Chapter 6

Compensatory fronto-parietal activity during working memory: an endophenotype of obsessive-compulsive disorder.

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Abstract

Background

Subtle deficits in executive functioning are present in patients with obsessive-compulsive disorder (OCD) and their first-degree relatives, suggesting involvement of the fronto-parietal circuits. The neural correlates of working memory may be a neurocognitive endophenotype of OCD.

Methods

Forty-three unmedicated OCD patients, 17 unaffected siblings and 37 matched comparison subjects performed a visuo-spatial n-back task, with a baseline condition (N0) and three working memory load levels (N1, N2, N3), during functional magnetic resonance imaging. Task-related brain activity was compared between groups in fronto-parietal regions-of-interest. Generalized psycho-physiological interaction analyses were used to study task-related changes in functional connectivity.

Results

OCD patients, compared with comparison subjects and siblings, showed increased error rates at N3 ($F=4.4$, $p=0.01$). Compared with comparison subjects, OCD patients showed task-related hyperactivation in left dorsal frontal areas and left precuneus, associated with better task performance. Siblings exhibited hyperactivation in a bilateral fronto-parietal network. Increased task load was associated with increased task-related brain activity, but in OCD patients and siblings this increase was smaller from load N2 to N3 than in comparison subjects ($\chi^2=7.3$, $p=0.03$). OCD patients, compared with siblings and comparison subjects showed increased task-related functional connectivity between frontal regions and bilateral amygdala.

Conclusions

These findings indicate that compensatory fronto-parietal brain activity in OCD patients and their unaffected relatives preserves task performance at low task loads, but is insufficient to maintain performance at high task loads. Fronto-parietal dysfunction may constitute a neurocognitive endophenotype for OCD possibly reflecting limbic interference with, and neural inefficiency within the fronto-parietal network.

Introduction

Obsessive-compulsive disorder (OCD) is a heritable (Nestadt et al., 2000;van Grootheest et al., 2005) neuropsychiatric disease characterized by intrusive thoughts (obsessions) and repetitive behaviors (compulsions) aimed at reducing the anxiety caused by the obsessions. The etiology of the disorder is not fully understood, and multiple genes and environmental factors are involved (van Grootheest et al., 2008). Disturbances in the fronto-striatal and fronto-parietal brain circuits have been implicated in the pathogenesis (Menzies et al., 2008a;Melloni et al., 2012), but in patients who have suffered from OCD for a number of years it may be difficult to differentiate between a pathological substrate underlying the disorder and abnormalities secondary to the disorder, for example as a result of performing compulsions for many years, or by its long-term treatment. A promising approach to elucidate the neurobiology of OCD is to search for intermediate markers of brain dysfunction (endophenotypes) that are closer to the underlying pathology than the heterogeneous clinical symptoms (Gottesman and Gould, 2003;Chamberlain and Menzies, 2009). An endophenotype reflects the genetic vulnerability to the disease, is relatively independent from disease severity and is more often present in unaffected first-degree relatives of patients than in the general population. Therefore, a family study design comparing patients and their unaffected first-degree relatives with unrelated comparison subjects enables identifying putative endophenotypes.

Deficits in several executive functions have been suggested as endophenotypes of OCD including decreased response inhibition (Chamberlain et al., 2007;de Wit et al., 2012;Rajender et al., 2011), cognitive inflexibility (Rajender et al., 2011;Chamberlain et al., 2007;Cavedini et al., 2010), and impaired performance on planning and working memory tasks (Cavedini et al., 2010;Delorme et al., 2007). Although functional neuroimaging studies probing executive functions have shown aberrant recruitment of frontal, parietal and cingulate cortices as well as striatal areas in OCD (Remijnse et al., 2005;van den Heuvel et al., 2005;Gu et al., 2008;Yucel et al., 2007;Fitzgerald et al., 2005;Kocak et al., 2011), few studies have directly compared OCD patients with unaffected first-degree relatives and comparison subjects. OCD patients share frontal and parietal white matter and grey matter abnormalities with first-degree relatives which are associated with decreased inhibitory control (Menzies et al., 2007;Menzies et al., 2008b) and both groups show altered frontal and parietal activation during reversal learning (Chamberlain et al., 2008) and response inhibition (de Wit et al., 2012). Another cognitive domain depending on fronto-parietal circuitry is working memory (Owen et al., 2005), which is hypothesized to be affected in OCD (Purcell et al., 1998;van der Wee et al., 2003;van der Wee et al., 2007;Savage et al., 1999).

To date no studies have reported on the functional neural correlates of working memory in unaffected relatives of OCD patients. In OCD patients, compared with comparison subjects, both increased and decreased working memory-related recruitment

of the anterior cingulate cortex (ACC)(van der Wee et al., 2003;Shin et al., 2006;Koch et al., 2012) and dorsolateral prefrontal cortex (dlPFC) (Shin et al., 2006;Nakao et al., 2009) has been observed. Other studies reported hyperactivity in premotor areas and inferior frontal gyrus (Koch et al., 2012;Henseler et al., 2008). Task performance in patients was impaired in some (van der Wee et al., 2003;Shin et al., 2006), but not in other of these studies (Henseler et al., 2008;Nakao et al., 2009;Koch et al., 2012). Visuo-spatial memory impairment in OCD has been more consistently reported than deficits in verbal memory (Kuelz et al., 2004), but others have argued that working memory deficits in OCD are not modality-specific but rather depend on executive demands, e.g., high task load and/or complexity (Harkin and Kessler, 2011). Moreover, inconsistencies in behavioral and neuroimaging studies may be partly explained by differences in methodology (e.g. verbal vs. spatial tasks, varying task complexity, variable statistical thresholding) and patient characteristics (pharmacological treatment, disease severity, comorbid depression). Also, executive function and recruitment of task-related fronto-parietal areas in OCD may be modulated by limbic activity. In a previous study on executive functioning across several anxiety disorders, including OCD, we showed that impaired task performance was related to decreased frontal and increased amygdala activity (van den Heuvel et al., 2011). Similarly, during response inhibition decreased dorsal cingulate activity was accompanied by increased limbic and striatal activity in OCD patients and increased functional connectivity between cingulate and limbic regions correlated with decreased performance (Kang et al., 2013).

