of SPMs for Stroop color-word interference (left panels) and ToL planning (right) in the Test sample (top) and Repetition Sample (bottom). Lists of significant clusters for both tasks and samples are depicted in **tables 7.2** and **7.3**. In both samples, the SPMs computed without IBI-regressors as confounders were highly similar to the SPMs obtained with the 7 IBI-regressors as nuisance effects. Although generally SPM maximum T-scores and number of suprathreshold voxels were lower after including heart rate regressors. To statistically assess the influence of including IBI-data in the GLM, we applied paired t-tests that compared the 1st level results of each subject with and without accounting for the 7 IBI-regressors. Despite this highly sensitive test, we found no significant differences at our statistical threshold of $p<0.05$, FDR corrected. For further exploration, we lowered the threshold to a more lenient value of $p < 0.005$, uncorrected, with no cluster extend limit. The results for the Stroop and ToL task, depicted in **figures 7.6** and **7.7**, revealed a general tendency to find more clusters when testing for larger fMRI activations when using a GLM without heart beat regressors. However, as can be observed by comparing the glass brain projections as well as the MRI slice overlays which indicate the anatomical location with lowest statistical p-value, the results for both tasks and for the Test and Repetition samples did not reveal a consistent pattern of differences at specific brain locations. Furthermore, the effect sizes of the difference contrast at the most significant voxel, as indicated by the bar graph inserts on the slice overlays, were very small (< 0.2).

Finally, we investigated if the impact of heart rate related fMRI changes on computed brain activations was larger in individuals with strong versus weak task related heart rate variation. For this, we computed correlations between task related heart rate change in each subject and the difference in activated number of voxels (at $p < 0.005$, uncorrected) as well as maximum statistical T scores as derived from a GLM with versus without heart rate regressors. The correlation values for both samples, listed in **table 7.4**, indicated no significant associations. For the Stroop task, there were close to significant positive correlations for number of activated voxel and maximum T scores, indicating a tendency for a higher reduction in number of activated voxels and maximum T scores after inclusion of IBI regressors in individuals with a stronger task related heart rate increase, but this was true only in the Repetition sample. For the ToL task there was a close

to significant negative correlation for activated number of voxels, indicating a tendency for an increase in number of suprathreshold voxels after inclusion of IBI regressors in individuals with a stronger task related heart rate increase, but this was found only in the Test sample.

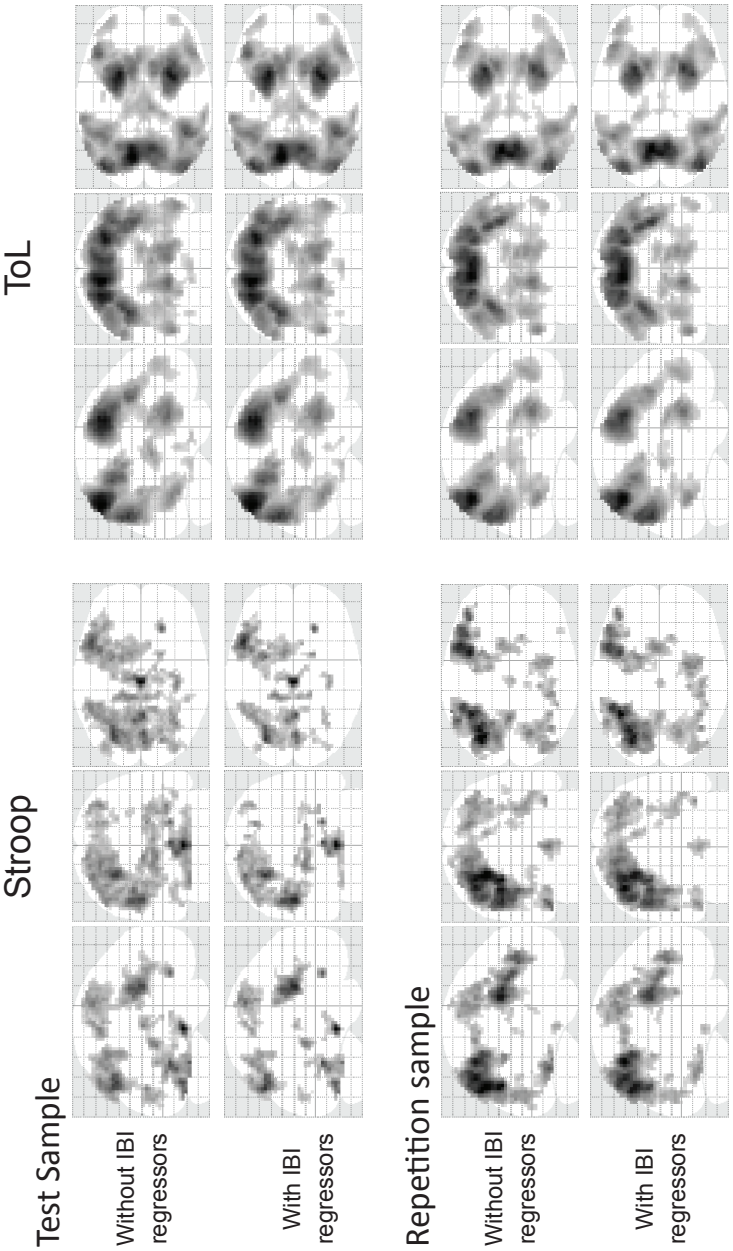


Figure 7.5. *Glass brain projections of SPMs for Stroop color-word interference (left) and Tol planning versus baseline (right) in the Test and Repetition samples. In each pane, top row projections indicate results without the IBI-regressors as confounders; bottom row projections indicate results obtained with the 7 IBI-regressors as nuisance effects.*

Table 7.2. Significant clusters from the SPMs for Stroop color-word interference

		Test sample										Repetition sample									
		Without IBI regressors					With IBI regressors					Without IBI regressors					With IBI regressors				
Anatomical location		MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels
		x	y	z			x	y	z			x	y	z			x	y	z		
L./R.	Brainstem	0	-21	-18	7.06	925	0	-21	-18	6.69	96	3	-18	-24	3.95	31	3	-21	-21	3.96	21
L./R.	Cerebellum	--	--	--	--	--	3	-51	-6	5.34	347	0	-54	-15	5.13	86	0	-54	-15	4.37	74
L.	Caudate/Glob. Pall.	-21	0	21	3.69	21	--	--	--	--	--	-18	-3	0	3.34	12	--	--	--	--	--
R.	Caudate	21	30	-6	5.03	39	21	30	-6	5.06	22	21	-3	18	3.47	14	21	-9	21	3.27	12
L.	Thalamus	--	--	--	--	--	-12	-18	18	3.98	25	--	--	--	--	--	--	--	--	--	--
R.	Thalamus	--	--	--	--	--	--	--	--	--	--	18	-21	15	3.33	13	--	--	--	--	--
L.	Parahippocampus	-9	-36	6	4.71	453	-18	-33	9	4.66	103	--	--	--	--	--	--	--	--	--	--
R.	Parahippocampus	--	--	--	--	--	30	-57	-3	3.83	34	--	--	--	--	--	--	--	--	--	--
L.	Occipital/Parietal	-27	-69	42	5.76	928	-27	-75	45	5.78	532	-27	-75	42	7.47	1365	-36	-51	51	7.63	2089
		--	--	--	--	--	-33	-81	12	3.72	21	-27	-93	0	3.56	18	--	--	--	--	--
R.	Occipital	21	-75	15	4.57	101	21	-75	15	3.65	24	33	-84	15	4.54	162	48	-78	0	4.14	132
R.	Parietal	21	-66	60	4.14	133	21	-66	60	3.45	10	21	-69	57	4.90	413	36	-75	27	3.72	22
		24	-60	42	3.56	19	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		30	-72	33	3.28	14	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
L.	Temporal	-42	-45	-9	3.65	41	--	--	--	--	--	-51	-57	-15	3.65	41	-51	-57	-15	4.69	128
R.	Temporal	45	-36	-12	3.54	20	--	--	--	--	--	42	-63	-12	3.54	20	42	-63	-12	4.61	15
R.	Precentral	36	-21	57	4.60	88	36	-21	57	4.20	40	42	-30	51	4.57	264	42	-30	51	4.37	88
L.	Frontal	-48	18	18	5.62	1188	-48	18	18	5.97	418	-39	9	27	6.90	785	-39	9	27	6.25	690
		--	--	--	--	--	-27	3	63	4.98	376	-30	6	51	4.65	404	-3	12	54	4.86	487
R.	Frontal	21	6	54	4.13	39	21	6	54	4.33	16	48	27	24	3.25	13	--	--	--	--	--

List of significant clusters from the SPMs for Stroop color-word interference in the Test sample (left) and Repetition sample (right). Left columns indicate results without the IBI-regressors as confounders; right columns indicate the results obtained with the 7 IBI-regressors as nuisance effects. MNI coordinates and T-score are listed for the voxels with largest effect size in each cluster.

Table 7.3. Significant clusters for Tol 4-steps planning versus baseline

		Test sample										Repetition sample									
		Without IBI regressors					With IBI regressors					Without IBI regressors					With IBI regressors				
Anatomical location		MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels
		x	y	z			x	y	z			x	y	z			x	y	z		
L./R.	Par./Occ./Temp./	-9	-72	57	13.70	4686	-12	-72	60	13.64	4563	-6	-63	54	14.82	5552	-6	-63	54	14.13	3778
	Thalamus/Caudate	-15	9	-3	8.27	1466	-15	9	-3	8.01	1335	--	--	--	--	--	-12	15	0	8.37	855
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-21	-33	18	5.18	80
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	18	-57	21	5.14	81
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	54	-57	-12	4.91	81
L./R.	Frontal/ Cingulate	27	6	54	12.61	2897	27	6	54	12.64	2843	-24	12	54	12.26	2562	-24	12	54	11.44	1172
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	24	12	51	11.28	1263
L.	Frontal	-33	57	9	4.83	142	-33	57	9	4.70	138	-39	51	6	6.17	198	-39	51	6	5.96	173
L.	Precuneus	-21	-57	21	3.66	24	-21	-57	21	3.50	15	--	--	--	--	--	--	--	--	--	--
L.	Temporal	-39	-12	-24	3.72	14	-39	-12	-24	3.17	11	--	--	--	--	--	--	--	--	--	--
	Brainstem	0	-33	-24	4.08	59	3	-33	-24	4.52	48	--	--	--	--	--	--	--	--	--	--
		-15	-18	-12	4.00	24	-15	-18	-12	3.59	13	--	--	--	--	--	--	--	--	--	--

List of significant clusters from the SPMs for Tol planning versus baseline in the Test sample (left) and Repetition sample (right). Left columns indicate results without the IBI-regressors as confounders; right columns indicate the results obtained with the 7 IBI-regressors as nuisance effects. MNI coordinates and T-score are listed for the voxels with largest effect size in each cluster.

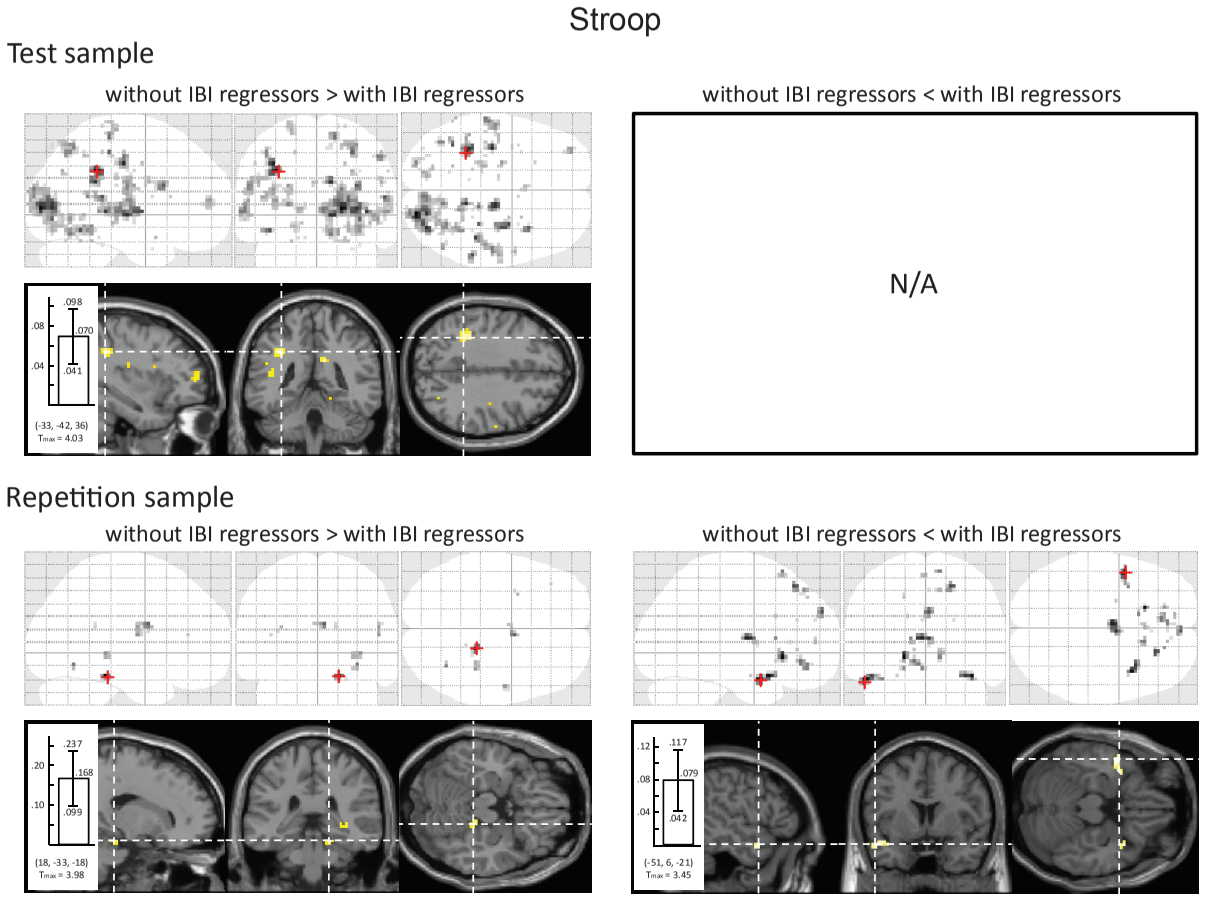
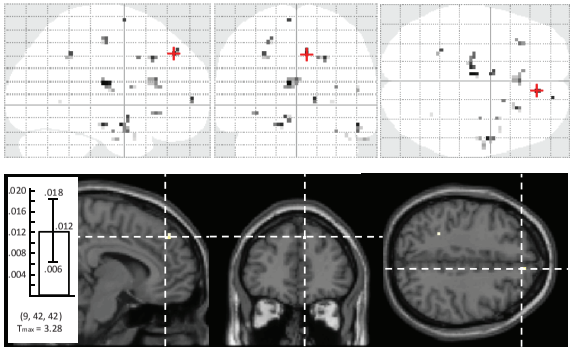


Figure 7.6. *Results of paired t-tests, for the Test sample (top) and Repetition sample (bottom), that compare brain activations during Stroop color-word interference in each individual as estimated with versus without accounting for the 7 IBI-regressors in the GLM. On the left, tests for regions with higher activation when IBI-regressors were not included; on the right, tests for regions with higher activation when IBI-regressors were included. Glass brain projections in each panel indicate the overall distribution of the observed clusters. MRI slice overlays show the voxel with largest statistical difference, with bar graph inserts on each sagittal slice depicting the contrast estimates and 90% confidence interval at this location (numbers within brackets below each bar indicate the MNI coordinates of the voxel and Tmax; the maximum statistical T-value).*

