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

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Abstract

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

Introduction

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

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

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

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

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

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

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

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

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

Materials and methods

Subjects

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

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

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

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

Protocol

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

Table 4.1. Twin sample demographics

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

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

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

PI-R-ABBR 2002 = mean Padua Inventory Abbreviated scores (SD) assessed in 2002; PI-R-ABBR current = mean Padua Inventory abbreviated scores (SD) at time of MRI examination; Y-BOCS severity lifetime/current = mean Yale-Brown Obsessive-Compulsive Scale severity scores (SD) across whole life span and at the time of MRI; Y-BOCS symptom lifetime/current: mean compound Yale-Brown Obsessive-Compulsive Scale symptom scores (SD) across whole life span and at the time of MRI; aggressive/checking, hoarding/saving, symmetry/ordering and washing/cleaning lifetime/current = mean Yale-Brown Obsessive-Compulsive Scale subcategory scores (SD) across the life span or at time of MRI (assessed using the Yale-Brown Obsessive-Compulsive Scale symptoms list).
Comorbidity lifetime/current (MINI) = mean comorbidity scores (SD) across whole life span or at the time of MRI (measured using the Mini International Neuropsychiatric Interview); Tic = mean tic scores (SD) at time of MRI; BDI = mean Beck Depression Inventory scores (SD) at time of MRI; STAI = mean State Trait Anxiety inventory scores (SD) at time of MRI; STAS = mean State Trait Anger Scale scores (SD) at time of MRI.

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

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

Tower of London

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

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

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

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

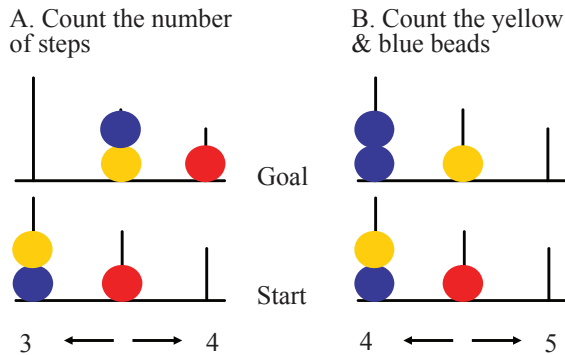


Figure 4.1. Examples of Tower of London stimuli used in the present study. (A) Planning condition; (B) baseline condition [adapted from van den Heuvel et al. (2005a)].