In the present study we aimed to investigate dysfunction of the fronto-parietal network as a potential endophenotype for OCD by studying unmedicated OCD patients, their unaffected siblings and unrelated matched comparison subjects while performing a visuo-spatial working memory task during functional magnetic resonance imaging (fMRI). We hypothesized decreased task performance at high load-levels in OCD patients and to a lesser extent in their unaffected relatives, coupled with aberrant recruitment of the fronto-parietal network. We expected interference of limbic regions with the task-related network.

Methods

Participants

Forty-four patients with OCD, 19 of their siblings and 38 healthy matched comparison subjects participated in the study. Results from a response inhibition task in these same samples were reported previously (de Wit et al., 2012). One comparison subject and one patient were excluded due to abnormalities of the structural scan. Two siblings were excluded, due to inadequate task performance at baseline. The remaining group consisted of 43 OCD patients, 17 siblings and 37 comparison subjects. Patients were recruited through outpatients services contributing to the Netherlands OCD Association study (Schuurmans et al., 2012), through Altrecht Academic Anxiety Center, and through

advertisements. Comparison subjects were recruited through advertisements. All subjects were screened for axis I diagnoses with the Structural Clinical Interview for DSM-IV (First et al., 1999). OCD symptom characteristics and severity were assessed with the Obsessive-Compulsive Inventory-Revised (OCI-R) (Foa et al., 2002) and the Yale-Brown Obsessive Compulsive symptom Severity scale (Y-BOCS)(Goodman et al., 1989), respectively. Current depressive symptoms were assessed with the Montgomery Åsberg Depression Rating Scale (MADRS)(Montgomery and Asberg, 1979), anxiety with the Beck Anxiety Inventory (BAI)(Beck et al., 1988), handedness with the Edinburgh Handedness Inventory (Oldfield, 1971) and verbal IQ was estimated with the Dutch reading test for adults (Schmand et al., 1991). Subjects were between 18-65 years old and had corrected-to-normal vision. Patients were included if they had a primary diagnosis of OCD without predominant hoarding. Psychiatric comorbidity (including tic disorders) was not an exclusion criterion. Exclusion criteria were psychotic symptoms, a major somatic illness, a history of major neurological illness and MRI contra-indications. All patients were off psychotropic medication for at least 4 weeks. Siblings did not have a diagnosis of OCD or any other current axis I diagnosis. Comparison subjects had no current axis I diagnosis and no family history of OCD. The protocol was approved by the local medical ethical committee (VU University Medical Center, Amsterdam) and all subjects gave written informed consent.

N-back task

Participants performed a visuo-spatial n-back working memory task designed after Gevins and Cutillo (1993) and programmed in E-prime 1.2. Participants watched a screen on which in each trial (every 2.8 seconds) a yellow dot randomly appeared at the left, right, bottom or top of a blue diamond, which corresponded to four similar locations on an MRI compatible response box (Current Designs, Inc., Philadelphia, USA). In the baseline condition (*0*-back or N0) participants were instructed to respond to the stimulus dot immediately by pressing the corresponding button. In the three increasing working memory load conditions subjects had to respond to the location of the dot with a delay of one (*1*-back or N1), two (*2*-back or N2) or three stimuli (*3*-back or N3), while simultaneously remembering new locations as the task continued. Conditions were presented in three blocks of 20 trials each, resulting in 60 trials per condition. Presentation of blocks was fixed, looping three times in an order of increasing difficulty (N0, N1, N2, N3) and started with a screen showing the current load-level. The main behavioral outcome measure was the percentage of correct responses for each load level. Prior to the experiment, participants were familiarized with the task during a practice session.

Image acquisition

Scanning was performed on a GE Signa HDxt 3.0-Tesla MRI-scanner (General Electric, Milwaukee, Wis, USA), at the VU University Medical Center, with an 8-channel circular polarized head coil. To reduce motion, the subject's head was immobilized with foam pads. Functional images with whole-brain coverage were acquired using a gradient echo-planar imaging sequence (repetition time 2100 ms; echo time 30 ms; 64x64 matrix; field of view 24 cm; flip angle 80°) with 40 ascending slices per volume (3.75x3.75 mm in-plane resolution; slice thickness 2.8 mm; inter-slice gap 0.2 mm). Three dummy scans were acquired before the task started. A structural T1-weighted scan was made for co-registration (256x256 matrix; voxel size 1x0.977x0.977mm; 172 sections).

Statistical analyses on clinical and behavioral data

Demographic and clinical data were analyzed with a standard statistical package (SPPS 15, Chicago), using one-way analysis of variance (ANOVA). Behavioral data were analyzed with repeated measures ANOVA with task load level as repeated factor and followed up by post-hoc two-sample T-tests. Associations between performance and clinical variables (Y-BOCS, MADRS and BAI) were investigated with correlations (Spearman's rho (rho_S)). If data did not meet parametric assumptions non-parametric tests were used as indicated. Significance was set at $p<0.05$ 2-tailed, trends at $p<0.1$.

Image processing and analyses

Imaging data were processed and analyzed using Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK). Standard preprocessing comprised reorientation, realignment and unwarping, co-registration, warping to Montreal Neurological Institute (MNI) standard space, reslicing to 3x3x3mm voxels and spatial smoothing with an 8 mm full-width-half-maximum Gaussian kernel.