ToL

Test sample

without IBI regressors > with IBI regressors

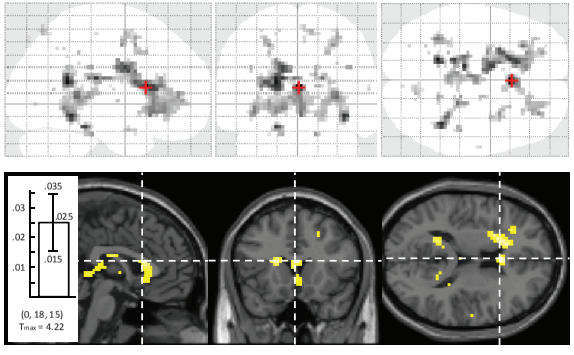


without IBI regressors < with IBI regressors

N/A

Repetition sample

without IBI regressors > with IBI regressors



without IBI regressors < with IBI regressors

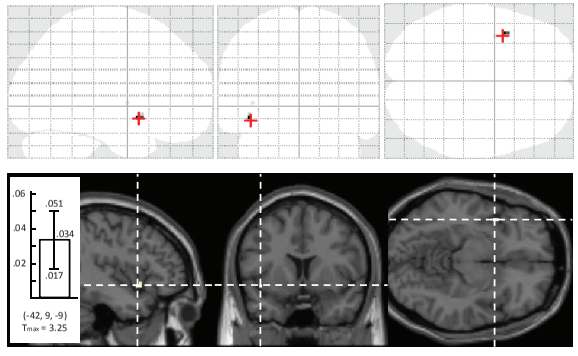


Figure 7.7. *Results of paired t-tests, for the Test sample (top) and Repetition sample (bottom), that compare brain activations for Tol planning versus baseline in each individual as estimated with versus without accounting for the 7 IBI-regressors in the GLM.*

Table 7.4. Correlations of 1st level results with task related IBI changes

	Test sample						Repetition sample					
task	# activated voxels			Maximum T score			# activated voxels			Maximum T score		
	N	*p*	Pearson r	N	*p*	Pearson r	N	*p*	Pearson r	N	*p*	Pearson r
Stroop	46	0.665	0.066	46	0.563	-0.088	45	0.083	0.261	45	0.077	0.266
ToI	46	0.067	-0.273	46	0.305	-0.155	45	0.485	0.114	45	0.238	0.180

Correlations between the task related heart rate change in each subject and the difference in number of suprathreshold voxels (# activated voxels: at p < 0.005, uncorrected) and maximum statistical T scores (Maximum T score) as derived from a GLM with versus without heart rate regressors. Left columns: results for the Test sample; right columns: results for the Repetition sample. N: number of subjects; Pearson r: Pearson's bivariate correlation coefficient; p: statistical significance of correlation. Significant positive correlations would indicate that inclusion of heart rate regressors results in a relatively larger reduction in number of activated voxels/maximum T scores for subjects with stronger task related heart rate increases and vice versa. Significant negative correlations would indicate a weaker reduction (or an increase) in number of activated voxels/maximum T scores after inclusion of heart rate regressors in subjects with stronger increases in heart rate and vice versa.

Discussion

In this study we found that heart rate was influenced by task difficulty. This is in line with previous evidence for substantial momentary variation in heart rate both at rest and during conditions of mild cognitive load, assumed to reflect a complex set of hormonal, thermoregulatory, hemodynamic and respiratory effects on neural control over the heart (Berntson et al., 1997). The increase in heart rate to ToL planning stimuli was quantitatively similar to the heart rate change noted after color-word incongruent Stroop stimuli, despite the fact that the ToL task was administered in an event-related design, rather than a blocked design as used for the Stroop. It must be emphasized however that the inter-stimulus time windows in the present ToL task were determined by the planning reaction times of the subjects which amounted to about 12 seconds for the 4-steps condition. These relatively long trial spacings provide a substantial amount of time to allow for significant heart rate changes.

The spontaneous variations in heart rate were highly correlated with the fMRI modulations during our cognitive paradigms. Similar correlations across large parts of the brain, but most robust over posterior regions, have been noted for functional recordings during rest (Chang et al., 2009; de Munck et al., 2008; Shmueli et al., 2007). Furthermore, the pattern of correlations across the 7 heart rate regressors in this study is highly consistent with the response function between heart rate and fMRI modulations during the resting state condition as reported by de Munck and colleagues [(2008), cf. fig. 4]. For the IBI regressor aligned in time with the fMRI data (0*TR) and a regressor delayed by 1*TR, positive associations

between heart rate and fMRI changes were observed, while for larger delays of 2, 3 and 4*TR, there were inverse associations. This essentially indicates that heart rate increases are initially followed, between 0 and 4.6 seconds, by enhanced fMRI signal, and thereafter, between 4.6 and 9.2 seconds, by reduced fMRI signal (and vice versa). Similar inverse relations were observed for the IBI regressors advanced in time by -2*TR, and in particular -1*TR. This would mean that fMRI signal increases (decreases) are generally followed up to 4.6 second later by decreased (increased) heart rate.

It should be kept in mind that the associations between heart rate and fMRI modulations observed in this study do not necessarily imply causal relations. For example, the possibility that shifting the IBI regressor backward or forward in time just results in bringing the low frequency component of the IBI changes either in or out of phase with respect to the low frequency component of the fMRI modulations may play a role. These low frequency fluctuations in IBI and fMRI signals may for example be caused by respiratory effects that are ignored in our study. However, the fact that the correlation pattern across the 7 heart beat regressors was similar during both tasks of this study, in particular for the unshifted regressor and the regressor delayed by 1*TR, and that IBI-fMRI response functions comparable to the present ones have been observed in the resting state study by de Munck et al. (2008), indicates that the observed heart rate-fMRI relations, at least partly, represent systematic effects.

Irrespective of the exact biological background, the presence of associations between heart rate and fMRI changes together with our finding that heart rate was influenced by task demands creates a theoretical potential for confounding of computed fMRI group effects. In the paradigms and control subjects we explored, however, the effect was small and not statistically significant. For both Stroop color-word interference and ToL planning, comparison of fMRI main effects after ignoring IBI information in the GLM with the main effects obtained after using IBI time series as nuisance regressors revealed the same general neurocircuitry engaged. In this context, it should be noted that the reduction of inter beat times after incongruent compared to congruent color-word stimuli for the Stroop and 4-steps planning versus baseline for the ToL were both in the order of about 8 milliseconds. Although statistically significant, these changes are only marginal relative to the standard deviations of IBI times in this study which were in the order of 130 ms. Overall, SPMs from the GLM with regressors did show a tendency towards small reductions in voxel T-scores and number of suprathreshold voxels compared to the SPMs obtained without heart beat regressors. This effect of heart rate regressors on the SPMs, although not statistically significant, does imply that their inclusion in the GLM can potentially influence final conclusions, in particular in studies dealing with weak-to-detect fMRI signal changes.

Our final conclusion is that there are substantial correlations between heart rate and fMRI signal changes across large parts of the brain during performance of cognitive tasks. However, even if heart rate is significantly modulated by task demands, the fMRI signals associated with heart rate variations only marginally impact on higher-order fMRI task effects. This conclusion is based on heart rate and fMRI data recorded during a commonly used cognitive task administered in a block design as well as a standard cognitive task presented in an event-related design, with relatively wide trial spacing. However, we expect similar results for cognitive test paradigms with closer trial spacing, in particular since smaller trial-to-trial onset times allow less time for significant heart rate changes. The absolute values of the observed IBI changes in this study were relatively small (around 8 ms). During tasks with higher emotional valence or in specific patient samples, heart rate changes and their effect on fMRI signals may be more pronounced. In particular for these cases we still recommend to evaluate the effect of including IBI data as nuisance regressors, also considering the small effort of deriving the regressors from the ECG.

8 Sex differences in gray and white matter structure: converging evidence from complementary methods

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Abstract

Imaging studies on sex differences in human brain structure have reported inconsistent findings. This might relate to suboptimal matching for age, family environment or family background. In addition, previous studies generally focussed on a single structural measure which provides an incomplete picture of male-female brain differences. We investigated sex differences in regional gray matter volume, white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs. Our results, which we confirmed in a unique subsample of 24 opposite sex twin pairs that optimally controls for genetic, intrauterine and familial environmental factors, provide a comprehensive and robust picture of sex differences in brain structure. Males showed larger gray matter volume and higher fractional anisotropy in, or surrounding, subcortical structures (hypothalamus, putamen, globus pallidus, thalamus) that have been linked to neuropsychiatric disorders with higher prevalence in males, and females had larger gray matter volume and a thicker cortex in the insula and anterior cingulate which have been related to neuropsychiatric disorders with higher prevalence in females. From this, we conclude that sex differences should be considered when studying the neurobiology of neuropsychiatric disorders that differ in prevalence or symptoms between the sexes.

Introduction

Sex differences in human brain anatomy are thought to play a crucial role in the differential sensitivity to psychiatric disorders between males and females (Abel et al., 2010; Parker and Brotchie, 2010; Rucklidge, 2010; Bekker and van Mens-Verhulst, 2007) as well as in sex differences in specific cognitive abilities (Halpern, 1997; Loring-Meier and Halpern, 1999; Mann et al., 1990; Burgaleta et al., 2011). The brains of males and females already begin to differ in an early developmental stage through the action of sex specific factors, such as hormonal, genetic and epigenetic factors (McCarthy and Arnold, 2011), and sex-specific maturation further continues during puberty and adolescence (Sisk and Zehr, 2005). Post-mortem and in vivo imaging studies of both children and adults consistently report that males have an approximately 9-12% larger brain volume than females. Apart from this global volume difference, regional sexual dimorphisms have also been found, primarily for areas with high numbers of sex steroid receptors. After correcting for total brain volume, males tend to have larger gray matter volumes in amygdala and hypothalamus, whereas females tend to have larger orbitofrontal, hippocampal and caudate volumes, for a review see Cosgrove et al. (2007) and Lenroot and Giedd, (2010). However, no difference in hippocampal and amygdalar volumes between males and females (Gur et al.,

2002) or larger hippocampal volume in males compared to females has also been reported (Good et al., 2001). Neuroimaging studies that investigated sex differences in cortical morphometry observed a thicker cortex in males in regions of the temporal lobe (Luders et al., 2006; Sowell et al., 2007; Lv et al., 2010), whereas in females a thicker cortex was observed for frontal (Im et al., 2006; Luders et al., 2004; Sowell et al., 2007; Lv et al., 2010), parietal (Im et al., 2006; Luders et al., 2004; Sowell et al., 2007; Lv et al., 2010) and occipital regions (Im et al., 2006; Luders et al., 2004; Lv et al., 2010). However, there are also studies that found no differences in cortical thickness between males and females (Salat et al., 2004; O'Donnell et al., 2005; Nopoulos et al., 2000; Crespo-Facorro et al., 2011).

Mixed results have also been reported for sex differences in regional white matter volumes. The corpus callosum has been found to be larger in females (Lacoste-Utamsing and Holloway, 1982), larger in males (Sullivan et al., 2001), or of similar size for males and females (Bishop and Wahlsten, 1997). Other regions reported to show significant sex differences include the anterior temporal lobe and internal capsule which was found to be larger in males and the posterior frontal lobe and optic radiation which was found to be larger in females (Good et al., 2001).

To examine sex differences in white matter microstructure in more depth, recent studies have applied diffusion tensor imaging (DTI). The most reported metric derived from DTI is fractional anisotropy (FA), which is a relative measure of the degree of water diffusion anisotropy within a voxel (Mori and Zhang, 2006; Beaulieu, 2002; Basser and Pierpaoli, 1996; Le Bihan et al., 2001) and can be interpreted as a proxy measure of white matter integrity. Higher fractional anisotropy in males has been reported for the internal capsule, thalamus, cingulate, occipito-parietal and temporal regions (Schmithorst et al., 2008; Chou et al., 2011; Menzler et al., 2011), whereas in females, higher fractional anisotropy has been found in the fronto-occipital fasciculus and parahippocampal regions (Chou et al., 2011). For other regions there are again inconsistencies. Higher fractional anisotropy in the left frontal lobe of females has been found (Szeszko et al., 2003), while other studies reported higher frontal fractional anisotropy in males (Schmithorst et al., 2008; Chou et al., 2011). Fractional anisotropy in the corpus callosum was found to be higher in males in some studies (Shin et al., 2005; Menzler et al., 2011) whereas others report higher fractional anisotropy in the corpus callosum of females (Chou et al., 2011; Schmithorst et al., 2008).

Inconsistencies across studies that investigated structural brain differences between the sexes may originate from differences in the analysis technique used (e.g., voxel-by-voxel comparison of fractional anisotropy versus tract-based spatial statistics (TBSS)). Previous studies also generally focussed on a single anatomical

parameter obtained from a specific measurement technique (e.g., gray matter volume, gray matter thickness, white matter volume or white matter integrity). The use of complementary techniques and converging evidence from anatomical measures (e.g., voxel-based morphometry (VBM) and cortical thickness for gray matter structure) would strongly improve the robustness of the findings. In addition and perhaps more importantly, previous studies generally lack a careful matching for age, family environment and family background which have shown to contribute substantially to individual differences in global as well as regional brain volume measures (Thompson et al., 2001; Toga and Thompson, 2005).

The present study aims to study sex differences in structural brain measures more thoroughly, by simultaneously investigating differences in regional gray matter volume, white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs, including 24 opposite sex twin pairs and 5 male-female sibling pairs close in age. In addition, these four brain measures were re-analyzed in the opposite sex twin pairs only, who are matched not only for age, but also for their early developmental environment, including the intrauterine environment, and part of their genetic background.

Based on previous findings we expect to find larger hypothalamic gray and internal capsule and temporal white matter volumes in males and larger caudate gray matter and frontal gray and white matter volumes in females. Regional sex differences detected by voxel-based mapping of gray and white matter volumes are expected to converge with sex differences in cortical thickness and white matter integrity. These sex differences should be confirmed when controlling for genetic and familial environmental factors.

Methods

Participants

Participants were recruited from an ongoing study in the Netherlands Twin Register (NTR) that investigates environmental and genetic influences on obsessive-compulsive (OC) symptoms (den Braber et al., 2010). We selected unrelated male-female pairs closely matched for age as well as Dizygotic-Opposite-Sex (DOS) twin pairs and male-female sibling pairs (maximum age difference 5 years) in the age range between 18 and 60 years. The DOS twin pairs and sibling pairs are very well matched, not only for age, but also for their early developmental environment and part of their genetic make-up. Exclusion criteria were brain damage, neurological disease, color blindness and contraindications for MRI (e.g., pregnancy, ferromagnetic fragments, clips and devices in the body and claustrophobia). In total, 69 male-female pairs (mean age 30.9, sd=0.71) participated

Table 8.1. Sample characteristics

	Males (*n* = 69)	Females (*n* = 69)	df	t-value	*p* -value
Demography					
Age	31.76±7.64	31.96±7.46	45	-.719	.476
Educational attainment (% low/middle/high)	10.1/30.4/59.4	8.7/34.8/56.5	2	.327*	.849
Global brain measures					
Gray Matter	750.49±61.69	662.47±55.65	68	11.373	<.001
White Matter	529.52±46.40	468.27±43.22	68	10.773	<.001
Total Intracranial Volume	1540.91±125.50	1355.83±114.34	68	11.935	<.001
Mean Fractional Anisotropy	0.30±0.01	0.29±0.01	68	2.791	.007
Mean Cortical Thickness	4.56±0.17	4.59±0.19	68	-1.084	.282

*Age: mean (±SD) age at time of MRI examination (in years); educational attainment (% low/middle/high): percentage of males and females with low, middle or high educational level. df: degrees of freedom. * tested using Chi-square statistics.*

in our MRI study, including 24 DOS twin pairs, 5 sibling pairs and 40 age-matched male-female pairs **(table 8.1)**.

Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, and neuropsychiatric illness as described elsewhere (den Braber et al., 2010). Educational attainment was assessed as the highest level of education of the participant, divided into 3 categories: 1) lower general and vocational education; 2) intermediate vocational and intermediate/higher general education; 3) higher vocational college and university. The ethical review board of the VU University medical centre approved the study protocol. All participants provided written informed consent.

Image acquisition

The MRI session consisted of an anatomical scan of about 6 minutes and a DTI scan of approximately 3 minutes. During the scan sessions, the participants remained inside the scanner and were asked to minimize head movement during and between consecutive runs. To reduce motion artifacts, each participant's head was immobilized using foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3D T1-weighted gradient-echo sequence (flip angle 8°; Repetition Time, TR = 9.69 ms; Echo Time, TE = 4.60 ms; matrix, 256x256 pixels;

voxel size, 1.00x1.00x1.20 mm). Diffusion tensor images were obtained in 32 directions by using single-shot echoplanar acquisition (flip angle 90°; Repetition Time, TR = 4834 ms; Echo Time, TE = 94 ms; matrix, 112x110 pixels; voxel size, 2.00x2.00x3.00 mm; b-value 1000 s/mm^2, 38 axial slices).

Data analysis

Regional gray matter and white matter volume differences between males and females were analyzed using VBM as implemented in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). T1-weighted MR images were segmented into gray matter, white matter and cerebrospinal fluid (CSF), and normalized to a group template (i.e., a specific template created from the 138 subjects that participated in this study) using the Diffeomorphic Anatomical Registration Through Exponential Lie algebra (DARTEL) algorithm, and subsequently warped from DARTEL space to the standard Montreal Neurological Institute (MNI) brain. To preserve volumetric information, a modulation step was added. Before statistical analysis, the resultant modulated images were spatially smoothed with an 8 mm isotropic Gaussian kernel.

To investigate sex differences in white matter integrity, fractional anisotropy maps were calculated from raw DTI scans using the Medical Image Navigation and Research Tool by INRIA (MEDINRIA, Asclepios Research Project - INRIA Sophia Antipolis, France). Fractional anisotropy maps were then co-registered with T1-weighted MR images and normalized using each subject's T1 to DARTEL to MNI warp parameters. Subsequently, data were spatially smoothed with an 8 mm isotropic Gaussian kernel and a voxel-by-voxel comparison of the fractional anisotropy values was performed in SPM8. As an alternative method statistical analysis of the fractional anisotropy data was carried out using TBSS (Smith et al., 2006), part of FSL (Smith et al., 2004), which projects all subjects' fractional anisotropy data onto a mean fractional anisotropy tract skeleton, before applying voxelwise cross-subject statistics. This was done in order to confirm the obtained voxel-by-voxel fractional anisotropy comparison and gives the opportunity to visualize WM differences on a true anatomical tract basis.

To investigate sex differences in cortical thickness delineation of gray and white matter surfaces were determined from MRI using FreeSurfer (Fischl and Dale, 2000) (http://surfer.nmr.mgh.harvard.edu/). Segmentation/boundary tessellation was checked for each scan by means of visual inspection, and manually adjusted when necessary. Subsequently, each individual's surface was registered onto the average surface provided by Freesurfer and spatially smoothed by 5 mm FWHM. Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface.

Statistical tests

Sex differences in demographic and global brain measures were tested using paired sample t-tests or Chi-square test (SPSS, Chicago, Illinois). Statistical results were considered significant at $p < 0.05$, Bonferroni corrected.

Differences in regional gray matter volume, regional white matter volume, fractional anisotropy maps, and cortical thickness between males and females were investigated using paired sample t-tests. Group differences are reported at an individual voxel threshold of $p < 0.05$, corrected for multiple comparisons. In addition, a paired sample t-test was performed on the DOS twin pairs only. For the confirmative analysis in this smaller subsample, group differences are reported at an individual voxel threshold of $p < 0.005$, uncorrected.

Results

Sample characteristics

Sample characteristics for the scanned males and females are summarized in the top of **table 8.1**. As expected, matched male-female pairs (excluding DOS twin pairs that are always identical for age) did not differ significantly in mean age ($t(45)=-.719$, $p=<.476$). Furthermore, males and females did not differ with respect to educational attainment ($\chi^2(2)=3.27$, $p=.849$).

Sex differences in global brain measures

Means, standard deviations and t-statistics for global brain volume measures are presented in the bottom of **table 8.1**. Males had larger total intracranial volumes (TIV), gray matter (GM) and white matter (WM). In addition, mean fractional anisotropy was higher in males. No significant differences in mean cortical thickness between the sexes was observed.

Sex differences in regional gray matter

Regional volumes (VBM: adjusted for total intracranial volume)

Table 8.2 (top) summarizes GM regions that were found to be larger in males compared to females, analyzed across all 69 pairs (left) and the DOS pairs only (right). In both analyses, these included 4 subcortical structures: hypothalamus, putamen, globus pallidus and thalamus pulvinar **(figure 8.1, top)**. Furthermore, clusters of larger cortical GM volume were found in the right precentral gyrus, right caudal middle frontal gyrus, left superior temporal gyrus and right cerebellum. From these, only the significant difference for the right precentral gyrus could be confirmed in the DOS sample.

Table 8.2. Sex Differences in Regional Gray Matter Volume

Test	Anatomical location	All pairs (69 males/69 females)				DOS pairs only (24 males/24 females)			
		MNI coordinates			T-value	MNI coordinates			T-value
		x	y	z		x	y	z	
males>females	right precentral	13.5	-30	64.5	3.90	15	-28.5	63	2.83
		24	-10.5	58.5	3.75	--	--	--	--
	right caudal middle frontal	34.5	18	49.5	3.38	--	--	--	--
	right hypothalamus	6	1.5	-12	8.52	6	1.5	-12	3.36
	left hypothalamus	-13.5	1.5	-16.5	5.60	-13.5	-1.5	-15	2.87
	right putamen	24	1.5	7.5	4.71	24	0	7.5	2.93
	left putamen	-24	1.5	9	5.07	-24	1.5	10.5	2.87
	right thalamus pulvinar	15	-33	7.5	4.57	--	--	--	--
	left thalamus pulvinar	-16.5	-34.5	3	4.45	-19.5	-34.5	6	2.89
	right globus pallidus	25.5	-19.5	-4.5	3.89	25.5	-18	1.5	2.89
	left superior temporal	-52.5	21	-13.5	4.73	--	--	--	--
	right cerebellum	49.5	-66	-52.5	3.87	--	--	--	--
females>males	right postcentral	40.5	-19.5	34.5	4.41	--	--	--	--
		22.5	-43.5	48	4.40	--	--	--	--
	left caudal anterior cingulate	-9	3	33	4.81	-10.5	-7.5	34.5	2.93
	right rostral middle frontal	43.5	36	16.5	4.50	46.5	34.5	13.5	2.83
	left insula	-31.5	-28.5	12	4.76	-31.5	-28.5	12	4.84
	left inferior temporal	-48	-25.5	-16.5	4.70	-48	-25.5	-16.5	3.43

Clusters with regional GM differences between males and females, analyzed across all 69 male-female pairs (left), and in the 24 DOS pairs only (right). Test: test for significant GM increases in males compared to females (males > females) or significant GM increases in females compared to males (females > males); Anatomical location: enlarged brain region; MNI coordinates (mm): location of voxel with largest effect size; T-value: t-statistics of voxel with largest effect size. Shaded rows indicate regions that showed both larger GM volume and thicker cortex.

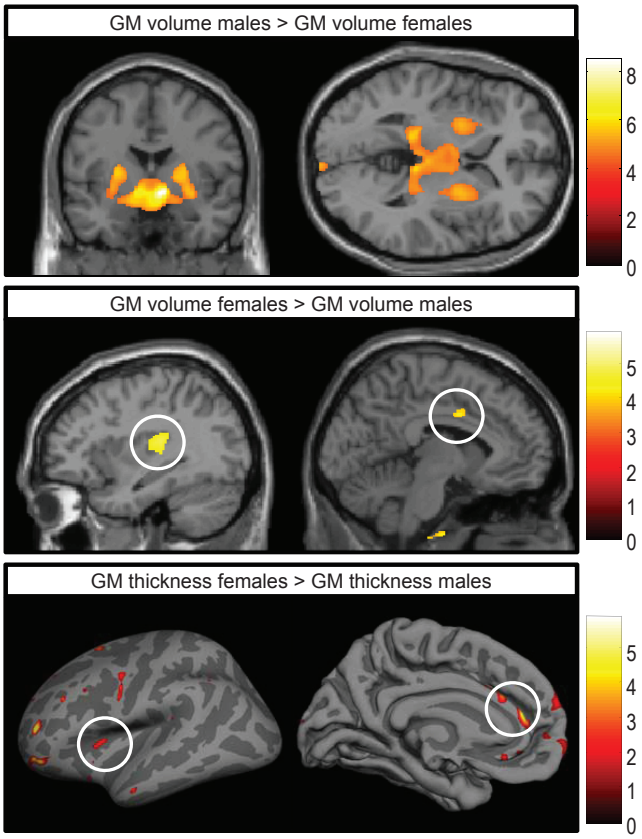

Figure 8.1. *Top: Subcortical brain regions showing larger gray matter volumes in males compared to females (including bilateral hypothalamus, putamen and thalamus pulvinar).*
Middle and bottom: Examples of brain regions showing both larger GM volume as well as higher cortical thickness in females compared to males (left middle encircled: left insular volume enlargement (lateral view); left bottom encircled: thicker left insular cortex (lateral inflated view, gyri in white, sulci in gray); right middle encircled: left anterior cingulate enlargement (medial view); right bottom encircled: thicker left anterior cingulate cortex (medial view, gyri in white, sulci in gray).

The bottom of **table 8.2** shows GM regions that are larger in females. These included the left caudal anterior cingulate gyrus, right rostral middle frontal gyrus, left insula and left inferior temporal gyrus. Two clusters of larger cortical GM were found in the right postcentral gyrus that could not be confirmed in the DOS pairs.

Cortical thickness (Freesurfer)

Table 8.3 (top) summarizes parts of the brain where males showed to have a thicker cortex compared to females, analyzed across all 69 pairs and for the DOS pairs only. In general, for both the left and right hemisphere these included more posterior structures of the brain, such as isthmus cingulate, inferior parietal cortex, precuneus, and lingual cortex, of which the latter two were confirmed in the DOS

pair analysis. For the left hemisphere, additional clusters were observed in the medial orbitofrontal cortex, parahippocampal cortex, middle temporal cortex and temporal pole, from which the latter one could be confirmed in the DOS pairs. For the right hemisphere, additional clusters were observed in the postcentral cortex, supramarginal cortex and lateral occipital cortex, from which the supramarginal cortex and lateral occipital cortex could be confirmed in the DOS pairs.

The bottom of **table 8.3** shows parts of the brain where females were found to have a thicker cortex. In general, for both the left and right hemisphere significant clusters were located in more anterior structures of the brain, such as the parahippocampal cortex, superior frontal cortex, caudal middle frontal cortex, parsorbitalis, lateral and medial orbitofrontal cortex, frontal pole, rostral and caudal anterior cingulate, insula, superior temporal cortex, and temporal pole. All of these were confirmed in the DOS pair analysis, except for the parahippocampal and medial orbitofrontal cortex. For the left hemisphere, additional clusters were observed in the lateral occipital cortex, rostral middle frontal cortex, precentral cortex, middle and inferior temporal cortex, fusiform cortex, and supramarginal cortex, which, except for the lateral occipital cortex, could be confirmed in the DOS pair analysis. For the right hemisphere, additional clusters were observed in the parsopercularis, postcentral cortex, isthmus cingulate, lingual cortex and inferior parietal cortex, from which the postcentral and inferior parietal cortex could be confirmed in the DOS pair analysis.

Interestingly, within some brain regions, including the right postcentral gyrus, left caudal anterior cingulate gyrus, left insula and left inferior temporal gyrus, females showed significant clusters of increased GM volumes as well as increased cortical thickness (middle and bottom row of **figure 8.1** and **table 8.2** and **8.3**, shaded rows).

Sex differences in regional white matter

Regional volumes (VBM: adjusted for total intracranial volume)

The male-female comparison for regional white matter volume revealed no significant sex differences.

White matter integrity – fractional anisotropy (voxel-based comparison/TBSS)

Table 8.4 (top) summarizes clusters of significant higher fractional anisotropy in males compared to females, analyzed across all 69 pairs (left) and for the DOS pairs only (right). In both analyses, significant clusters were observed in the superior longitudinal fasciculus, inferior longitudinal fasciculus, internal and external capsule, anterior thalamic radiation, corona radiata and corpus callosum. These results were confirmed by the analyses of fractional anisotropy using