Image acquisition

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

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

Data analysis

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

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

Statistical tests

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

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

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

Post hoc region of interest based comparison

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

Results

Questionnaire and interview data

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

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

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

Task performance

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

Functional imaging

Main task effect

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

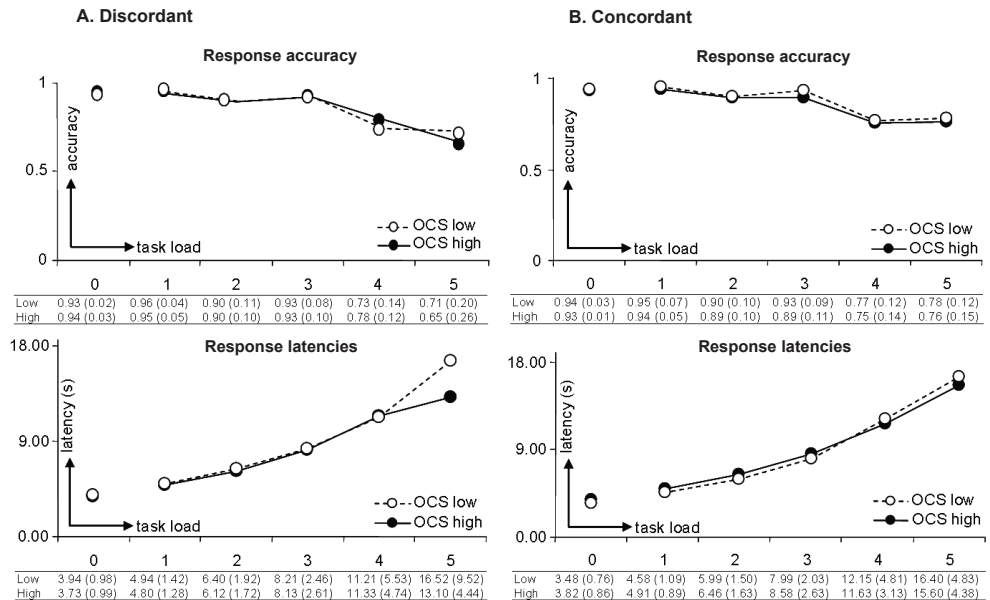


Figure 4.2. Tower of London task performance. (Top): Response accuracy (between 0 and 1) as a function of task load levels 1, 2, 3, 4 and 5 (task load 0 = baseline condition) in the (A) discordant group, (B) concordant group. (Bottom): Mean latencies (s) of correct responses as a function of task load.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

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

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

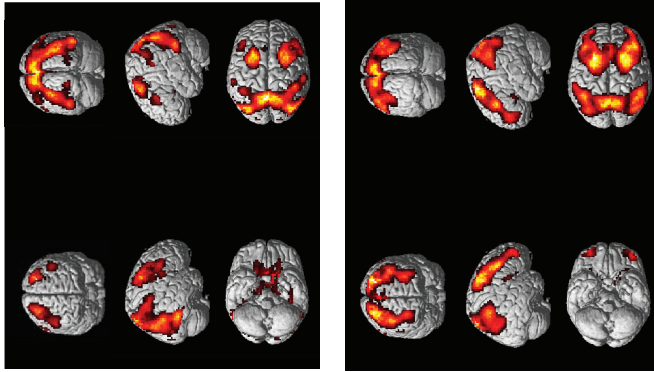
Table 4.3, left, and **figure 4.4** show clusters of obsessive-compulsive symptoms-related differences in brain activation between the discordant-high and low twins. For the ‘planning versus baseline’ contrast (**figure 4.4A**), twins scoring high on obsessive-compulsive symptoms compared with their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

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

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

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

Anatomical renderings



Glass brain projections

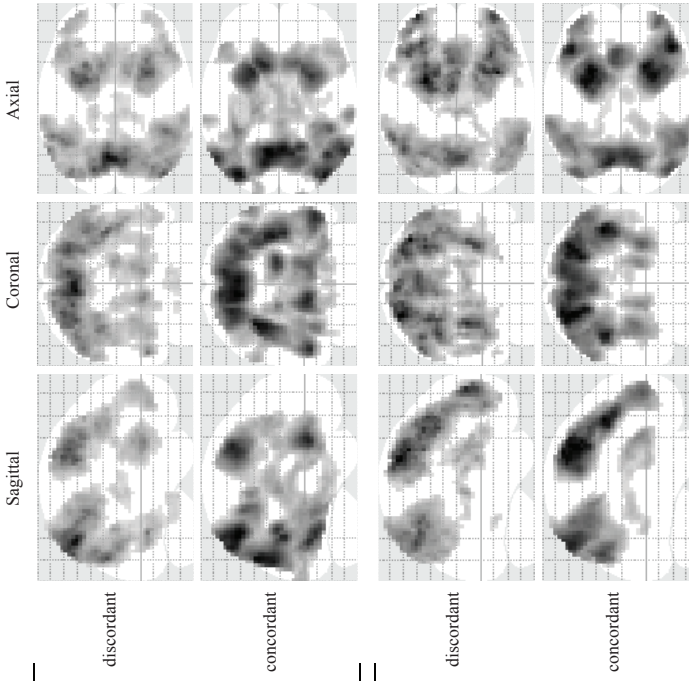


Figure 4.3. Brain regions showing increased functional MRI signal during Tower of London cognitive planning. Glass brain overviews depict brain activity patterns for 'planning versus baseline' (top) and 'task load' (bottom) contrasts in discordant and concordant twins. Anatomical renderings on the right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