Data were analyzed in context of the general linear model using a block design. At 1st level, for each individual participant a general task-related blood-oxygen-level-dependent (BOLD) response was computed by contrasting the summed response to N1, N2 and N3 with the response during baseline (contrast: [N123>N0]). Additionally, contrasts were computed for each load separately ([N1>N0], [N2>N0] and [N3>N0]) to characterize load-dependent effects (see analysis below). A high-pass filter (128 s cut-off period) was used to remove noise associated with low-frequency confounds.

Between-group differences were tested in 2nd level models using ANOVA and two sample T-tests, with the mean accuracy of N1, N2 and N3, added as a covariate, to ensure that group differences reflected a true measure of (susceptibility to) OCD instead of performance differences.

Regions-of-interest (ROIs) were derived from the whole-brain main effect of task across all subjects thresholded at $p<0.05$ with Family Wise Error correction (p_{FWE}).

Within the task-related clusters, local maxima with the best correspondence to those reported in an n-back meta-analysis (Owen et al., 2005) (Table 6.1) were used as peak coordinates around which a 10mm radius sphere was created with MarsBar (Brett et al., 2002). ROIs consisted of the bilateral dlPFC, premotor cortex and pre-supplementary motor area (premotor/pre-SMA), precuneus, inferior parietal cortex and one ROI for the ACC. To control for Type I errors a Bonferroni corrected *alpha* for the number of ROIs was calculated, taking into account the correlation between ROIs using the Simple Interactive Statistical Analysis Bonferroni Tool (http://www.quantitativeskills.com/sisa/calculations/bonfer.htm).The parameter estimates for the main effect of task in the nine ROIs showed a mean correlation coefficient of rho=0.68, resulting in an equivalent Bonferroni corrected *alpha* of 0.025. Therefore imaging results were considered significant at p_{FWE}<0.025 in the a priori ROIs and trend significant at 0.025< p_{FWE}>0.05 (Worsley et al., 1996). Whole-brain voxel-wise FWE-corrected results are reported as well.

Since working memory impairments in patients could be load-dependent (Callicott et al., 2000;Mannie et al., 2010;Koch et al., 2012), task load effects were characterized by extracting the mean parameter estimates for the [N1>N0], [N2>N0] and [N3>N0] contrasts from a "combined ROI" encompassing all ROIs, thereby representing the full task-related network, from the individual 1st level models. The percentual change in BOLD response from N1 to N2 and from N2 to N3 for each individual was computed and within- and between-group differences were tested in SPSS with one-sample T-tests and ANOVAs respectively.

To assess the effect of disease severity (patients only) and task performance (all groups) on task-related BOLD activity, Y-BOCS scores and mean accuracy scores were correlated to task-related activity (N123>N0) in a regression analysis in SPM.

To specifically test for task-related changes in connectivity between task-related seed regions and the amygdala, we used generalized psycho-physiological interaction analyses (gPPI)(McLaren et al., 2012). With gPPI all four task conditions and interactions could be modeled simultaneously, resulting in a better model fit compared to traditional PPI analyses (McLaren et al., 2012). ROIs with between-group differences during task-related brain activation (left dlPFC, left pre-SMA and left precuneus, see Table 6.3 in results section) were chosen as seeds and amygdala ROIs were based on the automatic anatomic labeling (AAL) atlas. For more detailed methodological information on the gPPI analyses see the supplementary methods section.

Table 6.1 Coordinates used for regions-of-interest (ROI), derived from peak activity during main effect of task across all subjects (N=97) and coordinates as reported in the meta-analysis by Owen et al.

Region	BA	side	Coordinates ROI			Coordinates Owen et al. 2005		
			x	y	z	x	y	z
Dorsolateral prefrontal cortex	46	L	-39	32	31	-	-	-
		R	39	38	28	36	36	24
Premotor/pre-SMA	6	L	-21	5	64	-	-	-
		R	30	5	58	16	4	57
						30	0	48
Precuneus	7	L	-9	-67	52	-	-	-
		R	12	-73	55	10	-58	54
						24	-60	52
						10	-48	64
Inferior parietal cortex	40	L	-45	-46	43	-34	-58	42
		R	42	-52	46	42	-50	36
Dorsal anterior cingulum	32/6	L/R	3	20	49	0	12	42

BA= Brodmann's area, L= left, R= right. ROI= region-of-interest, coordinates in MNI space

Results

Sample characteristics

The three study groups were matched on all demographic variables (see Table 6. 2). Patients had significantly higher scores on Y-BOCS, OCI-R, and MADRS compared to both comparison subjects and siblings. Comparison subjects and siblings did not differ on any clinical variable.

Behavioral results

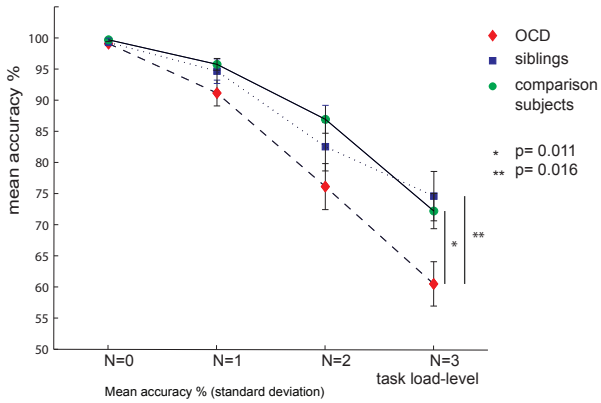
Friedman's ANOVAs (see Figure 6.1) showed that accuracy decreased with increasing task load across all groups ($\chi^2(3)=195.6$, $p<0.001$) and for each separate group (OCD: $\chi^2(3)=89.9$, $p<0.001$. siblings: $\chi^2(3)=36.3$, $p<0.001$, comparison subjects: $\chi^2(3)=71.6$, $p<0.001$). OCD patients showed decreased accuracy at N3 ($F(2,94)=4.68$, $p=0.01$), both compared to comparison subjects ($p=0.01$) and to siblings ($p=0.02$), but not at N1 or N2 ($p>0.1$). Siblings and comparison subjects had similar accuracy scores on all load levels ($p>0.1$). Reaction times did not significantly differ between groups at any task load level ($p>0.05$), with a trend for OCD patients to be slower at N1 ($\chi^2(2)=5.6$, $p=0.06$). Mean accuracy did not correlate with Y-BOCS severity ($rho_s=-0.12$, $p=0.44$),

Table 6.2: Demographic and clinical variables.