Table 8.3. Sex Differences in Cortical Thickness

		Left hemisphere								Right hemisphere							
		All pairs (69M / 69F)				DOS pairs only (24M / 24F)				All pairs (69M / 69F)				DOS pairs only (24M / 24F)			
Test	Anatomical label	MNI coordinates			T-value	MNI coordinates			T-value	MNI coordinates			T-value	MNI coordinates			T-value
		x	y	z		x	y	z		x	y	z		x	y	z	
males>females	medial orbitofrontal	-10.4	7.7	-14.9	5.67												
	isthmus cingulate	-5.7	-42.7	30.7	3.78					15.5	-51.0	6.5	3.69				
	postcentral									44.2	-24.8	40.0	4.15				
	parahippocampal	-20.1	-18.5	-26.3	3.48												
	lingual	-22.9	-54.8	0.0	4.16	-19.9	-64.1	-8.9	3.46	4.7	-53.2	3.8	5.50	19.5	-68.1	2.7	3.18
	temporal pole	-32.7	4.1	-14.1	4.78	-32.5	2.5	-14.5	4.27								
	middle temporal	-62.2	-26.0	-13.3	5.20												
	precuneus	-14.7	-49.0	39.1	3.90	-14.5	-49.7	39.1	4.08	5.6	-64.2	28.6	3.72	6.8	-55.7	18.9	2.98
	supramarginal									46.7	-33.7	28.7	3.72	37.2	-36.9	37.0	3.31
	inferior parietal	-38.9	-54.9	14.8	3.43					49.1	-50.2	25.6	4.13	48.2	-47.7	22.2	2.97
	lateral occipital									43.0	-67.0	5.5	4.30	42.5	-67.8	4.8	3.24
females>males	superior frontal	-12.1	54.6	25.2	4.91	-14.8	57.5	15.3	3.19	15.6	37.2	44.0	4.00	16.6	36.1	44.9	4.49
	rostral middle frontal	-36.2	48.9	12.3	5.34	-36.2	44.5	15.3	3.43								
	caudal middle frontal	-40.0	18.1	33.7	3.65					38.5	4.2	45.4	3.67	33.2	6.8	32.1	3.39
	parsorbitalis	-37.8	46.5	-9.7	5.20	-43.7	40.0	-10.9	3.40	43.9	36.9	-12.8	3.77	42.2	43.5	-7.7	2.97
	lateral orbitofrontal	-21.2	18.7	-18.0	4.12					16.9	30.6	-22.6	5.06	24.0	15.0	-18.5	4.37
	medial orbitofrontal	-7.2	28.0	-10.6	3.88					11.6	48.1	-3.4	3.94				
	frontal pole	-11.9	62.5	-5.7	5.65	-12.7	62.5	-5.1	3.75	10.5	64.4	-3.3	3.94				
	parsopercularis									53.0	24.0	15.7	4.01				
	precentral	-55.0	-2.1	36.5	4.27	-57.8	-4.8	11	3.26								
	postcentral									43.0	-28.7	62.1	3.78	48.8	-21.8	55.8	3.02
	rostral anterior cingulate	-7.8	37.3	12.6	5.89	-9.0	34.5	16.5	3.54	7.8	37.0	0.3	3.93				
	caudal anterior cingulate	-7.6	24.7	25.2	4.51	-9.0	16.8	30.7	3.47	7.8	33.8	17.5	4.52				
	isthmus cingulate									9.1	-39.3	26.3	3.70				
	insula	-30.4	10.3	11.7	4.12					32.9	13.1	-7.5	4.24	36.3	-2.0	-7.1	2.91
	superior temporal	-62.8	-26.8	5.1	3.47	-58.1	-6.7	-1.3	4.25	63.6	-36.6	12.7	4.58	50.7	2.5	-21.7	3.20
	middle temporal	-57.3	-5.8	-26.6	4.17	-57.3	-7.3	-24.4	3.29								
	inferior temporal	-45.5	-14.1	-35.8	3.59	-54.4	-26.7	-28.3	3.10								
	temporal pole	-34.6	14.4	-35.3	3.47	-33.6	13.8	-35.3	3.14	37.7	14.9	-35.4	3.87	40.7	13.1	-35.6	4.12
	lingual									14.8	-91.3	-6.8	3.79				
	parahippocampal	-28.1	-20.9	-28.4	3.80					29.5	-30.8	-18.1	3.67				
	fusiform	-33.5	-6.2	-34.0	3.76	-33.6	-37.3	-20.3	3.09								
	supramarginal	-49.3	-54.7	42.2	3.92	-49.1	-54.9	42.2	3.13								
	inferior parietal									40.3	-78.7	17.1	4.22	36.1	-75.9	35.2	4.32
	lateral occipital	-11.2	-101.2	6.8	3.64												

Clusters with cortical thickness differences between males and females for the left and right hemisphere, again analyzed across all 69 male-female pairs and in the DOS twin pairs only. Test: test for brain regions showing significant higher cortical thickness in males compared to females (males > females) or significant higher cortical thickness in females compared to males (females > males); Anatomical label: vertex label as provided by Freesurfer; MNI coordinates (mm): location of vertex with largest effect size; T-value: t-statistics of vertex with largest effect size. Shaded rows indicate regions that showed both thicker cortex and larger gray matter volume.

Table 8.4. Sex Differences in Fractional Anisotropy

		All pairs (69 males / 69 females)				DOS pairs only (24 males / 24 females)			
Test	White matter tract	MNI coordinates			T-value	MNI coordinates			T-value
		x	y	z		x	y	z	
males > females	left superior longitudinal fasciculus	-33	10.5	22.5	4.60	-31.5	13.5	25.5	3.22
	right superior longitudinal fasciculus	39	1.5	28.5	4.27				
	left inferior longitudinal fasciculus	-46.5	-24	-15	3.62	-48	-30	-15	3.51
	right inferior longitudinal fasciculus	49	-13	-4	4.63	52.5	-7.5	-4.5	3.72
	left internal capsule	-21	3	16	4.66	-24	6	15	2.87
	right internal capsule	21	3	16	4.20	24	0	15	2.99
	left external capsule	-30	2	15	4.65	-28.5	4.5	15	3.07
	right external capsule	30.5	-3	14	4.60	30	0	13.5	3.68
	left anterior thalamic radiation	-15	-13.5	13.5	4.15	-15	-33	7.5	3.82
	right anterior thalamic radiation	12	-22	12	3.64	10.5	-15	7.5	2.92
	left corona radiata	-24	12	15	3.01	-25.5	10.5	15	2.90
	right corona radiata	27	12	15	2.60				
	corpus callosum	1.5	16.5	21	4.72	18	27	18	2.94
females > males	left forceps minor	-12	39	-10.5	3.56	-12	42	-3	2.87
	right forceps minor	7.5	34.5	-12	3.21				
	right superior corona radiata	22.5	-15	34.5	3.32	28.5	-6	28.5	2.98
	left corticospinal tract	-4.5	-21	-28.5	3.74	-4.5	-21	-28.5	2.95
	right corticospinal tract	7.5	-19.5	-27	3.22	3	-19.5	-25.5	2.89
	right superior longitudinal fasciculus	45	-6	43	3.92				
	left inferior longitudinal fasciculus	-31.5	4.5	-34.5	4.00	-33	4.5	-31.5	3.01

Clusters with regional fractional anisotropy differences between males and females obtained from voxel-based analysis, measured across all 69 male-female pairs (left), and in the 24 DOS pairs only (right). Test: test for significant fractional anisotropy increases in males compared to females (males > females) or significant fractional anisotropy increases in females compared to males (females > males); White matter tract: white matter tract showing increased fractional anisotropy; MNI coordinates (mm): location of voxel with largest effect size; T-value: t-statistics of voxel with largest effect size.

TBSS (**figure 8.2**).

The bottom of **table 8.4** shows clusters of significant higher fractional anisotropy in females. Significant clusters were observed bilaterally in the forceps minor and corticospinal tract and in right superior corona radiata, right superior longitudinal fasciculus and left inferior longitudinal fasciculus. Most of these findings could be confirmed in the DOS pair analysis, except for the right forceps minor and right superior longitudinal fasciculus. Re-analyses of the fractional anisotropy data using TBSS provided no significant results.

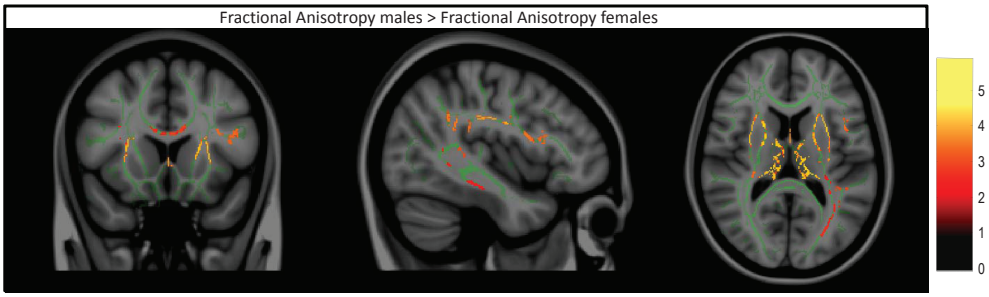

Figure 8.2. *Brain white matter tracts showing increased fractional anisotropy in males compared to females (in red/yellow) projected on a mean fractional anisotropy skeleton (in green) which represents the centers of all tracts common to the group (carried out using TBSS, part of FSL).*

Discussion

The present study aimed to create a more comprehensive picture of sex differences in structural brain measures, by investigating differences in regional gray and white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs, including 5 sibling pairs and 24 opposite sex twin pairs. In addition, data were re-analyzed in 24 opposite sex twin pairs only, in order to measure whether perfectly controlling for genetic, intrauterine and familial environmental factors would affect the results. Our analyses indicated that males have larger gray matter volumes and higher fractional anisotropy in, or close to, subcortical brain regions (e.g., hypothalamus, thalamus, putamen), whereas females have both larger gray matter volumes as well as greater cortical thickness in insular, anterior cingulate, postcentral and inferior temporal cortices. Results are discussed in more detail below, with a specific focus on regions that held in the opposite sex twin pair analysis and/or brain regions in which sex differences were confirmed by different structural brain measures (gray/white matter volume, fractional anisotropy, cortical thickness).

Sex differences in global brain measures

This study showed that males have significantly larger total intracranial volumes and a higher fractional anisotropy across the brain. The finding of larger global brain volumes in males is in line with the previous literature and is one of the most robust findings on sex differences in the brain, for review see Cosgrove et al. (2007) and Lenroot and Giedd, (2010). Higher mean fractional anisotropy in males has also been found previously (Kang et al., 2011). It has been reported that male brains possess higher neuronal densities, a higher number of neurons and fewer neuropil (i.e., neuronal and glial processes) (de Courten-Myers, 1999), with greater white matter volume available for the inter-neuronal connections (Gur et al., 1999; Allen et al., 2003). From this, one might expect males to have fewer but thicker, more organized, and possibly more myelinated fibers, which would explain the higher mean fractional anisotropy observed in males. No sex differences with regard to mean cortical thickness were found, which replicates previous findings (Im et al., 2006; Salat et al., 2004; Crespo-Facorro et al., 2011).

Sex differences in regional gray matter

Males showed more gray matter primarily in subcortical brain regions, such as hypothalamus, thalamus, putamen and globus pallidus, and higher cortical thickness in temporal regions (lingual gyrus and temporal pole) and parietal regions (inferior parietal and precuneus). Females, on the other hand, mainly had more gray matter and a thicker cortex in insular, anterior cingulate, postcentral and inferior temporal brain areas. A thicker cortex was also observed in frontal brain regions (e.g., superior frontal, rostral/caudal middle frontal, orbitofrontal). Interestingly, these regions contain high levels of androgen and estrogen steroid receptors (Goldstein et al., 2001) and therefore are more likely to exhibit sexual dimorphisms.

Our finding of a larger hypothalamus in males is consistent with previous studies (Goldstein et al., 2001; Bao and Swaab, 2010). This brain region contains significant populations of sex steroid receptors, plays a central role in the control of sexual and reproductive function, has been related to sexual orientation and plays a major role in sexual arousal and psychosexual identity (the personal self-representation of being a 'male' individual) (Swaab et al., 2002; Brunetti et al., 2008).

Together with the caudate nucleus, the putamen is regarded the main receptive component of the basal ganglia. Anatomically it is connected to the frontal cortex through a series of basal ganglia-thalamocortical loops that all run via the globus pallidus (Alexander et al., 1986; Haber, 2003). Together with the thalamus and supplementary motor areas the putamen and globus pallidus are principally involved in the so-called motor loop that plays an important role in the

programming and control of movement. Larger volumes of these regions, or higher neural densities have also been found by others (Giedd et al., 1997; Peper et al., 2009a). Interestingly, abnormalities within these regions have been linked to neuropsychiatric disorders that show a higher prevalence in males compared to females, such as tic disorders and schizophrenia (Shenton et al., 2001; Singer and Minzer, 2003).

Regional volume enlargements and higher cortical thickness in insular, anterior cingulate, and prefrontal regions in females have also been observed previously, especially in studies that focussed on cortical thickness measures (Good et al., 2001; Goldstein et al., 2001; Im et al., 2006; Luders et al., 2006; Sowell et al., 2007; Lv et al., 2010). These structures play a major role in emotion and interoceptive awareness (the sense of the physiological condition of the body). The insula has been associated with both detection and experiencing disgust (Wicker et al., 2003), whereas the orbitofrontal cortex (found to be thicker in females) is involved in emotional decision making (Bechara et al., 2000). The anterior cingulate, ventromedial prefrontal and lateral prefrontal cortices have been associated with integrating interoceptive information, and the insula has been found to play an important role in interoceptive attention (Critchley et al., 2004). Moreover, the perception of bodily state, and simultaneous activation of anterior cingulate, insular and prefrontal regions, was proposed as a crucial determinant for the processing and subjective experience of feelings (Pollatos et al., 2007). Interestingly, abnormalities within these brain regions have been found in neuropsychiatric disorders that show higher prevalences in females, such as depression and anxiety disorders and especially the latter is highly associated with altered bodily responses, including sweating, increased heart rate and blood pressure.

During a large part of human evolution males and females had different social roles (e.g., males: hunting, protect group from predators, make and use weapons; females: gather and prepare food and clothes, and care for the children). They also differ substantially in their optimal mating behavior (Buss, 2000; Eagly and Wood, 1999). Our finding of larger regional volumes in brain structures that play a central role in the control of sexual and reproductive function in males and in regions involved in more social emotional skills in females fits this evolutionarily perspective.

Sex differences in regional white matter

Our study did not reveal any difference in regional white matter volume. However, males and females did show significant differences in fractional anisotropy. In males higher fractional anisotropy was observed mainly in white matter tracts close to subcortical brain regions, such as internal and external capsule, anterior thalamic radiation, corona radiata, but also in corpus callosum, superior

longitudinal fasciculus and inferior longitudinal fasciculus. Higher fractional anisotropy surrounding subcortical brain regions is in line with previous reports (Chou et al., 2011; Menzler et al., 2011) as is the higher fractional anisotropy in the corpus callosum (Shin et al., 2005; Menzler et al., 2011). Although the latter finding has not been unequivocal (Schmithorst et al., 2008; Chou et al., 2011) we note that in our unique subsample of opposite sex twin pairs, that optimally controls for genetic, intrauterine and familial environmental factors, the higher corpus callosal fractional anisotropy holds for both the voxel-based comparison and TBSS.

Females showed higher fractional anisotropy primarily in frontal and temporal brain regions, including forceps minor and superior corona radiata, which is in line with results of a previous study (Szeszko et al., 2003), although higher fractional anisotropy in frontal regions has also been reported for males (Schmithorst et al., 2008; Chou et al., 2011). Of note, tract-based spatial statistics could not replicate these findings. However, it should be noted that most of the voxel-based comparison results were located near the white to gray matter boundary. Tract-based spatial statistics makes use of a mean fractional anisotropy tract skeleton that represents the centers of all tracts common to the whole group and usually does not include small tracts near this boundary. Fractional anisotropy differences in small white matter tracts near cortical regions could therefore be easily missed using tract-based spatial statistics.

The use of opposite sex twin pairs in the male-female comparison is a considerable strength of this study in terms of optimally controlling for genetic, familial environmental, and intrauterine factors. It has been hypothesized that females with a male co-twin might be exposed to higher testosterone levels than other women and experience a relative masculinization of the brain. Evidence for a larger total brain volume in opposite-sex female twins compared to same-sex female twins was reported in 9-year old children, but was not found in adults (Peper et al., 2009b). Masculinization would make our comparison more conservative, i.e., sex differences would be attenuated. As the sex differences in our opposite sex twin pairs were robust and highly consistent with the sex differences in the overall sample, the advantages of optimal matching in opposite sex twin pairs seem to outweigh the confounding by potential differences in intrauterine testosterone exposure.

