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Summary & Discussion

The main aim of this thesis was to explore whether environmental or genetic risk factors for OC symptoms affect the structure and functioning of the brain in the same or in different ways. To accomplish this, structural and functional MRI (fMRI) scans were obtained from monozygotic (MZ) twin pairs discordant or concordant for obsessive-compulsive (OC) symptoms. A comparison within MZ discordant twin pairs (one twin scores high and co-twin scores low for OC symptoms) was performed to identify neurobiological changes mediated by environmental risk factors for OC symptoms. A comparison between MZ twin pairs both scoring high and MZ twin pairs both scoring low for OC symptoms was performed to identify neurobiological changes mediated by genetic risk factors for OC symptoms. In addition, it was investigated whether fMRI changes associated with heart rate changes during the cognitive tasks could influence the interpretation of the fMRI data and it was explored if OC symptom related changes in gray matter volume were different for males and females. For the latter study, additional MRI data was obtained from a sample of opposite-sex twin and sibling pairs scoring concordant-high or concordant-low for OC symptoms. These data were combined with the MRI data obtained in the MZ (same-sex) twin sample.

Environmental and genetic risk factors for OC symptoms: Do they affect the same brain regions?

In chapters 3 to 6 the differential impact of environmental and genetic risk factors for OC symptoms on brain structure, and brain function during the performance of cognitive tasks, was investigated using the discordant/concordant MZ twin design. In **chapter 3** task performance and brain activation during the Tower of London planning paradigm were compared within 12 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 added 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. This sample of discordant/concordant MZ twins was also used to examine the differential impact of non-shared environmental versus genetic risk factors for OC symptomatology on inhibitory control related functional brain activation (Stroop and Flanker task: **chapter 5**) and on white matter structure (**chapter 6**). The findings of these studies, summarized in **table 10.1**, can be broadly divided into three different types: 1) environmental and genetic risk factors for OC symptoms affect different brain regions (**table 10.1**, uncolored cells); 2) environmental and genetic risk factors for OC symptoms affect the same brain regions (green cells); 3) environmental and genetic risk factors for OC

Table 10.1. Main findings of the studies that investigated the differential effect of environmental and genetic risk factors for OC symptoms on the brain

| Anatomical location | Planning related brain activity (Tower of London: chapter 3-4) | Response inhibition related brain activity (Stroop and Flanker: chapter 5) | White matter structure (fractional anisotropy: chapter 6) |
|-------------------------|--|--|--|
| premotor | environmentally mediated decrease genetically mediated decrease | | |
| medial frontal | | | environmentally mediated decrease genetically mediated increase |
| dorsolateral prefrontal | environmentally mediated decrease no genetic effects detected | environmentally mediated increase genetically mediated increase | environmentally mediated increase no genetic effects detected |
| inferior frontal | no environmental effects detected genetically mediated increase | | no environmental effects detected genetically mediated decrease |
| caudate nucleus | no environmental effects detected genetically mediated decrease | | |
| globus pallidus | no environmental effects detected genetically mediated decrease | | |
| anterior cingulate | environmentally mediated increase genetically mediated increase | environmentally mediated increase no genetic effects detected | |
| thalamus | | no environmental effects detected genetically mediated increase | |
| superior parietal | environmentally mediated decrease genetically mediated decrease | | |
| inferior parietal | environmentally mediated decrease genetically mediated increase | no environmental effects detected genetically mediated increase | environmentally mediated decrease genetically mediated increase |
| middle temporal | environmentally mediated increase genetically mediated decrease | environmentally mediated increase no genetic effects detected | environmentally mediated decrease genetically mediated increase |

Uncolored: brain regions affected by genetic risk factors for OC symptoms only or by environmental risk factors for OC symptoms only.

Green: brain regions affected by both environmental and genetic risk factors for OC symptoms.

Red: brain regions affected by environmental and genetic risk factors for OC symptoms, but in opposite directions.

symptoms affect the same brain regions but with opposite effects (red cells).