	OCD patients (n=43)		Siblings (n=17)		Comparison subjects (n=37)		Statistical analysis	
	mean	SD	mean	SD	mean	SD	$F_{(df=2, 94)}$	p
Demographic measures								
Age (years)	38.1	9.7	36.4	13.5	39.2	11.5	0.4	0.67
Gender (female:male)	21:22		5:12		20:17		$\chi^2 = 2.9$	0.24
Handedness (right:left)	36:7		12:5		32:5		$\chi^2 = 2.1$	0.35
Estimated verbal IQ	97.0	12.3	99.5	11.0	97.3	8.5	0.34	0.72
Subjects with comorbid axis I disorder	24[a]	56%	0		0			
Clinical measures								
Y-BOCS severity	21.3	6.1	0	0	0	0		
OCI-R, total score	24.5	11.8	2.9	3.1	3.2	4.8	65.0[b]	<0.001
checking subscore	6.3	3.7	0.5	0.8	0.5	0.9	57.5[b]	<0.001
washing subscore	3.1	3.9	0.18	0.4	0.3	0.6	18.5[b]	<0.001
symmetry subscore	4.7	3.7	0.8	1.3	0.8	1.5	35.4[b]	<0.001
MADRS (points)	11.2	8.0	1.6	3.3	0.9	1.5	53.5[b]	<0.001
BAI (points)	15.4	10.6	2.7	3.4	2.1	2.8	49.4 [b]	<0.001

SD, standard deviation; χ^2, Chi-square test (df=2), Y-BOCS= Yale-Brown Obsessive Compulsive Scale, OCI-R= Obsessive-Compulsive Inventory-Revised, MADRS= Montgomery Asberg Depression Rating Scale, BAI= Beck Anxiety Inventory. [a] 14 anxiety disorders, 10 mood disorders, 3 tic disorders, 2 eating disorders, 1 somatoform disorder, 1 alcohol dependence in partial remission. Total number of comorbid disorders is higher than 24, because several patients had more than one comorbid disorder. [b] Kruskal-Wallis test

but correlated negatively with MADRS scores (rho_S=-0.51, p<0.001 also when controlling for IQ rho=-0.49, p=0.001) and with BAI scores (rho_S=-0.51, p<0.001). Partial correlations revealed that BAI scores, corrected for MADRS, still correlated with performance (rho_S=-0.41, p<0.007), but MADRS scores, corrected for BAI scores, did not (rho_S=-0.25, p>0.05).



Mean accuracy % (standard deviation)

	N=0	N=1	N=2	N=3
OCD	99.1 (1.7)	91.2 (13.6)	76.2 (24.3)	60.6 (23.3)
Siblings	99.3 (1.3)	94.7 (8.1)	82.6 (16.0)	74.7 (16.3)
Comparison subjects	99.7 (1.7)	95.8 (5.8)	87.0 (13.7)	72.3 (17.2)

Figure 6.1: Performance for three groups at different load levels.

Main effect of task and group-by-task effects

A robust effect of task (N123>N0) was found in each group (Figure 6.2 panel A and Table S6.1), including activation of the previously reported bilateral fronto-parietal network (Owen et al., 2005). OCD patients, compared with comparison subjects, showed increased task-related activity in left dlPFC and at trend-level in the left premotor/pre-SMA and left precuneus (Table 6.3). Siblings, compared with comparison subjects, showed increased activity in bilateral dlPFC, left premotor/pre-SMA, ACC, bilateral precuneus and bilateral inferior parietal cortex. Compared with OCD patients, siblings showed increased activity in the ACC. No regions showed increased activation in comparison subjects compared to either siblings or OCD patients. Whole-brain analyses showed no additional between-group differences. Adding age, gender, and IQ as nuisance covariates to the analysis did not change the results. Separate analyses excluding OCD patients with comorbid depression (N=12) or anxiety (N=14) also yielded similar results (data not shown).

Task load effects

Activation of the task-related network (combined ROI) at each load level is shown in Figure 6.2 panel B. OCD patients showed a 59% median increase in activation from N1 to N2 ($p=0.002$) and a non-significant 3% decrease from N2 to N3 ($p=0.78$). Siblings showed 63% median increase in activation from N1 to N2 ($p=0.01$), but no significant increase from N2 to N3 (3%, $p=0.76$). The comparison group showed an increase both from N1 to N2 (106%, $p<0.001$) and from N2 to N3 (21%, $p=0.002$). Percent changes from N2 to N3 significantly differed between groups (Kruskall-Wallis $\chi^2=7.26$, $p=0.03$), between patients and comparison subjects (Mann-Whitney $U=535$, $p=0.01$)

Table 6.3 Significant group interactions between 43 OCD patients, 17 siblings and 37 comparison subjects (HC) for task-related activity (contrast N123>N0)