In summary, by simultaneously investigating differences in regional gray matter volume, white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs, and by confirming our findings in a unique subsample of opposite sex twin pairs that optimally controls for genetic, intrauterine and familial environmental factors we were able to create a more comprehensive and robust picture of sex differences in brain structure. Our data

shows males to have larger gray matter volumes and higher fractional anisotropy in, or surrounding, subcortical structures. These brain structures are involved in the control of sexual and reproductive function (hypothalamus) and the programming and control of movement (putamen, globus pallidus, thalamus) and have been associated with neuropsychiatric disorders that show a higher prevalence in males (tic disorders, schizophrenia). Conversely, females were characterized by larger gray matter volumes and greater cortical thickness in brain regions importantly involved in emotion and interoceptive awareness (insula, anterior cingulate) and associated with neuropsychiatric disorders that have a higher prevalence in females (depression, anxiety disorders). The observed sex differences in regional brain structure provide a rich source of information for understanding the behavioral differences that exist between males and females. Sex differences should always be considered in studies on the neurobiology of neuropsychiatric disorders that differ in prevalence or symptoms between the sexes.

9 Obsessive-compulsive symptom related sex differences in brain structure

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A. den Braber, E.J.C. de Geus, D.I. Boomsma, and D. van 't Ent. Obsessive-compulsive symptom related sex differences in brain structure (under review, Biological Psychology).

Abstract

Neuroimaging studies have indicated abnormalities in cortico-striato-thalamo-cortical circuits in obsessive-compulsive disorder patients compared to controls. However, differences have been observed between studies regarding the direction of anatomical changes, which may reflect heterogeneity in the patient groups. Since sex differences in human brain anatomy are very evident and obsessive-compulsive symptomatology and its developmental trajectories tend to be distinct in males and females, we investigated if sex is a potential source of heterogeneity. To investigate this hypothesis, magnetic resonance imaging scans of 31 males scoring high for obsessive-compulsive symptoms, 41 low-scoring males, 58 high-scoring females and 73 low-scoring females were analyzed and the interaction of obsessive-compulsive symptoms by sex on gray matter volume was assessed using voxel-based morphometry. An obsessive-compulsive symptoms by sex interaction was observed for the left middle temporal gyrus (larger in males with obsessive-compulsive symptoms, but no effect in females), the right middle temporal gyrus (larger in males with obsessive-compulsive symptoms, but reduced in females with obsessive-compulsive symptoms) and right precuneus (larger in females with obsessive-compulsive symptoms, but reduced in males with obsessive-compulsive symptoms). These observed differences acted to reduce or hide a main effect in our study and therefore might, in part, explain the different outcomes in the mixed sex-samples previously studied. Our findings illustrate the importance of taking sex into account when investigating the neurobiology of obsessive-compulsive symptoms.

Introduction

Obsessive-compulsive (OC) symptoms are characterized by recurrent, persistent, and intrusive anxiety provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions) performed to reduce anxiety and/or distress caused by the obsessions. Well known obsessions are fear of contamination, pathological doubt, need for symmetry, and somatic, sexual and aggressive obsessions. Compulsions include checking, washing, counting, symmetry/precision and hoarding behavior. When a person has these obsessions and/or performs compulsions for more than one hour a day and these thoughts and rituals significantly interfere with daily life routines, the person fulfills the criteria for obsessive-compulsive disorder (OCD). The life-time prevalence of OCD is 0.5-2% (American Psychiatric Association, 1994; Grabe et al., 2000), but obsessions are much more prevalent in the general population – as high as 72% (Rachman and de Silva, 1978; Salkovskis and Harrison, 1984) and the prevalence of OC symptoms reaches up to 20% (Fullana et al., 2009).

Over the last two decades, neuroimaging studies have indicated several neurobiological changes underlying the psychological and behavioral dysfunction of OCD. Results from structural and functional magnetic resonance imaging (sMRI/fMRI) studies mainly point to volume differences and altered regional brain activation in the ventral prefrontal cortex (PFC), dorsolateral prefrontal cortex (DLPFC), basal ganglia, anterior cingulate cortex (ACC), and thalamus (Menzies et al., 2008a; Radua and Mataix-Cols, 2009; Radua et al., 2010; Rotge et al., 2009). These findings have contributed to the widely accepted neuroanatomical model of OCD involving the direct and indirect cortico-striato-thalamo-cortical (CSTC) loops (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). The direct loop functions as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors. The indirect loop functions as a negative feedback loop important for inhibiting and switching between behaviors. It has been hypothesized that an imbalance between these loops, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate OC symptomatology (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000).

In spite of the convergence on the same brain regions, inconsistencies in the direction of OCD effects have been reported for volumetric differences of the implicated brain areas (e.g., larger vs. smaller) as well as their metabolism (e.g., hypo- or hyperactivation) (Friedlander and Desrocher, 2006; Menzies et al., 2008a). These inconsistencies might be due to methodological differences between studies (e.g., differences in sample size, scanning modalities/parameters and analysis methods) but they may also reflect heterogeneity in the patient groups scanned. For instance, we have shown that brain regions were affected differently in subjects characterized by high environmental risk for OC symptoms than in those characterized by high genetic risk for OC symptoms (den Braber et al., 2010; den Braber et al., 2011).

Here we hypothesize that sex is a second potential source of heterogeneity. Sex differences in human brain anatomy are very evident. The brains of males and females already begin to differ in an early developmental stage through the action of sex specific factors, such as hormonal, genetic and epigenetic factors (McCarthy and Arnold, 2011), and sex-specific maturation continues during puberty and adolescence (Sisk and Zehr, 2005). Postmortem and in vivo imaging studies of both children and adults consistently reported that males have approximately 9-12% larger brain volumes than females. Apart from this global volume difference, regional sexual dimorphisms have also been reported, primarily for areas with high numbers of sex steroid receptors. After correcting for total brain volume, males tend to have larger gray matter volumes in amygdala and hypothalamus, whereas females tend to have larger orbitofrontal, hippocampal and caudate volumes (for review see: Cosgrove et al., 2007; Lenroot and Giedd, 2010).

These sex-specific differences in the healthy brain also highlight the need to evaluate sex differences in the association of brain structure with neuropsychiatric disorders, especially those that differ in prevalence and/or symptoms between males and females, like OCD (Labad et al., 2008; Noshirvani et al., 1991; Castle et al., 1995; Lensi et al., 1996; Tukel et al., 2004; Bogetto et al., 1999). The present study examines differences between males and females with low or high OC symptomatology focusing specifically on gray matter volumes. We hypothesize to find sex-moderation of OC symptomatology effects in brain areas that were already implicated in OC symptoms in mixed male-female samples and that have high levels of sex steroid receptors, i.e., the striatum, the thalamus, the insula, the ACC, and frontal, temporolimbic and parietal areas. We further explore the existence of 'crossed line interactions' where an opposite effect of OC symptomatology in males and females may have acted to hide a main effect in mixed-sex samples.

Methods

Participants

Participants were recruited from an ongoing study in the Netherlands Twin Register (NTR) that investigates environmental and genetic influences on obsessive-compulsive (OC) symptoms (den Braber et al., 2010). Surveys were sent to twin families including the Padua Inventory Abbreviated (PI-R-ABBR) (Cath et al., 2008; van Oppen et al., 1995). Completed PI-R-ABBR questionnaires were returned by 20.204 subjects (including 9512 twins and 2403 siblings). From this sample we selected twin and sibling pairs in the age range between 18 and 60 years who both scored very high, very low or very discordant for OC symptoms. A subject was classified as high-scoring for OC symptoms if the PI-R-ABBR score was ≥15. A subject was classified as low-scoring for OC symptoms if the PI-R-ABBR score was ≤7. These PI-R-ABBR cut-off scores were derived from sensitivity and specificity measurements in an independent sample of OCD patients when compared to clinical controls (n=120; mean scores 20.7, SD 8.1; sensitivity 0.74 and specificity 0.72 at the best cut-off point of 16 (Cath et al., 2008)). Exclusion criteria were brain damage, neurological disease, color blindness and contraindications for MRI (e.g., pregnancy, ferromagnetic fragments, clips and devices in the body and claustrophobia). A final of 203 subjects participated in our MRI study, including 58 high-scoring females, 31 high-scoring males, 73 low-scoring females and 41 low-scoring males **(table 9.1)**.

Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, an adapted form of the

Table 9.1. Sample characteristics

	Males		Females			
	high (n = 31)	low (n = 41)	high (n = 58)	low (n = 73)	p OCS main effect	p OCS*SEX
Sample characteristics						
Age (years (SD))	30.52 (8.19)	31.32 (8.68)	35.90 (10.42)	36.55 (9.62)	0.665	0.984
Educational attainment (%low/middle/high)	12.9/38.7/48.4	7.3/26.8/65.9	17.2/36.2/46.6	8.2/28.2/63.0	0.039 #	0.337
Total intracranial volume (cc (SD))	1564.32 (122.71)	1524.21 (125.76)	1358.78 (109.99)	1332.04 (105.90)	0.064	0.003*
	mean (SD)	mean (SD)	mean (SD)	mean (SD)		
Obsessive-compulsive symptoms						
PI-R-ABBR (0-48)	19.90 (3.58)	4.00 (1.85)	20.62 (4.98)	4.38 (2.15)	<0.001*	0.733
Y-BOCS severity lifetime (0-40)	8.65 (6.87)	3.24 (4.85)	10.33 (7.22)	4.19 (5.88)	<0.001*	0.979
Y-BOCS symptoms:						
Agressive/Checking (0-14)	2.52 (2.11)	0.88 (1.05)	3.17 (2.50)	0.96 (1.17)	<0.001*	0.256
Hoarding/Saving (0-2)	0.61 (0.67)	0.20 (0.51)	0.60 (0.70)	0.22 (0.42)	<0.001*	0.842
Symmetry/Ordering (0-5)	0.81 (1.10)	0.24 (0.54)	1.03 (1.40)	0.32 (0.72)	<0.001*	0.362
Washing/Cleaning (0-12)	1.23 (1.65)	0.46 (0.90)	2.12 (2.32)	0.56 (1.11)	<0.001*	0.065
Sexual/Religious (0-8)	1.42 (1.63)	0.49 (0.75)	1.88 (1.86)	0.47 (0.73)	<0.001*	0.168
Somatic (0-2)	0.55 (0.72)	0.49 (0.22)	0.72 (0.77)	0.29 (0.54)	<0.001*	0.698
Comorbidity						
MINI :						
Depression (n)	1	0	1	0	0.191	0.341
Panic disorder(n)	0	0	2	0	0.191	0.205
Agoraphobia (n)	0	0	3	1	0.321	0.383
Social phobia (n)	1	1	2	0	0.321	0.319
Post-traumatic stress disorder (n)	0	0	1	0	0.438	0.640
Generalized anxiety disorder (n)	1	1	11	0	0.001 #	<0.001 #
Tic (0-8)	0.23 (0.62)	0.20 (0.51)	0.34 (0.71)	0.07 (0.25)	0.061	0.088
BDI (0-39)	2.13 (2.08)	1.59 (1.95)	4.62 (4.81)	1.64 (2.47)	0.001*	0.007*
STAI (0-60)	11.68 (5.95)	9.02 (5.77)	13.95 (7.46)	9.41 (7.37)	0.002*	0.340
STAS (0-30)	0.23 (0.88)	0.00 (0.00)	0.43 (1.90)	0.12 (0.55)	0.094	0.491

Age: age at time of MRI examination (in years); Educational attainment (% low/ middle/ high): percentage of OC symptom high-scoring males, OC symptom low-scoring males, OC symptom high-scoring females and OC symptom low-scoring females with low, middle or high educational level.

PI-R-ABBR: mean Padua Inventory Abbreviated scores (SD); Y-BOCS severity lifetime: mean Yale–Brown Obsessive–Compulsive Scale severity scores (SD) across whole life span; Y-BOCS symptoms: mean number of symptoms indicated within the aggressive/checking, hoarding/saving, symmetry/ordering, washing/cleaning, sexual/religious and somatic symptom dimensions.

*MINI (depression, panic disorder, agoraphobia, social disorder, post-traumatic stress disorder, generalized anxiety disorder): number of subjects with current comorbid disorder (measured using the Mini International Neuropsychiatric Interview); Tic: mean tic scores (SD) at time of MRI; BDI: mean Beck Depression Inventory scores (SD) at time of MRI; STAI: mean State Trait Anxiety Inventory scores (SD) at time of MRI; STAS: mean State Trait Anger Scale scores (SD) at time of MRI. * significant at <0.05 bonferroni corrected; # significant difference in distribution between groups, tested using Chi-square, Fisher exact test.*

Yale-Brown Obsessive-Compulsive Scale (Goodman et al., 1989a; Goodman et al., 1989b), to measure both life-time and current OC symptoms and severity, the State Trait Anxiety Inventory and State Trait Anger Scale (Spielberger et al., 1970; Spielberger et al., 1983), and the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) to test for possible comorbidities. Comorbidities tested by the MINI include depression, panic disorder, agoraphobia, social phobia, post-traumatic stress disorder and generalized anxiety disorder. Depressive symptoms were furthermore measured using the 13-item Beck Depression Inventory Short Form (BDI-R) (Beck et al., 1961; Beck et al., 1974). In addition, participants were screened for the eight most common tics (head shaking, eye blinking, other facial tics, shoulder raising, expressing swear words/ foul language/dirty words, sound making, growling and throat clearing/coughing/ sniffing). The ethical review board of the VU University medical centre approved the study. All participants provided written informed consent.

Image acquisition

The MRI session consisted of an anatomical scan of about 6 minutes. During the scan session, subjects were asked to minimize head movement. To reduce motion artifacts, each participant's head was immobilized using foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3D T1-weighted gradient-echo sequence (flip angle 8°; Repetition Time, TR = 9.69 ms; Echo Time, TE = 4.60 ms; matrix, 256x256 pixels; voxel size, 1.00x1.00x1.20 mm).

Data analysis

MRI data were analyzed using SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). T1-weighted MR images were segmented into gray matter, white matter and cerebrospinal fluid, and normalized to a group template (i.e., a specific template created from the 203 subjects that participated) using the Diffeomorphic Anatomical Registration Through Exponential Lie algebra (DARTEL) algorithm, and subsequently warped from DARTEL space to the standard Montreal Neurological Institute (MNI) brain. To preserve volumetric information, a modulation step was added. Before statistical analysis, the resultant modulated images were spatially smoothed with an 8 mm isotropic Gaussian kernel.

Statistical tests

Differences in survey- and interview-based variables were tested using a mixed-model analysis of variance (ANOVA; Mixed Models Linear menu item in SPSS) with sex (male versus female) and OC symptom status (high versus low) as two fixed factors and family as a random factor to account for family dependence (as the

data contained twins and siblings). Differences in educational attainment and comorbidity were analyzed using Chi-square statistics (crosstabs; Chi-square, Fisher exact option in SPSS). Statistical results were considered significant at $p < 0.05$, Bonferroni corrected.

Differences in regional gray matter volume were tested using the general linear model (full-factorial ANOVA) implemented in SPM8. The design consisted of the two independent factors OC symptom status (high or low-scoring) and sex (male or female) and was used to determine the main effect of OC symptom status and the OC symptom status by sex interaction effect. To account for family dependence brain maps of a twin and co-twin of each concordant pair were entered as repeated measures to account for within-twin pair correlations of brain structure. The main effect of OC symptom status was assessed to acquire a general idea of the volumetric brain differences between OC symptom high and low-scoring subjects. The interaction effect of OC symptoms status by sex was assessed by the F-ratio from the ANOVA and could be interpreted as OC symptom related brain changes that are different in males and females. For significant interactions we plotted the weighted mean voxel intensities and 90% confidence intervals for the most significant coordinate in the region separately for the high-scoring males, low-scoring males, high-scoring females and low-scoring females, in order to reveal what could explain the observed interaction. Post hoc tests comparing 'high-males versus low-males' and 'high-females versus low-females' for each coordinate derived from the interaction analysis were considered significant at $p < 0.01$.