1) Environmental and genetic risk factors for OC symptoms affect different brain regions

Results (as summarized in **table 10.1**) suggest that environmental risk factors for OC symptoms mainly affect white matter structure and planning related activity patterns in the dorsolateral prefrontal part of the brain, whereas genetic risk factors for OC symptoms mainly affected white matter structure and/or planning related activity patterns in inferior frontal and striatal brain regions. Interestingly, all of these brain regions are implicated in the widely accepted neuroanatomical model of obsessive-compulsive disorder (OCD) involving the direct and indirect cortico–striato–thalamo–cortical (CSTC) loops where 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 (**figure 1.1**, thesis general introduction) (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). The dorsolateral prefrontal part of the brain is implicated in the dorsal loop of the CSTC in which disturbances may result in perseveration, reduced mental control and impaired response inhibition. In contrast, the inferior frontal part of the brain, is implicated in the ventral loop of the CSTC in which disturbances may result in tactless, impulsive and disinhibited behavior. So, although the results indicate that partly different regions were affected by environmental and genetic risk factors for OC symptomatology, both classes of risk factors strikingly converge on the dorsal and ventral CSTC loops, and a disturbance in either one of these brain regions could therefore result in OC behavior.

2) Environmental and genetic risk factors for OC symptoms affect similar brain regions

The results also indicated that some brain regions were affected by both environmental and genetic risk factors for OC symptoms. These included, the anterior cingulate (more activated in OC symptom high-scoring subjects during planning), superior parietal (less activated in OC symptom high-scoring subjects during planning) and dorsolateral prefrontal regions (more activated in OC symptom high-scoring subjects during response inhibition). It might be that these brain regions, commonly affected in all high-risk subjects, are the most closely related to the behavioral abnormalities observed in OCD. This appears to make sense for the anterior cingulate, 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 OCD (Aouizerate et al., 2004). It also appears to make sense for dorsolateral prefrontal regions, since dysfunction of this brain structure is associated with preservative, disinhibited behaviors, which OCD patients particularly show during the completion of their compulsions (Friedlander and

Desrocher, 2006). However, the results are not yet conclusive. An environmentally as well as genetically mediated increase in anterior cingulate was only observed during planning (Tower of London) and an environmentally as well as genetically mediated increase in dorsolateral prefrontal activity was only observed during a task measuring inhibitory control (Stroop). Moreover, the studies that investigated effects of environmental and genetic risk factors for OC symptoms on white matter structure and planning related brain activity indicated that the dorsolateral prefrontal region was only affected by environmental risk factors for OC symptoms. The observed planning related increase in anterior cingulate activity and response inhibition related increase in dorsolateral prefrontal activity could, alternatively, also be related to the specific demands of the tasks and act as compensatory mechanisms. During the Stroop task participants were asked to indicate the ink color of a printed word but to suppress processing of word meaning. The dorsolateral prefrontal cortex plays an important role in the inhibition of behaviors and is thought to be hypoactivated in OCD patients. The finding of increased dorsolateral prefrontal activity during the Stroop in OC symptom high-scoring subjects could therefore also reflect an adjustment to perform well, which is in line with the finding that OC symptom high-scoring subjects did not perform worse compared to OC symptom low-scoring subjects.

3) Environmental and genetic risk factors for OC symptoms affect the same brain regions but in opposite directions

White matter structure and/or planning related activity patterns in medial frontal, inferior parietal and middle temporal brain regions were found to be affected by both environmental and genetic risk factors for OC symptoms. However, alterations in these regions were observed in opposite directions in subjects at high environmental risk compared to subjects at high genetic risk (e.g., inferior parietal: decreased fractional anisotropy & decreased task-related activation vs. increased fractional anisotropy & increased task-related activation). Both medial frontal, parietal and temporal regions have been implicated in the neuroanatomical model for OCD, predominantly through their functional connections with the ventral prefrontal and dorsolateral prefrontal cortex. A change in the anatomy and functional activity of these regions in either direction (e.g., too much or too little) may lead to an imbalance between the direct and indirect pathways of the ventral and dorsal frontal-striatal loops, which subsequently may induce OC-like behavior.

Overall, these results indicate that environmental and genetic risk factors for OC symptoms can affect the brain differently, which highlights the importance to separate symptoms originating from a genetic predisposition from symptoms that are environmentally mediated, when studying the neurobiology of this disorder. One question that remains is whether the concordant MZ twin design

is the most optimal design for identifying brain regions that are under the influence of genetic risk factors for OC symptoms. Based on genetic model fitting in a large unselected group of Dutch twin pairs, it was shown that shared environment did not play a significant role in OC symptomatology (van Grootheest et al., 2007). From this it was assumed that a similarity in OC symptomatology in MZ twin pairs reflects their genetic resemblance. However, when deliberately selecting affected MZ twin pairs, some rare pairs could be included in which the OC symptoms in both subjects were triggered by non-shared (unique) environmental events (e.g., one twin has had a severe illness and the co-twin lost a child). Self-report questionnaires completed by the twins 1-3 weeks prior to MRI scanning, included questions on specific life-events. When exploring this data in retrospect it was found that 4 of the 23 concordant-high twin pairs indicated that they both experienced a negative life-event (e.g., sexual assault, violent crime, severe illness). There was no information available on the exact time these negative events took place relative to the onset of the OC symptoms, and on the impact of these specific events, so although they could have triggered OC symptoms, in genetically susceptible subjects, their occurrence might also be unrelated to the symptoms.