Region-of-interest	BA	side	Interaction	Coordinates			k_e	Z	p_{FWE}
				x	y	z			
dlPFC	46	L	OCD>HC	-36	35	28	20	3.52	0.007
			Siblings>HC	-39	35	31	56	4.71	<0.001[a]
		R	Siblings>HC	39	38	25	9	3.40	0.011
Premotor/	6	L	OCD>HC	-15	-1	64	1	2.89	0.041[b]
pre-SMA			Siblings>HC	-21	8	67	87	5.20	<0.001[a]
			Siblings>OCD	-21	8	64	12	3.42	0.01
		R	no interactions						
Precuneus	7	L	OCD>HC	-6	-67	58	5	2.96	0.034 [b]
			Siblings>HC	-12	-70	61	37	3.99	0.002
		R	Siblings>HC	15	-79	55	8	3.16	0.022
IPC	40	L	Siblings>HC	-54	-46	46	4	3.49	0.009
				-42	-55	46	3	3.02	0.033 [b]
		R	Siblings>HC	42	-46	46	11	3.41	0.013
dACC	32/6		Siblings>OCD	0	20	46	39	3.46	0.009

[a] also significant at p<.05 whole-brain Family Wise Error corrected [b] significant at trend-level p_{FWE}>0.025. BA= Brodmann's area. L= left, R= right. dlPFC= dorsolateral prefrontal cortex, pre-SMA= pre-supplementary motor area, IPC= inferior parietal cortex, dACC= dorsal anterior cingulate cortex. Coordinates in MNI space. K_e=cluster size. Z= Z score. p_{FWE}= p-value with family wise error correction for the search volume. Coordinates in MNI space

and at trend-level between siblings and comparison subjects (Mann-Whitney U=214, p=0.06). The percent change in activation from N1 to N2 showed group differences at trend-level (Kruskall-Wallis χ^2=4.85, p=0.09) driven by a larger increase in activity in the comparison group than in OCD patients (Mann-Whitney U=575, p=0.03). Visual inspection (see Supplemental Figure S6.1) showed that observed task load-related changes were not driven by any particular ROI.

Effects of task-performance and disease severity on brain activity

Y-BOCS scores did not correlate with task-related activation of the fronto-parietal network in OCD patients. In comparison subjects mean accuracy correlated positively with task-related activation in bilateral inferior parietal cortex and precuneus and right premotor/pre-SMA. In OCD patients and siblings no correlations between brain

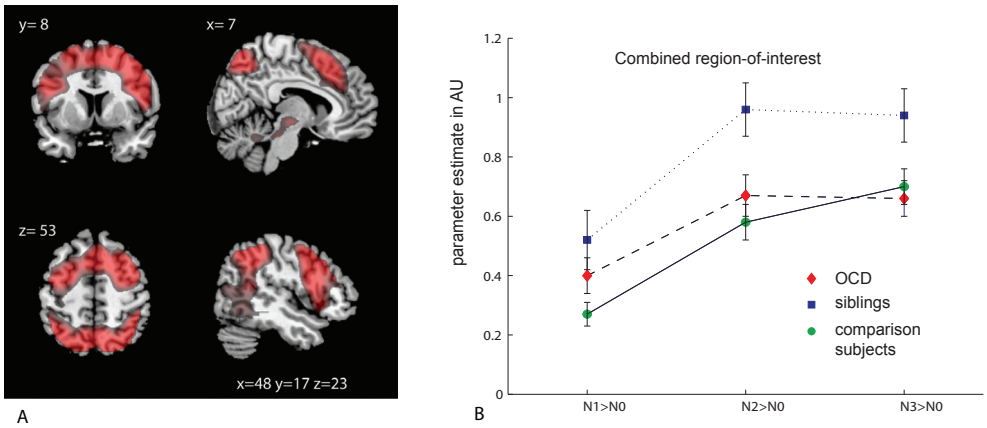


Figure 6.2:

Panel A: Main effect of task (contrast N123>N0) across all participants (N=97), thresholded at T=4.71, p<0.05 Family Wise Error corrected. The figure shows activation in a bilateral medial and lateral fronto-parietal network.

Panel B: Parameter estimates (in arbitrary units aU) for each task load level for the combined region-of-interest (see supplemental Figure S6.2 for the separate regions-of-interest) show hyperactivation at the lower load levels and no increase in activation from N2 to N3 in OCD patients and siblings vs. comparison subjects.

activity and performance were found. To further assess the effects of task-performance on brain activation in OCD patients, we performed a post-hoc analysis in which we compared task-related brain activation between OCD patients with high and low accuracy (based on a median-split on mean accuracy scores (median=81%)). OCD patients with low accuracy had lower estimated IQ, higher depression and anxiety scores and tended to have more comorbidity (Supplemental Table S6.2). An ANOVA with 4 groups (OCD low accuracy, OCD high accuracy, siblings, comparison subjects) revealed that task-related hyperactivation in left dlPFC and left premotor/pre-SMA was present in the high accuracy OCD group but not in the low accuracy group (see Figure 6.3 and Supplemental Table S6.3).

Functional connectivity between fronto-parietal seeds and bilateral amygdala

OCD patients showed increased task-related functional coupling between the left pre-SMA/premotor seed and bilateral amygdala compared with comparison subjects and between the left dlPFC seed and the right amygdala compared with both comparison subjects and siblings (see Table 6.4). There were no between-group differences for the left precuneus seed. Siblings did not differ from comparison subjects. Between-group differences in connectivity between the left pre-SMA and right amygdala were primarily driven by the OCD patients with low task accuracy (see Figure 6.4). Increased

connectivity with the amygdala was most evident at N3 for the low accuracy group, whereas OCD patients with high accuracy and siblings did not show significant connectivity, and comparison subjects showed an inverse correlation between pre-SMA and amygdala activity. Connectivity between left pre-SMA and right amygdala did not correlate with BAI scores ($p>0.05$).