All comparisons were performed with adjustments for total intracranial volume (i.e., the covariate; TIV). Because sex was partially confounded with comorbidity, in particular anxiety and depression (see **table 9.1**), data were re-analyzed with total score on the 13-item Beck Depression Inventory Short Form as an additional covariate. Volumetric changes for the main effect of OC symptoms and the OC symptom by sex interaction effect were assumed significant at $p<0.001$ uncorrected with a minimal cluster size of 10 voxels.

Results

Sample characteristics

Sample characteristics are summarized in **table 9.1**. As expected, OC symptom high-scoring subjects (regardless of sex), had significantly higher scores for measurements on OC symptomatology, including OC symptoms as measured with the PI-R-ABBR as well as OC symptom severity measured with the Yale-Brown Obsessive-Compulsive Scale severity questionnaire. In addition, OC symptom

Table 9.2. Regional Gray Matter Differences in OCS high compared to OCS low-scoring subjects

A. Covaried for total intracranial volume									B. Covaried for total intracranial volume and Beck Depression Inventory scores					
Test	Anatomical Location	BA	MNI coordinates			T-value	*p* -value	# voxels	MNI coordinates			T-value	*p* -value	# voxels
			x	y	z				x	y	z			
High>Low	Right precentral	6	66	-1.5	36	3.85	<0.001	75	66	-1.5	36	3.37	<0.001	11
	Left middle temporal	21	-70.5	-28.5	-3	3.98	<0.001	89	-70.5	-28.5	-3	3.96	<0.001	78
		21	-63	-39	-4.5	3.73	<0.001	151	-63	-39	-4.5	3.57	<0.001	57
High<Low	Left dorsolateral prefrontal	9	-28.5	13.5	34.5	3.46	<0.001	18	-28.5	13.5	34.5	3.45	<0.001	17
	Left insula	13	-46.5	-1.5	7.5	3.65	<0.001	73	-46.5	0	13.5	3.70	<0.001	67
	Right substantia nigra		13.5	-21	-13.5	3.39	<0.001	11	13.5	-21	-12	3.44	<0.001	22

A. Clusters with regional gray matter differences between OC symptom high and low-scoring subjects.

Test: test for significant gray matter increases (high > low) or decreases (high < low) in OC symptom high relative to OC symptom low-scoring subjects; BA: Brodmann area; MNI coordinates (mm): location of voxel with largest effect size; T-value: T-value of voxel with largest effect size; p-value: cluster p-value; # voxels: number of voxels in cluster.

B. Clusters with regional gray matter differences between OC symptom high and low-scoring subjects, after adjusting for Beck-depression inventory scores.

high-scoring subjects were more often diagnosed with current co-morbid disorders, mainly anxiety and depression. Furthermore, an interaction between 'OC symptom score' and 'sex' was found for anxiety and depression. This was due to higher levels of co-morbid anxiety and depression in high-scoring females compared with high-scoring males.

Main effect of obsessive-compulsive symptoms

Differences in gray matter volumes between OC symptom high-scoring subjects and OC symptom low-scoring subjects, regardless of sex, are presented in **table 9.2 (left)**. OC symptom high-scoring subjects had increased gray matter volumes in right precentral and left middle temporal gyrus and decreased gray matter volumes in left dorsolateral prefrontal gyrus, left insula, and right substantia nigra. Same results were obtained when depression scores were covaried for **(table 9.2, right)**.

Interaction effects: OC symptoms x Sex

To examine whether OC symptom related brain changes were different for males and females, the interaction effect of OC symptoms by sex was investigated. To reveal what could explain these interactions, weighted mean intensities (contrast estimates) and 90% confidence intervals were plotted for each significant coordinate derived from the interaction analysis **(figure 9.1)**. Post hoc tests, indicated that our finding of a regional enlargement in the left middle temporal gyrus in subjects scoring high for OC symptoms was completely driven by a larger gray matter volume for this region in OC symptom high-scoring males **(table 9.3, left)**. In addition, a region within the right middle temporal gyrus was found to be larger in OC symptom high-scoring males, but reduced in OC symptom high-scoring females. This opposite finding in males and females acted to hide the OC symptom main effect in the right middle temporal gyrus. A region within the right precuneus was found to be larger in OC symptom high-scoring females, but reduced in OC symptom high-scoring males. This region was again not found in the OC symptom main effect. Same results were obtained when depression scores were covaried for **(table 9.3, right)**.

Discussion

This study investigated if sex could be a potential source of heterogeneity in brain imaging outcomes on OC symptomatology. To assess how OC symptomatology affects the brain we first compared gray matter volumes from subjects with high OC symptom scores with those from subjects with low OC symptom scores, regardless of sex. Regions that were found to be larger in subjects with high OC symptom scores, included the right precentral and left middle temporal gyrus,

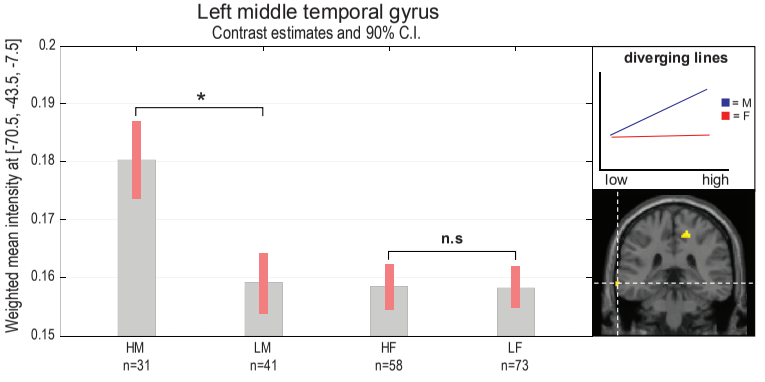
Table 9.3. Interaction effect of OCS*SEX in regional Gray Matter

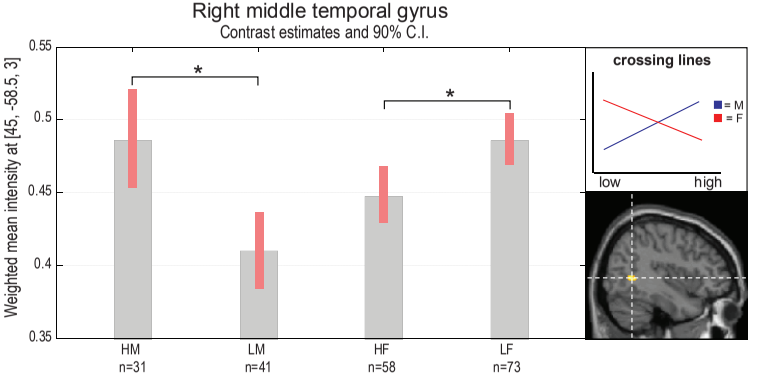
A. Covaried for total intracranial volume								B. Covaried for total intracranial volume and Beck Depression Inventory scores					
Anatomical Location	BA	MNI coordinates			F-value	*p*-value	# voxels	MNI coordinates			F-value	*p*-value	# voxels
		x	y	z				x	y	z			
Larger gray matter volumes in males with high OC symptomatology versus males with low OC symptomatology, with no effect or a reversed effect of OC symptomatology in the females													
Left middle temporal	21	-70.5	-30	-3	12.50	<0.001	12						
	21	-70.5	-43.5	-7.5	14.40	<0.001	33	-70.5	-43.5	-7.5	16.15	<0.001	57
Right middle temporal	39	45	-58.5	3	16.57	<0.001	104	45	-58.5	3	18.52	<0.001	153
Larger gray matter volumes in females with high OC symptomatology versus females with low OC symptomatology, with no effect or a reversed effect of OC symptomatology in the males													
Right precuneus	7	18	-37.5	54	17.53	<0.001	227	18	-37.5	54	17.73	<0.001	249

A. Clusters showing OC symptom related brain changes that are different in males and females. F-value: F-value of voxel with largest effect size.

B. Clusters showing OC symptom related brain changes that are different in males and females, after adjusting for Beck-depression inventory scores.

Larger gray matter volumes in males with high OC symptomatology versus males with low OC symptomatology, with no effect or a reversed effect of OC symptomatology in the females





Larger gray matter volumes in females with high OC symptomatology versus females with low OC symptomatology, with no effect or a reversed effect of OC symptomatology in the males

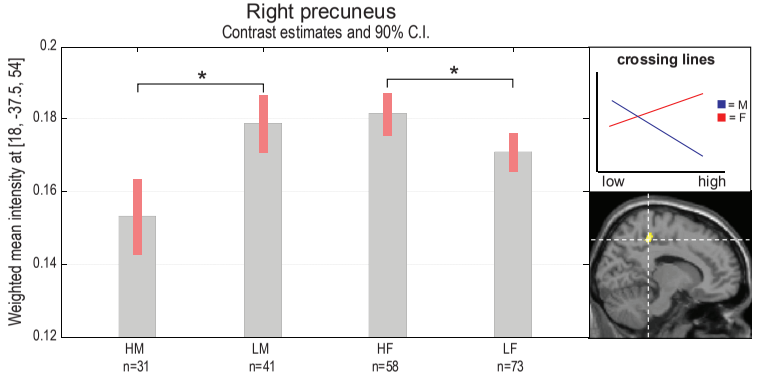


Figure 9.1. *OC symptom status related brain changes that are different in males and females. Bar graphs indicate the weighted mean intensities (contrast estimates) and 90% confidence intervals for each coordinate derived from the OC symptom status by sex interaction test, separately for the OC symptom high-scoring males (HM), OC symptom low-scoring males (LM), OC symptom high-scoring females (HF) and OC symptom low-scoring females (LF). * = Post hoc tests ('high-males versus low-males' or 'high-females versus low-females') significant at p < 0.01; n.s. = Post hoc tests not significant. Diverging lines can act to reduce or hide the main effect. Crossing lines are likely to hide the main effect.*

whereas the left dorsolateral prefrontal gyrus, the left insula and right substantia nigra were found to be reduced in OC symptom high-scoring subjects. Our finding of a reduced dorsolateral prefrontal gyrus is consistent with previous studies (see meta-analysis by Radua and Mataix-Cols (2009)). Together with the substantia nigra, this region has been implicated in the the dorsolateral prefrontal-striatal loop of the CSTC network (Cummings, 1993), that has been associated with OC symptomatology (Mataix-Cols and van den Heuvel, 2006). A reduced insular volume also replicates previous findings (Pujol et al., 2004; Soriano-Mas et al., 2007) and this structure has been mainly linked to OC symptomatology through its involvement in the neurocircuitry of disgust (Husted et al., 2006).

The main focus of this paper was whether OC symptom related brain changes are different for males and females and whether this difference may have acted to hide or reduce a main effect in our study, or in the mixed sex-samples previously studied. In order to investigate this, the interaction effect of OC symptoms by sex on gray matter volume was assessed. This OC symptoms by sex interaction analysis indicates that our finding of increased left middle temporal volume in OC symptom high-scoring subjects, was completely driven by a larger gray matter volume for this region in OC symptom high-scoring males. The OC symptom by sex interaction analysis also revealed opposite effects of OC symptomatology in males and females for the right middle temporal gyrus and right precuneus. OC symptom high-scoring males were found to have a larger right middle temporal gyrus, whereas in high-scoring females this region was reduced. Conversely, the right precuneus was found to be larger in OC symptom high-scoring females, but reduced in OC symptom high-scoring males. These opposite findings in males and females acted to hide the OC symptom main effect. The middle temporal gyrus is involved in verbal memory and auditory processing (Binder et al., 1994; Boly et al., 2004; Grasby et al., 1993), and precuneus function has been associated with higher order cognitive processes, including visuo-spatial processing, episodic memory retrieval and planning (Cavanna and Trimble, 2006). Both regions have been implicated in the neuroanatomical model for OCD, predominantly through their functional connections with the dorsal and ventral prefrontal cortex. However, results from structural and functional imaging studies have provided inconsistent results regarding the direction of anatomical and functional changes for these specific brain regions (Menzies et al., 2008a).

Based on our results we hypothesize that the use of mixed-sex samples with unequal distribution of males and females between studies (e.g., more males in one study versus more females in another study) may have contributed to these opposite findings. With regard to middle temporal and precuneus volumes, a review of the current literature supports our hypothesis directly. For example, Kim and colleagues, observed increased gray matter in the right temporal gyrus

in a sample that included a higher number of males compared to females (patients: 17 males versus 8 females) (Kim et al., 2001), whereas Tagao and colleagues observed decreased gray matter for this region in a sample that included a higher number of females compared to males (patients: 9 males versus 14 females) (Togao et al., 2010). In addition, with respect to the right precuneus, Pujol and colleagues found a tendency towards decreased gray matter for this region in a sample that included more males than females (patients: 40 males versus 32 females) (Pujol et al., 2004). This finding was replicated, and found to be significant, in a patient sample including 21 males and 9 females (Soriano-Mas et al., 2007). This latter study also assessed the feasibility of classifying single subject cases of MRI data as OCD patients or healthy controls using their whole brain anatomy, and found that including gender in their analyses improved their classification accuracy (Soriano-Mas et al., 2007) which further supports our finding that OC symptom related brain changes can be different in males and females.

Previous studies have shown that OC symptomatology and its developmental trajectories tend to be distinct in males and females, where females tend to report more contamination obsessions and cleaning compulsions (Labad et al., 2008; Noshirvani et al., 1991; Castle et al., 1995; Lensi et al., 1996; Tukel et al., 2004) whereas symmetry, religious and sexual obsessions (Labad et al., 2008; Lensi et al., 1996; Tukel et al., 2004) and an earlier onset of the disorder is more common in males (Labad et al., 2008; Noshirvani et al., 1991; Castle et al., 1995; Tukel et al., 2004; Bogetto et al., 1999). Interestingly, distinct neural correlates for these specific OC symptom dimensions have been found, both in brain structure as well as in brain function during specific symptom provocation (Mataix-Cols et al., 2004; van den Heuvel et al., 2009). The 'male' symmetry/ordering dimension was found to be negatively correlated with regional gray matter volume in motor cortex, insula and parietal cortex (which includes the precuneus) and positively correlated with temporal gray and white matter volume. The finding of a smaller precuneus and a larger middle temporal lobe in the males with high OC symptomatology, with opposite or no effects in females, suggest that the brain regions differentially affected in males and females may be intimately connected to the difference in patterns of OC symptomatology between the sexes.

In summary, this study shows that OC symptom related changes in the left middle temporal gyrus, right middle temporal gyrus and right precuneus are different for males and females. These findings might, in part, explain inconsistencies in the previous literature and show the importance of taking sex into account when investigating the neurobiology of OC symptoms.

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Appendices

I Invitation letter sent to twins and siblings
II Additional information letter sent to twins and siblings
III MRI brochure
IV MRI questionnaire and medication list
V Confirmation letter sent to participating twins and siblings
VI Informed consent
VII Instruction brochure for buccal cell samples collection

Appendix I

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Ons kenmerk MRt/adb	Uw kenmerk	Telefoon	E-mail

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Aankondiging van het onderzoek:
'Tweelingfamilieonderzoek naar gedrag, hersenstructuur en functie'

Geachte mevrouw, mijnheer,

De komende tijd gaat het Nederlands Tweelingen Register (NTR) een nieuw onderzoek doen naar de structuur en de functie van de hersenen bij een- en twee-eiige tweelingen en hun broer/zus. Dit onderzoek wordt uitgevoerd door onderzoekers van het NTR in samenwerking met de Vrije Universiteit (VU) en het Academisch Medisch Centrum (AMC). Wij nodigen hiervoor tweelingen en hun broer/zus uit die veel of juist weinig op elkaar lijken in hun antwoorden op eerdere vragenlijsten.