To be more sure that results obtained from the concordant MZ twin pair comparison are related to genetic risk factors for OC symptoms it is useful to collect OC symptom scores from the parents of these twins, where a high score on these symptoms in parents of high-scoring twins strengthens the assumption that these symptoms are indeed mediated by genetic risk factors (de Geus et al., 2007). The twin pairs included in this study were selected based on their sum scores on the 12-item Padua Inventory Revised Abbreviated (PI-R-ABBR), a self-report inventory measuring OC symptoms. In 2002 and 2008, the PI-R-ABBR was also sent to the parents of these twin pairs, but only if they were registered in the Netherlands Twin Register. From 13 (out of 23) concordant-high-scoring twin pairs, and from 14 (out of 28) concordant-low-scoring twin pairs, PI-R-ABBR data was available from at least one of the parents. When selecting the highest PI-R-ABBR score from each set of parents, parents from concordant-high-scoring twins show a mean(SD) OC symptom score of 13.9(3.46) and a median of 14, whereas parents from concordant-low-scoring twins show a mean(SD) OC symptom score of 8.5(5.13) and a median of 7.5. These parental data support the idea that a genetic contrast was created by selecting low and high concordant MZ twins.

An alternative to detect brain regions that are specifically affected by genetic risk factors for OCD, is by comparing brain structure and function between subjects selected to be at high or low genetic risk for OCD based on genetic variants found to be highly associated with the disorder. Over the last years, association and linkage studies have been performed in order to identify the genetic variants that contribute to the risk for OCD [for review see (Nicolini et al., 2009; Pauls, 2010;

de Mathis et al., 2011]). Most of these studies pointed towards functional deficits of genes involved in serotonergic, glutamatergic and dopaminergic neural signaling. However, with the exception of the glutamate transporter gene *SLC1A1* (Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a; Wendland et al., 2009), none of the examined candidate genes have been consistently replicated, and none of the association and linkage studies performed to date include sample sizes large enough to achieve genome-wide significance. So, a first challenge will be to create a more comprehensive picture of the genetic variants associated with OCD. In order to achieve this, genome-wide association studies are probably necessary. These genome-wide association studies provide more power to identify risk genes of relatively small effects, compared to the linkage and candidate gene studies previously performed, but ask for very large samples of affected individuals to obtain genome-wide significance. To date such studies have not yet been conducted for OCD. Once a more homogeneous picture of the genetic variants associated with OCD is created, neuroimaging studies comparing the brains of subjects at high genetic risk with those at low genetic risk for OCD based on these identified genetic variants can be performed to detect the brain regions specifically affected by genetic risk factors for the disorder.

Methodological issues

When comparing brain structure and function between subjects scoring high and low on psychiatric symptoms, results could be confounded by several factors. Here, it was investigated whether fMRI changes associated with heart rate changes during the cognitive tasks could influence the interpretation of the fMRI results obtained from the discordant/concordant twin comparison and if sex differences could be a potential source of the heterogeneity in previous MRI studies on OCD.

Heart rate

In order to explore the extent to which fMRI signal changes between cognitive task conditions were influenced by between-condition differences in heart rate, in **chapter 7** simultaneous electrocardiogram and BOLD fMRI recordings were performed in a group of subjects during the color-word Stroop task and the Tower of London cognitive planning task. It was found that there are substantial correlations between heart rate and fMRI signal changes across large parts of the brain during the performance of these cognitive tasks. However, even when heart rate was significantly modulated by task demands, the fMRI signals associated with heart rate variations did not significantly impact on higher-order fMRI task effects, indicating there is no need for taking heart rate variations into account when analyzing these specific fMRI task effects. However, the observed heart rate variations in this study were relatively small. In studies that make use of a paradigm

were heart rate variations between the task conditions are more pronounced (e.g., a task with higher emotional valence), it might still be useful to include heart rate data as a confounder. Inclusion of heart rate might also be useful in group comparison studies where one group shows a stronger heart rate change between stimuli. The OC symptom high-scoring twins included in our study did not show a significant stronger heart rate change between stimuli compared to low-scoring twins.