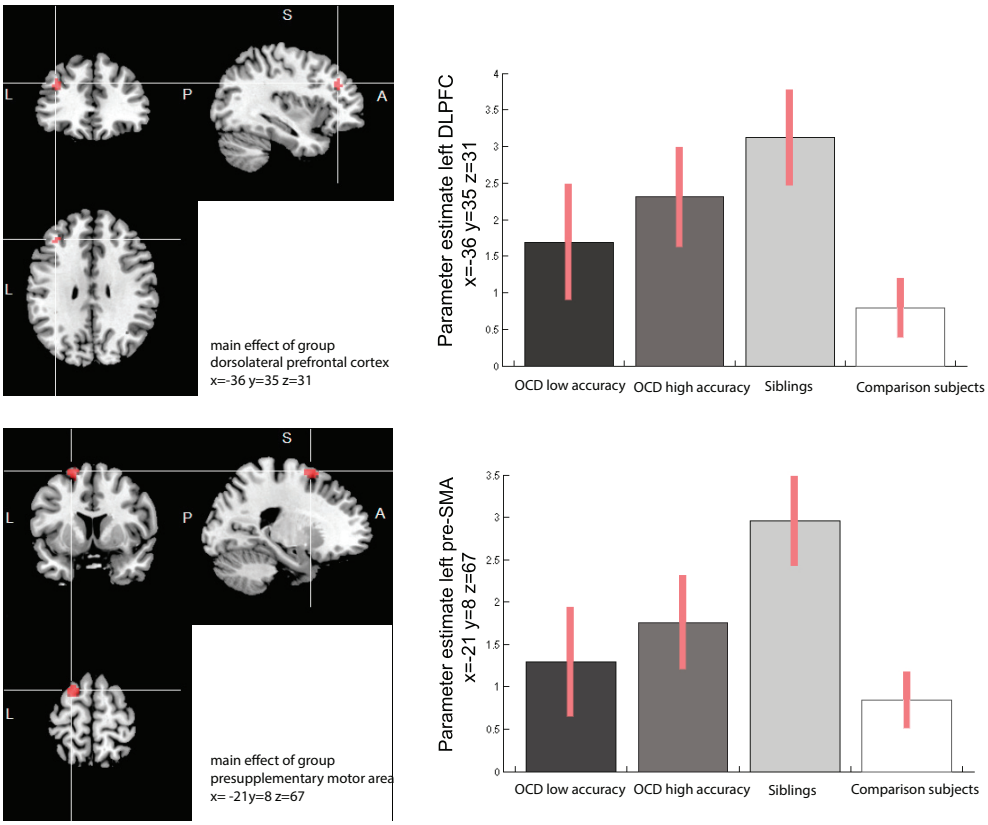


Figure 6.3: ANOVA on task-related activity (contrast N123>N0) with four groups. The OCD group was median split on task performance (accuracy) in a low performing group (OCD low accuracy) and high performing group (OCD high accuracy). Thresholded at $p<0.05$ with Family Wise Error correction for the search volume.

Table 6.4 Between-group differences from generalized psycho-physiological interaction (gPPI) analyses.

Seed region			MNI Coordinates			Main effect of group			OCD>comparison subjects			OCD>siblings		
		ROI	x	y	z	k_e	Z	p_{fwe}	k_e	Z	p_{fwe}	k_e	Z	p_{fwe}
Left premotor/pre-SMA	L	Amygdala	-30	-4	-14	1	2.56	0.047 [a]	4	3.20	0.007			ns
	R	Amygdala	30	-1	-14	10	3.38	0.006	26	3.98	0.001			ns
Left dlPFC	L	Amygdala						ns						
	R	Amygdala	30	-4	-14	2	2.87	0.027	1	2.63	0.043 [a]	17	3.29	0.007
Left precuneus	L	Amygdala						ns						
	R	Amygdala						ns						

Task-related changes in functional connectivity for three seed regions with the bilateral amygdala. Main effect of group (ANOVA) and post-hoc group comparisons. Siblings vs. comparison subjects and siblings or comparison subjects> OCD did not show significant results. Amygdala ROIs had a mean correlation of rho=0.75, resulting in a Bonferroni corrected alpha of $p<0.042$, [a] significant at trend-level, $p_{FWE}>0.042$, L=Left R=Right ROI=Region-of-interest. K_e= cluster extent p_{FWE}= p-value with family wise error correction, dlPFC= dorsolateral prefrontal cortex, pre-SMA= pre-supplementary motor area

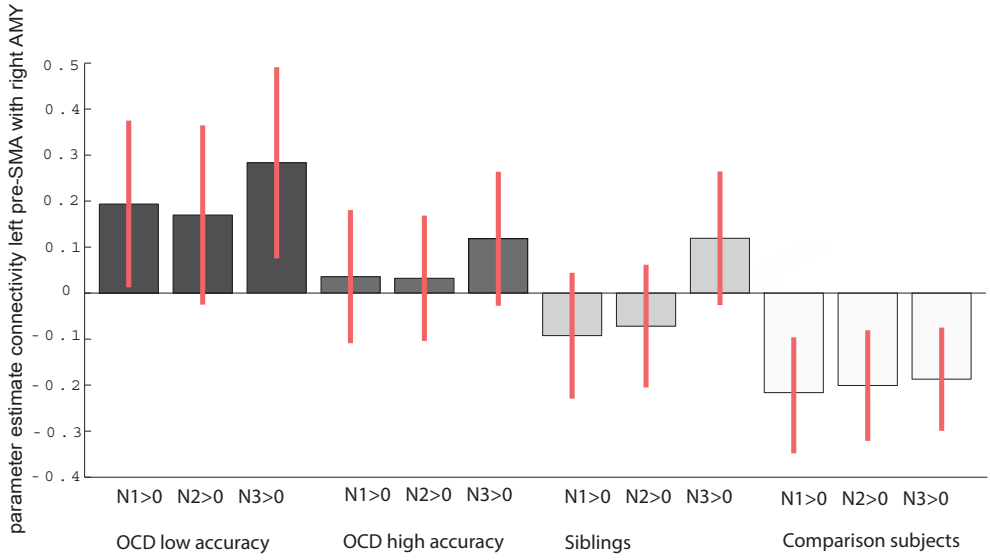


Figure 6.4:

Parameter estimates (in arbitrary units) for connectivity between the left pre-supplementary motor area/ premotor and the right amygdala (30 2 -14) for OCD patients with low and high accuracy, siblings and comparison subjects.