Hierbij willen wij u graag uitnodigen mee te doen. Het onderzoek bestaat uit het maken van een MRI-scan bij u en uw (tweeling)broer/-zus, het meten van de hartslag (ECG) gelijktijdig met het maken van de MRI-scan en het afnemen van een aantal vragenlijsten en een interview.

Met MRI (Magnetic Resonance Imaging) kan een opname van de hersenen gemaakt worden. De MRI-techniek is *niet-invasief*, dat wil zeggen er komt geen straling, contrastmiddel of toediening van enige andere substantie aan te pas. De vragenlijsten kunt u gedeeltelijk thuis invullen. Het interview bestaat uit vragen over gezondheid, het opgroeien, het meemaken van belangrijke levensgebeurtenissen en een aantal vragen over repetitief gedrag. Hieronder verstaan we gedachten of handelingen die zich steeds weer herhalen.

Binnenkort zal ik u bellen om eventuele vragen die u heeft naar aanleiding van deze brief te beantwoorden, en met het verzoek of u aan dit onderzoek mee zou willen doen. U kunt ook contact met mij opnemen (tel: / email: . Zou u dit in het bijzonder willen doen wanneer u onlangs nog van telefoonnummer bent veranderd?

Als u mee wilt doen aan het onderzoek ontvangt u na het telefoongesprek uitgebreide informatie thuis. Naar aanleiding van de uitgebreide informatiebrief kunt u zich opgeven voor deelname aan het onderzoek. Het onderzoek vindt plaats in het AMC ziekenhuis en duurt voor 3 personen in totaal ongeveer 4 uur. Voor dit onderzoek is het belangrijk dat u en uw (tweeling)broer/-zus allen meedoen.

Wij hopen dat u zult willen meedoen aan dit onderzoek! U krijgt, als u daar prijs op stelt, een foto van uw eigen hersenen zoals gemaakt tijdens de MRI-scan. Daarnaast ontvangt u een vergoeding voor de reiskosten en als kleine attentie een cd- of boekenbon van 15 euro.

Wij danken u voor het lezen van deze brief en hopen u bij dit onderzoek te mogen verwelkomen.

Met vriendelijke groet,

Mw. Anouk den Braber, onderzoeker
Dhr. Dr. Dennis van 't Ent, universitair docent/ onderzoeker
Mw. Prof. dr. Dorret Boomsma, hoogleraar
Dhr. Prof. dr. Eco de Geus, hoogleraar

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

Appendix II

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
			2
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
MRt/adb			

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije **Universiteit** *amsterdam*

Informatie over het 'Tweelingfamilieonderzoek naar gedrag, hersenstructuur en functie'

Geachte mevrouw / heer……..,

Onlangs hebben wij gevraagd of u mee wilt doen aan een nieuw onderzoek bij een- en twee-eiige tweelingen en hun broer/zus naar gedrag, hersenstructuur en functie. Ik heb u daarover gesproken aan de telefoon. Hierbij stuur ik u de beloofde informatie over het onderzoek.
Het onderzoek bestaat uit het maken van een serie opnames van de hersenen met een MRI-scan bij uzelf en uw (tweeling)broer/-zus, het meten van de hartslag gedurende de MRI-scan, het maken van een monduitstrijkje voor DNA-onderzoek, het invullen van een aantal vragenlijsten en een mondeling interview.
De MRI-scan wordt gemaakt in het AMC (Academisch Medisch Centrum) in Amsterdam. Tijdens het bezoek aan het AMC wordt u ook gevraagd naar gezondheid, naar gedrag, en naar allerlei gebeurtenissen in uw leven die voor u belangrijk zijn geweest. De vragenlijst en het materiaal voor het monduitstrijkje krijgt u thuisgestuurd.
In deze brief vindt u meer informatie over het onderzoek. We hopen dat u na het lezen van deze brief en de bijlagen uw medewerking aan dit onderzoek wilt verlenen.

Achtergrond
Onderzoekers van het Nederlands Tweelingen Register (NTR) willen graag meer weten over sekseverschillen in de hersenmechanismen die betrokken zijn bij repetitief gedrag. Hieronder verstaan we steeds terugkerende gedachten en/of handelingen die bij het merendeel van de bevolking voorkomen, zoals herhaald handen wassen, uitvoerig de sloten van uw huis controleren als u weggaat, of dingen rechtzetten omdat het anders niet symmetrisch is. Mensen verschillen onderling sterk in de mate waarin ze dergelijk gedrag vertonen, zelfs binnen tweelingparen, en het vermoeden bestaat dat de vorm en de grootte van sommige hersengebieden samenhangen met dit soort gedrag. Bovendien bestaat het vermoeden dat deze verschillen terug te voeren zijn op de werking van het erfelijke materiaal (DNA). Om dit te onderzoeken willen wij graag een MRI-scan uitvoeren en erfelijk materiaal verzamelen. Het erfelijke materiaal (DNA) wordt ook gebruikt om na te gaan (indien u een tweeling bent) of u een een- of twee-eiig tweelingpaar bent. U ontvangt hier, als u dit op prijs stelt, de uitslag van. Daarnaast vragen wij u om toestemming het materiaal te mogen bewaren voor toekomstig onderzoek.

Het onderzoek waarvoor uw medewerking wordt gevraagd
Wij willen bij u en uw (tweeling)broer/-zus een hersenscan afnemen. De hersenscans worden gemaakt met behulp van een techniek die Magnetic Resonance Imaging heet, of kortweg MRI. Hoe deze techniek werkt staat hieronder beschreven. Tijdens het maken van de MRI-scan van de hersenen willen we de hartslag registreren. Het is voor dit onderzoek belangrijk dat beide broers/zussen van het tweelingpaar en eventueel nog een extra broer/zus aan de MRI-scan deelnemen.

Voor het verkrijgen van erfelijk materiaal (DNA) zullen wij u vragen thuis monduitstrijkjes te maken met wattenstaafjes en dit mee te nemen naar het AMC op de dag van het onderzoek. Dit is een gemakkelijk en pijnloos onderzoek. Wanneer u besluit deel te nemen aan het onderzoek ontvangt u informatie over hoe dit precies in zijn werk gaat.

Wat zou het belang van het onderzoek voor uzelf kunnen zijn?
Dit onderzoek heeft op zich geen specifiek belang voor uzelf, behalve dat u, als u daar prijs op stelt, een hersenfoto van uzelf krijgt. Indien er iets bijzonders te zien zou zijn op de scanfoto's, wordt u hiervan op de hoogte gesteld, en zo nodig voor verder onderzoek doorverwezen. Het algemene belang dat u dient door mee te doen, is dat we door inzicht in de mechanismen betrokken bij normale vormen van repetitief gedrag, ook meer gaan begrijpen van buitensporig of ziekelijk repetitief gedrag. Wij hopen dan ook de ziekelijke vormen van repetitief gedrag beter te kunnen bestrijden, wanneer we de achtergronden beter kennen.

De gang van zaken tijdens het onderzoek
Voorafgaand aan de scan wordt de algemene procedure uitgelegd, en heeft u de gelegenheid om vragen te stellen. Na deze introductie begint een van de tweeling of hun broer/zus met de MRI-scan. U ligt gedurende ongeveer 60 minuten in een scanner die voortdurend opnamen van de hersenen maakt. Tijdens het scannen voert u een aantal opdrachten uit en wordt uw hartslag geregistreerd. Uw (tweeling)broer/-zus doet dan mee aan het interview. Het interview bestaat uit vragen over gezondheid, het opgroeien, het meemaken van belangrijke levensgebeurtenissen en een aantal vragen over repetitief gedrag. Na een korte pauze wisselt u van plaats. Het bezoek duurt voor 3 personen ongeveer 4 uur.

Magnetic Resonance Imaging (MRI)
Bij MRI wordt gebruik gemaakt van een magneetveld en radiogolven. Met deze techniek kan een opname van de hersenen gemaakt worden. De MRI-techniek is *niet-invasief*; dat wil zeggen dat er geen straling, contrastmiddel of toediening van enige andere substantie aan te pas komt. Voor zover bekend heeft deze techniek *geen* nadelige effecten op de gezondheid. Uit veiligheidsoverwegingen moeten wij zwangere vrouwen en personen met metaal in of aan hun lichaam (pacemakers, vaatclips) uitsluiten van dit onderzoek. Metalen voorwerpen op het lichaam (sieraden, piercings) moeten worden afgedaan. Ook is het van belang dat u geen last heeft van claustrofobie (angst voor kleine ruimtes).

De MRI-scan vindt plaats in het MRI-gebouw van het AMC. Bijgevoegd vindt u een informatiefolder van het AMC, waarin wordt uitgelegd hoe u zich kunt voorbereiden op een MRI-onderzoek.

Als u besluit deel te nemen aan het onderzoek moet u ermee instemmen dat u wordt geïnformeerd over eventuele toevalsbevindingen. Dit betekent dat u geïnformeerd wordt over afwijkingen, zoals iets wat zou kunnen wijzen op een hersenafwijking, die bij toeval worden geconstateerd in uw data. In dat geval zal er nader contact met u worden opgenomen.

Hartslagregistratie
Door middel van 4 zogenoemde elektroden – dit zijn geleidende stickers die op de borstkas geplakt worden – kan de elektrische activiteit van het hart (de hartslag) geregistreerd worden. Deze techniek heet elektrocardiografie (ECG) en is *niet-invasief*. Voor zover bekend heeft deze techniek *geen* nadelige effecten op de gezondheid. Door het ECG te meten tijdens het maken van de MRI-scan, kan de door hartslag veroorzaakte 'ruis' in de hersenactiviteit, uit het beeldsignaal gefilterd worden. Dit geeft een nog betrouwbaarder beeld van de hersenactiviteit.

Vertrouwelijkheid van de gegevens
Alle persoonlijke gegevens zullen strikt vertrouwelijk worden behandeld en gecodeerd worden verwerkt bij een wetenschappelijke rapportage.

Vrijwilligheid van deelname
Deelname is vrijwillig en u kunt zich op elk moment, ook na ondertekening van het toestemmingsformulier, zonder opgave van redenen uit het onderzoek terugtrekken zonder dat dit een goede verstandhouding in de weg zal staan.

Verzekering
Omdat er aan de deelname aan dit onderzoek geen of verwaarloosbare extra risico's zijn verbonden heeft de medisch-ethische toetsingscommissie van het VU medisch centrum vrijstelling verleend van de verplichting tot het afsluiten van een risicoverzekering.

Nadere informatie
Mocht u na het lezen van de brief nog aanvullende informatie willen ontvangen of komen er vragen bij u op, dan kunt u altijd contact opnemen met de uitvoerende onderzoeker van het onderzoek, mw. A. den Braber, tel. , of e-mail . Indien u er prijs op stelt informatie over dit onderzoek in te winnen bij een onafhankelijk arts, die niet bij de uitvoering van het onderzoek betrokken is, dan is Prof. Dr. A.J.L.M. van Balkom, arts en psychiater bij de afdeling Psychiatrie VUmc / GGZ Buitenamstel, tel. , bereid uw vragen te beantwoorden.

Vergoeding
U ontvangt, als u hier prijs op stelt, een afdruk van uw eigen hersenscan. Daarnaast ontvangt u een vergoeding voor de gemaakte reiskosten en een cadeaubon van 15 euro voor uw medewerking aan dit onderzoek.

Tenslotte
Als u wilt meedoen, moet er voorafgaand aan het onderzoek een toestemmingsformulier (Informed Consent) worden getekend. Dit formulier wordt getekend door uzelf en de onderzoeker. Na afloop van het onderzoek ontvangt u een verslag met daarin de algemene onderzoeksresultaten.

Binnenkort wordt u telefonisch benaderd om eventuele vragen die u heeft naar aanleiding van deze brief te beantwoorden, en met het verzoek of u aan dit onderzoek mee zou willen doen. Wanneer u geïnteresseerd bent in het onderzoek, kunt u gedurende dit gesprek uw voorkeursdatum aangeven voor het scanonderzoek dat plaatsvindt in het MRI-gebouw van het AMC. De scans vinden meestal plaats in de avonduren en in het weekend. Buiten deze tijden om is het wat moeilijker om een afspraak te maken. Als u geen mogelijkheden ziet om een afspraak te maken op bovengenoemde tijden zullen wij kijken of de afspraak eventueel kan plaatsvinden op een andere dag of tijdstip. Wij willen u er nogmaals op attenderen, dat het voor dit onderzoek belangrijk is dat zowel u als uw (tweeling)broer/-zus aan de MRI-scan deelnemen. Wij danken u voor het lezen van deze informatie en hopen u binnenkort bij dit onderzoek te mogen verwelkomen.

Met vriendelijke groet,

Mw. Anouk den Braber, onderzoeker
Dhr. Dr. Dennis van 't Ent, universitair docent/onderzoeker
Mw. Prof. Dr. Dorret Boomsma, hoogleraar
Dhr. Prof .dr. Eco de Geus, hoogleraar

Bijlagen:
I: Informatiefolder MRI
II: Vragenlijst MRI en medicijngebruik

NB Wilt u de vragenlijst voor het MRI-onderzoek en de vragenlijst naar medicijngebruik invullen en alvast overleggen met uw (tweeling)broer/-zus over welke dagen u allen mee zou kunnen doen? De onderzoeker zal de vragenlijsten samen met u doornemen gedurende het telefoongesprek.

Appendix III

Informatiefolder MRI

MRI: Magnetic Resonance Imaging

Waar?

Indien u mee wilt werken aan dit onderzoek wordt voor u een afspraak voor een MRI-scan gemaakt. Het onderzoek zal plaatsvinden in het MRI-gebouw van het AMC (zie onderstaande route beschrijving).

Het MRI-gebouw

Het onderzoek vindt plaats in gebouw F0 (begane grond). U loopt via de hoofdingang het ziekenhuis binnen, rechts van u bevindt zich de receptie, links een wachtruimte. U loopt rechtdoor langs de receptie tot u zich in een hal bevindt met aan uw linkerhand een foodcorner met terras. U loopt langs de foodcorner en gaat meteen naar links, u loopt alsmaar rechtdoor en volgt de bordjes MRI, tot u in de wachtruimte van de MRI-faciliteit aankomt. Hier zult u door één van onze onderzoekers worden verwelkomd.

Wat is MRI?

MRI of kernspin-tomografie is de nieuwste methode om te zien wat er binnen uw lichaam gebeurt. Via MRI kan de onderzoeker zich een beeld vormen van wat zich in uw lichaam afspeelt, zonder dat daarvoor een ingreep nodig is en zonder dat gebruik wordt gemaakt van röntgenstralen. MRI geeft informatie die niet op een andere manier te verkrijgen is. Het onderzoek is pijnloos en er zijn geen na- of bijwerkingen.

Hoe werkt MRI?