Sex differences

The brains of males and females are different with respect to global as well as regional volumes (Cosgrove et al., 2007; Lenroot and Giedd, 2010). However, previous findings with respect to regional volumetric differences are not yet conclusive. For example, some studies found larger hippocampal volumes in females (Giedd et al., 1997; Filipek et al., 1994), whereas others found larger hippocampal volumes in males (Good et al., 2001), or no difference in hippocampal volume between the sexes (Gur et al., 2002). We 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 a large sample of carefully matched male-female pairs, including opposite-sex twin and sibling pairs (**chapter 8**). The findings indicated that males have larger gray matter volumes and higher fractional anisotropy in, or surrounding, subcortical structures that are involved in the control of sexual and reproductive function (hypothalamus) and the programming and control of movement (putamen, globus pallidus, thalamus). In females larger gray matter volumes and greater cortical thickness were found in brain regions involved in the processing of emotion and interoceptive awareness (insula, anterior cingulate). Sex differences have also been observed with respect to the type of OC symptoms and its developmental trajectories. Females tend to report more contamination obsessions and cleaning compulsions, whereas symmetry, religious and sexual obsessions and an earlier onset of the disorder is more common in males (Labad et al., 2008; Noshirvani et al., 1991; Castle et al., 1995; Lensi et al., 1996; Tükel et al., 2004; Bogetto et al., 1999). This led to the question whether sex could be a potential source of heterogeneity between studies investigating the neurobiology of OCD. To address this question, the interaction of OC symptoms by sex on gray matter volume was assessed using voxel-based morphometry (**chapter 9**). For this analysis all high and low-scoring males and high and low-scoring females from the MZ twin sample and the opposite-sex twin and sibling pairs were included. The results indicated an OC symptom by sex interaction for the left middle temporal gyrus (larger in males with OC symptoms, but no effect in females), the right middle temporal gyrus (larger in males with OC symptoms, but reduced in females with OC symptoms) and right precuneus (larger in females with OC symptoms, but reduced in males

with OC symptoms). These observed differences acted to reduce or hide a main effect and thereby stress the importance of taking sex into account when investigating the neurobiology of OC symptoms.

The studies exploring the differential impact of environmental or genetic risk factors for OC symptoms on brain structure and functioning in chapters 3, 4, 5 and 6 of the present thesis did not take sex into account when comparing OC symptom high to OC symptom low-scoring subjects. In order to additionally test whether genetic and environmental risk factors for OC symptoms assert differential effects in the male and female brain, a full factorial design could be applied with twin pair type (discordant or concordant), OC symptom status (high or low-scoring) and sex (male or female) as three independent factors. However, this requires large samples of MZ discordant and concordant twin pairs. This study included a relatively small number of MZ male pairs (discordant: 6 pairs, concordant-high: 6 pairs and concordant-low: 8 pairs). Thus, larger samples of, especially, discordant and concordant MZ male pairs are needed. In addition, for future studies it might be of interest to test whether sex-specific sets of genetic variants for OCD derived from previously performed association studies (de Mathis et al., 2011) assert differential effects in the male and female brain.

Overall conclusions

- Environmental and genetic risk factors for OC symptoms affect the structure and functioning of the brain in different ways.
- Environmental risk factors for OC symptoms affect dorsolateral prefrontal white matter structure and dorsolateral prefrontal activity during a planning task that requires the subject to achieve a goal through a series of intermediate steps (Tower of London).
- Genetic risk factors for OC symptoms affect inferior frontal white matter and inferior frontal activity during a planning task.
- Fractional anisotropy and planning related activity in inferior parietal and middle temporal regions are affected by environmental and genetic risk factors for OC symptoms, but in opposite directions.
- Although different regions were affected by environmental and genetic risk factors for OC symptomatology, both classes of risk factors strikingly converge on the ventral and dorsal CSTC loops, confirming the importance of these brain regions in OC behavior.
- Increased anterior cingulate activity during a planning task is seen in OC symptom high-scoring twins from both the discordant and concordant sample, and may act as a compensatory mechanism to keep planning performance intact.

- Increased dorsolateral prefrontal activity during a task demanding inhibitory control (Stroop task) is seen in OC symptom high-scoring twins from both the discordant and concordant sample, and may act as a compensatory mechanism to keep inhibitory control intact.
- During performance of the Stroop task and Tower of London task there are substantial correlations between heart rate and fMRI signal changes across a large part of the brain, but these only marginally impact on higher-order fMRI task effects, indicating there is no need to correct for variations in heart rate in these fMRI paradigms.
- OC symptom related changes in gray matter volume are partly different for males and females, showing the importance of taking sex into account when investigating the neurobiology of OC symptoms.