Discussion

We investigated the neural correlates of spatial working memory as a possible endophenotype for OCD. Both OCD patients and their unaffected siblings showed task-related hyperactivity in the fronto-parietal network compared to healthy participants. OCD patients showed increased activity in left dlPFC, left premotor/pre-SMA and left precuneus, whereas siblings showed more extensively increased bilateral fronto-parietal recruitment. At the most demanding level of the task, OCD patients showed decreased task performance and failed to further increase activation in the task-related network. Siblings performed adequately, also at higher task load.

Fronto-parietal dysfunction as an endophenotype for OCD

Altered functioning of the fronto-parietal network in OCD patients is consistent with structural (van den Heuvel et al., 2009;Menzies et al., 2008b;Pujol et al., 2004) and functional neuroimaging studies (Melloni et al., 2012). Working memory-related hyperactivation of the dlPFC (Nakao et al., 2009), parietal cortex (Henseler et al., 2008;Shin et al., 2006), premotor, inferior frontal cortex (Koch et al., 2012) and ACC (van der Wee et al., 2003;Shin et al., 2006) has been reported previously in OCD patients. Fronto-parietal hyperactivity during working memory in unaffected relatives of OCD patients is a novel finding. Previous studies on structural brain changes in

OCD patients and their unaffected relatives found altered fronto-parietal white matter fractional anisotropy in OCD patients and to a lesser extent in their siblings (Menzies et al., 2008b) and a relationship between fronto-parietal volume changes and impaired response inhibition in patients and siblings (Menzies et al., 2007). Functional neural correlates of impaired response inhibition in OCD patients and siblings, as was recently reported by our group, included decreased inferior parietal and inferior frontal brain activity in patients and increased activity in left premotor/pre-SMA in patients and bilaterally in siblings (de Wit et al., 2012). Finally, impaired frontal and parietal activation in patients and relatives was found during reversal learning (Chamberlain et al., 2008). Since our sibling group was similar to the comparison group on all clinical variables we may conclude that between-group differences reflected genetic susceptibility to OCD, without being confounded by subclinical OCD symptoms. Fronto-parietal hyperactivity in patients was not related to disease severity and remained significant after exclusion of patients with comorbid depression and anxiety, further indicating that it may constitute an endophenotype reflecting trait rather than state characteristics.

Compensatory neural recruitment

Consistent with the literature (Schneider-Garces et al., 2010;D'Esposito et al., 2000;Jansma et al., 2000), comparison subjects showed a task load-dependent increased activation of the working memory network, but activation in OCD patients and siblings reached a ceiling at N2. A similar pattern was described previously in the ACC of OCD patients during working memory (Koch et al., 2012) and has also been reported in schizophrenia (Callicott et al., 2000) and in unaffected relatives of depressed patients (Mannie et al., 2010). Additionally, studies in healthy aging showed that fronto-parietal hyperactivation is related to preservation of working memory performance at low task loads, and that at higher task loads the limits of neural resources are reached and performance falls short (Schneider-Garces et al., 2010;Reuter-Lorenz and Cappell, 2008). In our study, siblings showed most extensive general task-related hyperactivation coupled with intact performance, suggesting successful compensatory neural recruitment. Moreover, OCD patients with normal performance also showed task-related hyperactivation, in contrast to patients with a performance deficit. Since the latter group had lower estimated IQ and higher depressive and anxious symptoms, we may conjecture that these factors are inversely related to their ability for compensatory neural recruitment, consistent with a previous report that comorbid depression influences executive function in OCD (Moritz et al., 2003). Task-related neural recruitment may be influenced by the efficiency of top-down control over the limbic circuitry, which has previously been reported in depression (Johnstone et al., 2007) and which is supported by the gPPI findings showing that OCD patients, especially those with impaired task performance, have increased task-related connectivity between prefrontal regions and the amygdala. Increased connectivity with the amygdala may reflect increased uncertainty about their task-performance (Stern et

al., 2013). This effect could be state dependent, but as the sibling group did not show a negative coupling between prefrontal areas and the amygdala, as was present in the comparison group, it may also be interpreted as vulnerability trait for OCD. Increased functional connectivity between prefrontal areas and amygdala has also been reported in social anxiety disorder (Goldin et al., 2009).

Compensatory up-regulation of brain activity in OCD has been suggested by several authors across different task paradigms (van den Heuvel et al., 2005;Yucel et al., 2007;Henseler et al., 2008) and may constitute a non-specific mechanism to maintain adequate cognitive performance. Compensatory fronto-parietal up-regulation during planning was also reported in twins scoring low vs. high on subclinical OCD symptoms (den Braber et al., 2010). Although not directly comparable, it may be consistent with increased fronto-parietal recruitment in unaffected siblings (low OCD symptoms) vs. OCD patients (high OCD symptoms) observed in the present study. Some other studies found decreased activity during performance of cognitive tasks in OCD (Gu et al., 2008;van den Heuvel et al., 2005;Kocak et al., 2011), which may be explained by suboptimal IQ matching or depressive symptoms and comorbidity in the OCD group possibly hampering compensatory activity.