Bij MRI wordt gebruik gemaakt van magneetvelden en radiogolven. Tijdens het onderzoek wordt u in een magnetisch veld geplaatst. De waterstofatomen in uw lichaam richten zich in dit veld en daardoor reageren zij op de radiosignalen van het MRI-systeem. De waterstofatomen worden daardoor zelf kleine zendertjes waarvan de signalen weer kunnen worden opgevangen, geordend en via een computer omgezet in een afbeelding van de onderzochte lichaamsdelen.

Wanneer komt u niet in aanmerking?

Het onderzoek wordt gestoord door metalen voorwerpen. Wanneer bij u een pacemaker is geïmplanteerd, kunt u dus geen MRI-onderzoek ondergaan. Ook wanneer een chirurgische clip is aangebracht op een van de bloedvaten in het hoofd, zal het over het algemeen beter zijn geen MRI-onderzoek van het hoofd te doen. Tegenwoordig worden andere chirurgisch ingebrachte metalen voorwerpen, zoals heupkopprothesen, metalen pennen en schroeven niet als een beletsel gezien voor onderzoek. In twijfelgevallen kunt u dit bespreken met uw onderzoeker.

Voorbereiding

In het algemeen hoeft u voor een MRI-onderzoek geen dieet te houden of speciale richtlijnen te volgen. Wij verzoeken u echter wel op de dag van het onderzoek geen alcoholische dranken te nuttigen en 2 uur voor aanvang van het onderzoek geen cafeïnehoudende koffie te drinken. Omdat het onderzoek wordt gestoord door metalen voorwerpen mag u die niet meenemen in de onderzoeksruimte. Oogmake-up bevat ook metalen. Wij verzoeken u om uw

ogen voor dit onderzoek niet op te maken. U kunt uw bezittingen achterlaten in de kleedkamer.

Neem dus in geen geval de volgende voorwerpen in de magnetische ruimte mee:

- Creditcards (bevatten magnetische code)
- Munten (kleingeld)
- Sieraden
- Horloges
- Manchetknopen
- Gehoorapparaten
- Alle andere metalen voorwerpen

Het onderzoek

Het MRI-onderzoek vindt plaats in een ruimte, die is afgeschermd tegen radiogolven van buitenaf. U ligt op een onderzoekstafel die tijdens het onderzoek in de magneet van het toestel wordt geschoven. Dit is geen afgesloten ruimte. Indien u claustrofobisch bent, of niet goed tegen kleine ruimtes kunt, neemt u dan contact op met uw onderzoeker.
Het is belangrijk dat u zich tijdens het onderzoek ontspant. Probeert u zo rustig mogelijk te blijven liggen, want bewegingen beïnvloeden het resultaat in negatieve zin. Tijdens het onderzoek voelt u absoluut niets. Het monotone getik dat u hoort is afkomstig van het omschakelen van de radiospoelen. Het zijn normale geluiden bij een MRI-onderzoek. De duur van het MRI-onderzoek is ongeveer een uur.

Vragen

Voor alle vragen die u eventueel heeft, kunt u terecht bij uw uitvoerend onderzoeker:
Mw. A. den Braber
Tel:
E-mail :

Appendix IV

Vragenlijst voor MRI-onderzoek

MRI is een onderzoeksmethode waarbij gebruik gemaakt wordt van een sterk magneetveld. In zeldzame gevallen kan dit magneetveld een gevaar vormen. Om ieder risico in dit opzicht uit te sluiten, verzoeken wij u onderstaande vragen in te vullen. Gedurende het telefoongesprek zal de onderzoeker deze lijst nog samen met u doornemen.

Heeft U:

- Een pacemaker of (oude) pacemakerdraden? □ ja □ nee □ ?
- Een medicijnpomp (bijv. insulinepomp)? □ ja □ nee □ ?
- Een neuro-stimulator?
- Een uitwendige prothese (bijv. kunstarm)? □ ja □ nee □ ?
- Eén of meerdere piercings op uw lichaam? □ ja □ nee □ ?
- Tatoeages of permanente make-up? □ ja □ nee □ ?
- Tandtechnische constructies (beugels, draadjes e.d.)? □ ja □ nee □ ?
- Medicijnpleisters (nicotine-, hormoonpleisters e.d.)? □ ja □ nee □ ?

Heeft u ooit een operatie ondergaan aan:

- Het hoofd (bv. plaatsen vaatclip of pompje)? □ ja □ nee □ ?
- Het hart (bv. kunstklep)? □ ja □ nee □ ?
- De ogen (bv. geïmplanteerde lenzen)? □ ja □ nee □ ?
- De oren (gehoordbeentjesprothese, gehoorapparaat)? □ ja □ nee □ ?
- De botten (waarbij platen en schroeven zijn gebruikt)? □ ja □ nee □ ?
- Anderszins? □ ja □ nee □ ?
  Zo ja, aan…….

Bent u (oud) metaalbewerker of bankwerker? □ ja □ nee
Bestaat er kans op metaalsplinters in uw oogkas? □ ja □ nee

Heeft u last van:

- Engtevrees/claustrofobie? □ ja □ nee
- Kortademigheid (bij plat liggen)? □ ja □ nee

Wat is uw gewicht? ……kg
Wat is uw lichaamslengte? ……cm

In te vullen door vrouwen:
Bent u (mogelijk) zwanger? □ ja □ nee

Medicijngebruik:

Ik gebruik **wel / geen** medicijnen (doorhalen wat niet van toepassing is).

Als u medicijnen gebruikt, wilt u dan uw medicijngebruik op de volgende pagina noteren? Schrijft u alstublieft de naam van de medicijnen en de dosering zoals aangegeven op de verpakking nauwkeurig over en geef de reden van gebruik aan.

Naam medicijn	Dosering gram/dag	Start datum	Eind datum	Reden van gebruik

Heeft u nog opmerkingen naar aanleiding van het invullen van de lijst?

_____
_____
_____
_____

NB Houdt u deze vragenlijst ingevuld bij de hand gedurende het telefoongesprek! Ook verzoeken wij u deze vragenlijst mee te nemen op de dag van het onderzoek.

Hartelijk dank voor uw medewerking!!

Appendix V

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
			-
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
MRt/adb			

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Betreft: Bevestiging deelname aan het 'Tweelingfamilieonderzoek naar gedrag, hersenstructuur en functie'

Geachte mevrouw / heer……..,

Wij zijn blij dat u mee wilt doen aan het onderzoek naar gedrag, hersenstructuur en functie, uitgevoerd door de Vrije Universiteit in samenwerking met het Nederlands Tweelingen Register (NTR). Hierbij sturen wij u de bevestiging voor de afspraak, die we telefonisch met u gemaakt hebben.

Datum:
Tijd:
Plaats: Academisch Medisch Centrum (AMC), Amsterdam

In de bijlage vindt u een routebeschrijving naar het AMC en een plattegrond van het terrein. Als u met de auto komt, raden wij u aan om te parkeren op het parkeerterrein direct naast de ziekenhuisingang en de ingang van de polikliniek (P1). Als P1 vol is, wordt u doorverwezen naar het parkeerterrein P4-B, aan de andere kant van het AMC. P4-B ligt direct bij de ingang van de faculteit. Borden verwijzen u vervolgens naar het ziekenhuis. Denkt u er vooral aan uw parkeerkaartje uit de auto mee te nemen. Het onderzoek vindt plaats in gebouw F0 (begane grond). U loopt via de hoofdingang het ziekenhuis binnen, rechts van u bevindt zich de receptie, links een wachtruimte. U loopt rechtdoor langs de receptie tot u zich in een hal bevindt met aan uw linkerhand een foodcorner met terras. U loopt langs de foodcorner en gaat meteen naar links, u loopt alsmaar rechtdoor en volgt de bordjes MRI, tot u in de wachtruimte van de MRI-faciliteit aankomt. Hier zult u door één van onze onderzoekers worden verwelkomd.
Wilt u, als u naar het AMC komt, de ingevulde vragenlijsten (bijlage III) meenemen en ook de thuis gemaakte monduitstrijkjes. Hoe u de monduitstrijkjes maakt, leest u in de bijgevoegde brief (bijlage IV) met instructiefolder 'Het monduitstrijkje: hoe verzamelt u DNA met een wattenstaafje'.

Tenslotte willen wij u vragen het toestemmingsformulier (bijlage V) goed door te lezen, in te vullen en te ondertekenen. Zonder uw toestemming mogen wij uw gegevens niet gebruiken.

Om u zo snel mogelijk te kunnen vergoeden voor de gemaakte reiskosten (inclusief parkeergeld) en voor het onderzoek, zouden wij u willen vragen uw rekeningnummer en sofi-nummer mee te nemen naar de testdag. Als u met de trein komt vragen wij u uw treinkaartje te bewaren.

Wilt u op de dag van het onderzoek geen alcohol drinken en 2 uur voor aanvang van het onderzoek geen cafeïnehoudende dranken drinken? Verder zijn er geen bijzondere voorbereidingen nodig voor het onderzoek.

Wij willen u bij deze heel hartelijk danken voor uw medewerking en zien u graag tegemoet op bovenstaande datum.

Met vriendelijke groet,

Mw. Anouk den Braber, onderzoeker
Dhr. Dr. Dennis van 't Ent, universitair docent/ onderzoeker
Mw. Prof. dr. Dorret Boomsma, hoogleraar
Dhr. Prof. dr. Eco de Geus, hoogleraar

Mocht u op bovengenoemde datum onverwachts verhinderd zijn, kunt u contact opnemen met Mw. A. den Braber voor een nieuwe afspraak:
Tel.

Bijlagen:
I: Route beschrijving AMC
II: Plattegrond AMC
III: Zelf-invul vragenlijsten
IV: Brief: Monduitstrijkje bij deelname
V: Toestemmingsformulier

Appendix VI

Toestemmingsformulier (*Informed Consent*):

- Ik verklaar hierbij op voor mij duidelijke wijze, mondeling en schriftelijk, te zijn ingelicht over de aard, methode en doel van het onderzoek. Mijn vragen zijn naar tevredenheid beantwoord. De schriftelijke informatie behorende bij deze verklaring is mij overhandigd. Ik stem geheel vrijwillig in met deelname aan dit onderzoek. Ik behoud daarbij het recht deze instemming weer in te trekken zonder dat ik daarvoor een reden behoef op te geven. Ik ga ermee akkoord dat ik en mijn huisarts/ behandelaar worden geïnformeerd over eventuele toevalsbevindingen die in mijn onderzoeksgegevens worden geconstateerd. Ik ga ermee akkoord dat er wat cellen van mijn wangslijmvlies bewaard worden voor DNA onderzoek onder een codenummer. Ik ga ermee akkoord dat tijdens het maken van de MRI-scan mijn hartslag geregistreerd wordt door middel van elektrocardiografie.

Naam deelnemer: _____

Datum: _____

Handtekening: _____

- Ik heb mondelinge en schriftelijke toelichting verstrekt op het onderzoek. Ik verklaar mij bereid nog opkomende vragen over het onderzoek naar vermogen te beantwoorden.

Naam onderzoeker: _____

Datum:_____

Handtekening: _____

Appendix VII

INSTRUCTIE

HET MONDUITSTRIJKJE:
hoe verzamelt u DNA
met een wattenstaafje

VRIJE UNIVERSITEIT NEDERLANDS TWEELINGEN REGISTER

NEDERLANDS TWEELINGEN REGISTER

NTR01101DNAUINSTR

Vrije Universiteit Amsterdam
Biologische Psychologie
Van der Boechorststraat 1
1081 BT Amsterdam

telefoon:
e-mail:
www.tweelingenregister.org

NEDERLANDS TWEELINGEN REGISTER

Waarom monduitstrijkjes?

Met behulp van tweelingen en hun families onderzoekt het Nederlands Tweelingen Register de invloed van erfelijke factoren en omgevingsfactoren op individuele verschillen in gedrag en gezondheid. Door middel van vragenlijst- en laboratoriumonderzoek kan worden nagegaan hoe groot deze erfelijke en omgevingsinvloeden zijn. Als er erfelijke invloeden zijn, kan met deze methoden echter niet bepaald worden welke genen verantwoordelijk zijn voor verschillen in gedrag en gezondheid. Daarvoor is erfelijk materiaal, het zogenaamde DNA, nodig.

Met een monduitstrijkje kan op een eenvoudige en pijnloze manier erfelijk materiaal worden verzameld.
Een monduitstrijkje wordt gemaakt door met een wattenstaafje zachtjes langs de binnenkant van de mond te wrijven. De cellen van het wangslijmvlies worden zeer vaak vernieuwd. Daarom zijn deze cellen bij uitstek geschikt voor verzameling van erfelijk materiaal.
De cellen kunnen ook worden gebruikt om te bepalen of een tweeling één- of twee-eiig is. De tweelingen die aan dit onderzoek meedoen krijgen deze uitslag te zijner tijd thuisgestuurd.

INSTRUCTIE

Wat zit er in de enveloppe?

U heeft 5 buisjes ontvangen: een wat dikkere buis met 16 wattenstaafjes en 4 dunne buisjes met alleen een beetje vloeistof.

Wat te doen met het materiaal?

De monduitstrijkjes moeten 4 keer worden afgenomen, verspreid over twee dagen (2 keer per dag; bijv. voor de ochtend- en avondmaaltijden). Niet van te voren de mond spoelen, tanden poetsen of eten!

Per afname gebruikt u 4 wattenstaafjes:

- 1 staafje voor de binnenkant van de bovenlip en het tandvlees van de bovenkaak.
- 1 staafje voor de binnenkant van de onderlip en het tandvlees van de onderkaak.
- 1 staafje voor de binnenkant van de linkerwang.
- 1 staafje voor de binnenkant van de rechterwang.

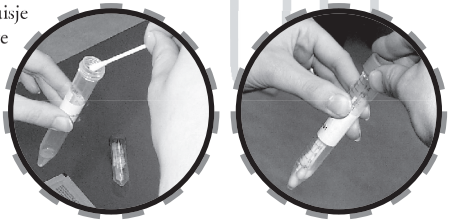
U wrijft per wattenstaafje ongeveer 10-20 seconden zorgvuldig en met enige druk (het hoeft niet hard; het mag geen pijn doen).

Na het wrijven deponeert u het staafje, met het watje naar beneden, in de vloeistof in één van de dunne buisjes. Doe alle 4 staafjes in ditzelfde dunne buisje. De andere dunne buisjes zijn voor de volgende monduitstrijkjes.
Wilt u het deksel van het buisje goed dichtdraaien? (Niet te hard om barsten van de buis te voorkomen).
Als u na twee dagen alle vier de afnames heeft gedaan, heeft u de dikke buis niet meer nodig. Deze kunt u dus weggooien.

Wilt u de vier dunne buisjes met de monduitstrijkjes vervolgens direct in de bijgesloten antwoordenveloppe met de gewone post aan ons opsturen? Let op: als het vriest, de enveloppe niet in een buitenbrievenbus gooien.

Nadat het erfelijk materiaal is geanalyseerd, zullen eventuele restanten voorlopig opgeslagen worden voor mogelijk aanvullende bepalingen ten behoeve van het onderzoek naar leefgewoonten, gedrag en gezondheid. Wanneer u dit niet wilt, stelt u ons dan daarvan s.v.p. op de hoogte. In dat geval zullen wij het overgebleven materiaal vernietigen.

Indien u nog vragen heeft, kunt u contact opnemen met ons secretariaat. Onze gegevens staan op de achterzijde van deze brochure.



NEDERLANDS TWEELINGEN REGISTER