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

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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 prefrontal 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-aminobutyric 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 dopamine₁ influence on the direct loop of the ventromedial frontal-striatal circuit and a stronger inhibitory dopamine₂ 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.

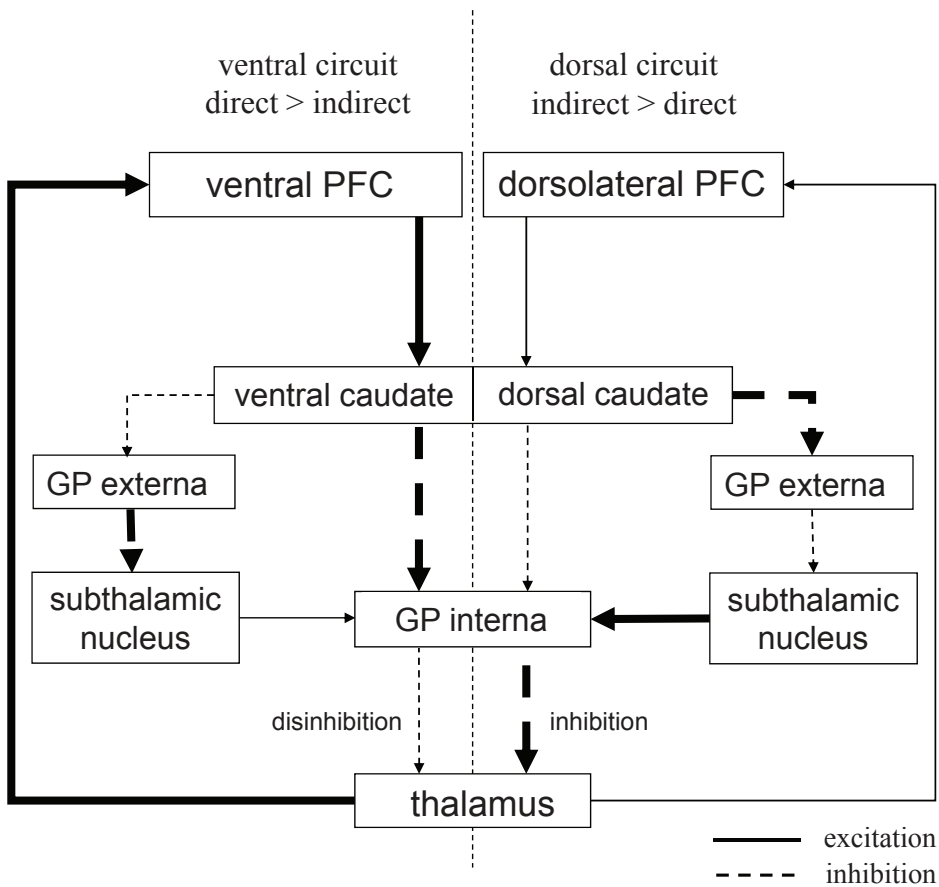


Figure 1.1. A widely accepted neuroanatomical model of OCD involving the direct and indirect CSTC loops. It is hypothesized that an imbalance between these loops, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate OC symptoms (adapted from Mataix-Cols and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

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)	<i>lunch, dinner or tea with cake</i>
Structural MRI, functional MRI and DTI (± 60 min)	Explaining and practising fMRI tasks on personal computer (± 15 min)
<i>lunch, dinner or tea with cake</i>	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/mm ²	±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.

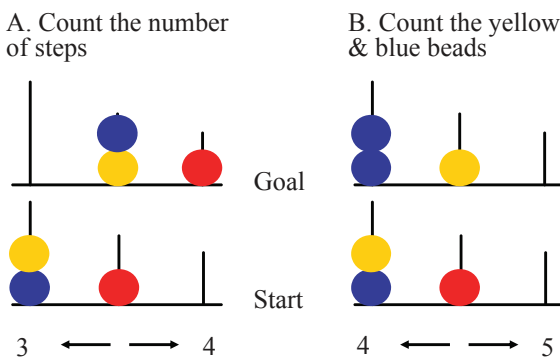


Figure 2.1. Examples of Tower of London stimuli; (A) Planning condition; (B) baseline condition.

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.