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

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

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

Post hoc region of interest comparisons

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

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

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

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

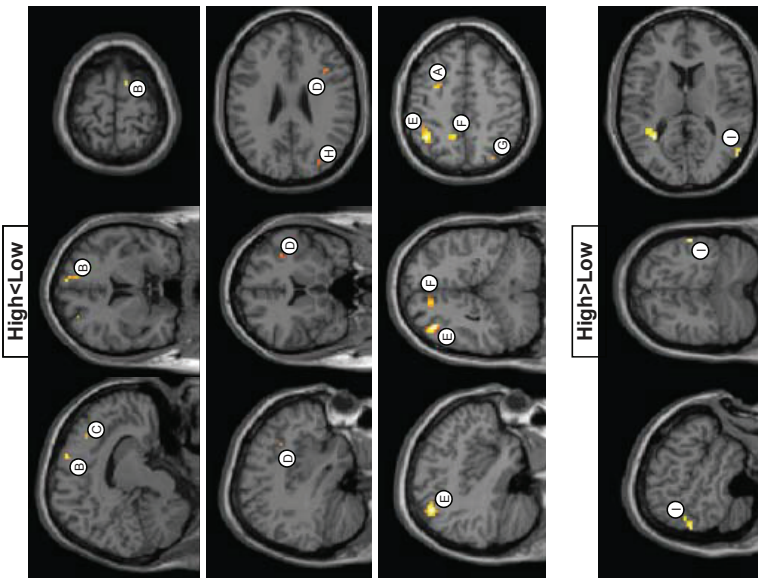
Test	Cluster label ^a	Anatomical location	BA	MNI coordinates (19 pairs)			Z score	p-value	No. of voxels	MNI coordinates (8 pairs) ^b			Z score	No. of voxels	Euclidean distance (mm)
				x	y	z				x	y	z			
<i>'planning vs. baseline'</i>															
high<low	A	Left premotor cortex	6	-27	3	54	3.41	<0.001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

a Clusters with regional brain activation differences between discordant-high and discordant-low twins for the 'planning versus baseline' and 'task load' contrasts.

Test = test for significant decreases (high<low) or increases (high>low) in twins with high relative to low obsessive–compulsive symptoms scores; cluster label = alphabetical cluster label as displayed in anatomical overlays of figure 4.4A ('planning versus baseline' contrast) and B ('task load' contrast); BA = Brodmann area; MNI coordinates (19 pairs) (mm) = location of voxel with largest effect size for the 19-pair comparison; Z score = z-value of voxel with largest effect size; p-value = cluster P-value; no. of voxels = number of voxels in cluster.

b Montreal Neurological Institute coordinates (mm) of voxel with largest effect size for the post hoc within-pair comparison in the 8 pairs still extremely discordant at the time of scanning; Z score = z-value of voxel with largest effect size for the post hoc 8 pair comparison; no. of voxels = number of voxels in cluster for the post hoc 8 pair comparison; Euclidean distance (mm) = distance between the coordinates derived from the original 19 pair comparison and the coordinates derived from the additional 8 pair comparison.

A. Planning vs. baseline



B. Task load

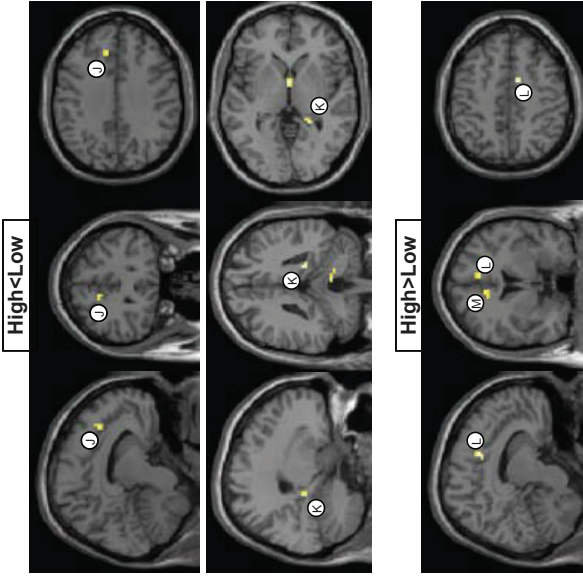


Figure 4.4. Brain regions showing reduced (top: high<low) and increased (bottom: high>low) functional MRI signal in twins with high obsessive-compulsive symptoms versus twins with low scores from the discordant group. (A) 'planning versus baseline' contrast; (B) 'task load' contrast.

