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Obsessive-compulsive symptom related sex differences in brain structure

This chapter is submitted as:

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; Tükel 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

| Sample characteristics | Males | | Females | | P | OCS*SEX |
|---|------------------|------------------|------------------|------------------|--------------------|---------------------|
| | high (n = 31) | low (n = 41) | high (n = 58) | low (n = 73) | | |
| 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: | | | | | | |
| Aggressive/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 | | | | | | | | | | | | | | |
|---|------------------------------|----|-----------------|-------|-------|---------|---------|----------|-----------------|-------|------|---------|---------|----------|
| 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,

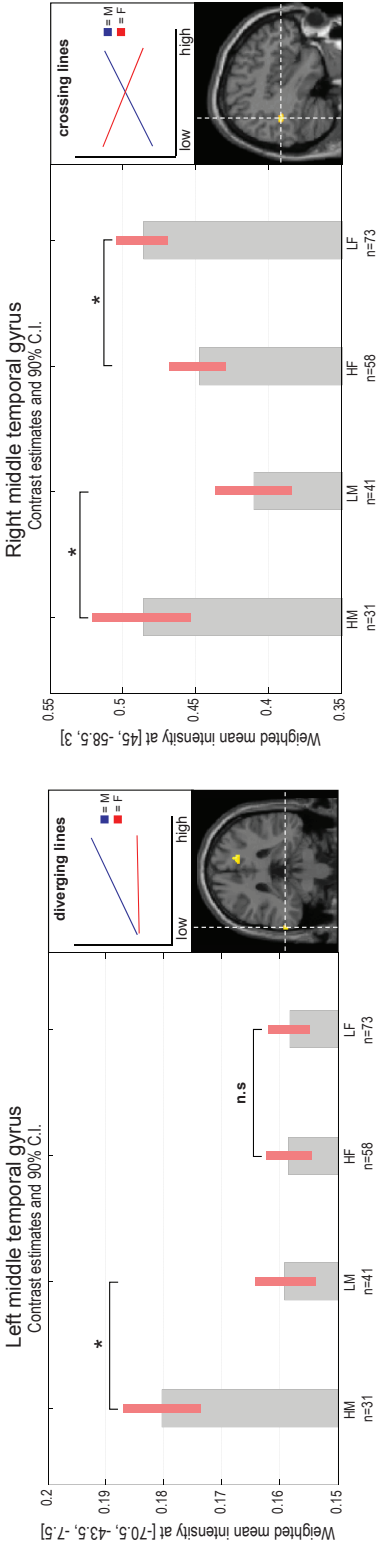
Table 9.3. Interaction effect of OCS*SEX in regional Gray Matter

| A. Covaried for total intracranial volume | | | | | | | | | | |
|--|----|-----------------|-------|------|---------|---------|----------|--|---------|---------|
| Anatomical Location | BA | MNI coordinates | | | F-value | p-value | # voxels | B. Covaried for total intracranial volume and Beck Depression Inventory scores | | |
| | | x | y | z | | | | MNI coordinates | F-value | p-value |
| 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 |
| Right middle temporal | 39 | 45 | -58.5 | 3 | 16.57 | <0.001 | 104 | 45 | -58.5 | 3 |
| 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

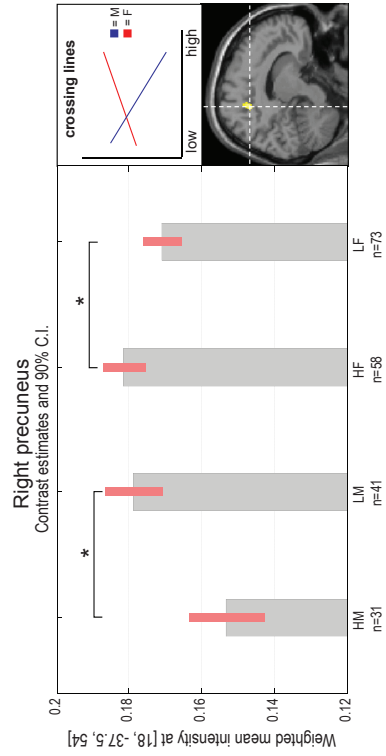


Figure 9.1. OC symptom status related brain changes that are different in males and females. Bar graphs indicate the weighted mean intensities (contrast estimates) and 90% confidence intervals for each coordinate derived from the OC symptom status by sex interaction test, separately for the OC symptom 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) (Tagao 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; Tükel et al., 2004) whereas symmetry, religious and sexual obsessions (Labad et al., 2008; Lensi et al., 1996; Tükel 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; Tükel 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.