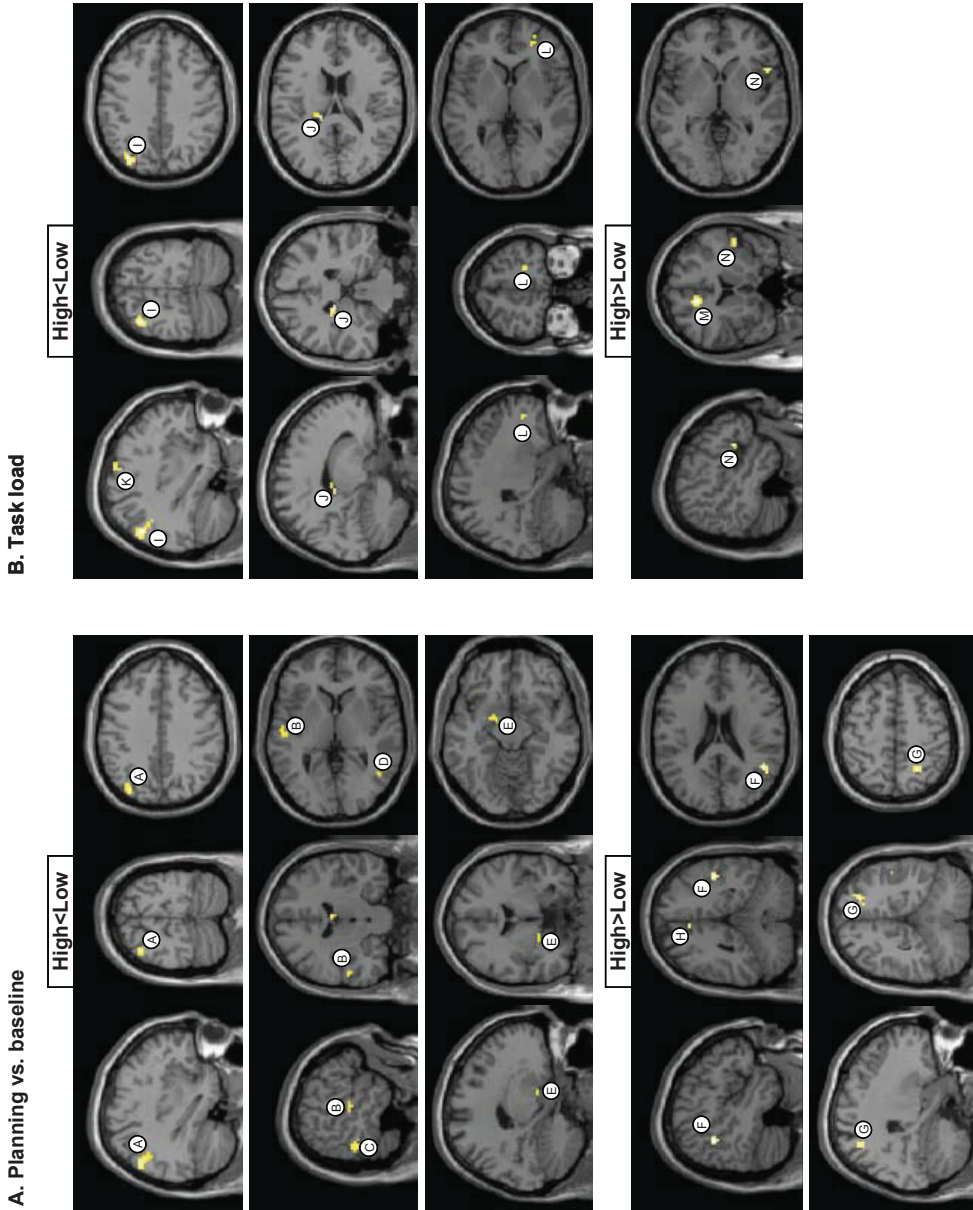
Table 4.4. Brain activation differences between twins scoring high and low on obsessive–compulsive symptoms from the concordant group

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

a Clusters with regional brain activation differences between concordant-high and concordant-low twins for the 'planning versus baseline' and 'task load' contrasts.

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

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



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

Discussion

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

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

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

Regions affected by environmental risk

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

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

Regions affected by genetic risk

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

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

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

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

Regions affected by environmental and genetic risk

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

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

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

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

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

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

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

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

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

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