If up-regulated brain activation in OCD patients and their siblings indeed reflects a compensatory mechanism, the question arises what causes inefficiency in the fronto-parietal network. Although our data cannot directly answer this question, there are several explanations that are not mutually exclusive. There is some evidence for subtle structural or biochemical alterations in one or more brain regions necessary for working memory performance in OCD (Szeszko et al., 2005;Yucel et al., 2007). Additionally, functional connectivity between frontal and parietal regions (Salazar et al., 2012), or between the fronto-parietal network and the default mode network or limbic circuitry may be altered in OCD (Stern et al., 2012). Dysfunction of the fronto-parietal network seems to impair not only spatial working memory, but several other cognitive functions. In the same sample we also found hyperactivation of the pre-SMA related to impaired response inhibition (de Wit et al., 2012). One might suggest that OCD patients need to compensate for a failure to inhibit irrelevant stimuli which impairs working memory performance (Gazzaley et al., 2005). The latter suggestion is supported by data that show decreased inhibitory activity while suppressing a distracter coupled with compensatory medial frontal activation during retrieval in OCD patients performing a delayed matching to sample task (Ciesielski et al., 2012).

Clinical implications

Although fronto-parietal hyperactivity was not correlated with symptom severity, its presence in unaffected siblings suggests it may be related to resilience to disease development or progression. Future studies should investigate whether strengthened compensatory hyperactivation of the circuit adds to improved cognitive functioning

in OCD and even reduction in OC symptoms. Patients may benefit from training of selective attention or from neuro feedback using real-time fMRI to up-regulate fronto-parietal circuits. Recently a proof of concept study showed promising results for this approach in patients with major depressive disorder (Linden et al., 2012). Another possible clinical implication, although somewhat speculative, is the use of task-related brain activation in the predicting treatment success after CBT; up-regulated frontal brain activity was found to be a positive predictor for response to CBT in social anxiety disorder (Klumpp et al., 2013) and specific phobia (Paquette et al., 2003).

Strengths and limitations

The sample consisted of a well-matched large group of un-medicated OCD patients and comparison subjects. The sibling group tended to have more males, however adding gender as a covariate did not change the results. OCD patients with comorbidity were included to study a representative sample (Schuurmans et al., 2012). Depressive, but especially anxious symptoms influenced performance and decreased performance was related to imaging results, stressing the importance to take clinical characteristics into account when interpreting results (Moritz et al., 2003). Patients were included regardless of a family history of OCD, therefore it remains unresolved if fronto-parietal dysfunction is related to familial or non-familial OCD or both. No information was collected on what participants subjectively experienced during scanning (e.g., distress, uncertainty or compulsions) and how this may have influenced their task performance. Finally, differences between patients and comparison subjects were detected at group level and their use as a potential biomarker in individual patients awaits empirical confirmation, e.g. using machine learning methods (Hoexter et al., 2013;Weygandt et al., 2012).

Conclusions

This study provides evidence that increased recruitment of the fronto-parietal network constitutes an endophenotype for OCD. Our results suggest that fronto-parietal hyperactivation in patients and unaffected first-degree relatives is probably a compensatory mechanism related to inefficient processing within the fronto-parietal network. Additionally, increased task-related fronto-limbic connectivity in OCD patients compromises cognitive performance, which emphasizes the role of limbic interference in OCD.

Supplements for chapter 6

Generalized Psycho-Physiological Interaction Analyses

Seed regions of the left dorsolateral prefrontal cortex, left pre-supplementary motor area and left precuneus were 6 mm spheres, drawn around the same peak voxel as the corresponding region of interest (ROI). The physiological variable was created by extracting the mean deconvolved time course from the seed region. Psycho-physiological interaction (PPI) interaction terms were computed as the cross product of the physiological variable and each task regressor (N0, N1, N2, N3). This resulted in three 1st level models (one per seed region) with nine regressors: four task conditions, four PPI interaction terms and the time course of one seed region. Contrasts between the PPI interaction at each load level and baseline (e.g., [PPI N1>PPI N0], [PPI N2>PPI N0], [PPI N3>PPI N0]) were brought to 2nd level in a full factorial model with three groups and three load levels. Performance was added as covariate of no interest. The between-group comparisons were restricted to altered connectivity between the seed regions with the bilateral amygdala. Amygdala ROIs were based on Automatic Anatomic Labeling masks in the Wake-Forest University Pick atlas and were considered significant at $p<$ 0.042 (family-wise error corrected), after a similar Bonferroni correction for correlated outcome measures as was performed for the main effects of task analysis, based on a correlation between right and left amygdala parameter estimates of rho = 0.75.

Supplementary Table S6.1. Peak activity in regions of interest for main effect of task (N123>N0) for each separate group.

		OCD Patients (*n* = 43)					**Siblings (*n* = 17)**					**Comparison Subjects (*n* = 37)**				
Region-of-interest	side	Coordinates			Z	p_{FWE}	Coordinates			Z	p_{FWE}	Coordinates			Z	p_{FWE}
		x	y	z			x	y	z			x	y	z		
Dorsolateral prefrontal cortex	L	-39	32	28	6.15	<0.001	-39	26	31	5.67	<0.001	-39	26	31	4.81	0.014
	R	39	38	28	6.72	<0.001	39	38	25	5.42	0.001	39	38	28	7.46	<0.001
Premotor cortex/ pre-supplementary motor area	L	-15	-1	64	6.61	<0.001	-18	8	67	5.94	<0.001	-18	2	64	5.32	<0.001
	R	27	5	61	7.80	<0.001	12	14	55	5.70	<0.001	27	11	52	7.39	<0.001
												18	8	64	6.63	<0.001
Precuneus	L	-12	-76	49	6.75	<0.001	-33	-61	49	4.91	0.023	-6	-67	49	5.77	<0.001
	R	12	-70	55	7.27	<0.001	18	-73	58	5.16	0.005	15	-73	52	7.25	<0.001
							30	-67	52	6.24	<0.001					
Inferior parietal cortex	L	-39	-52	46	7.05	<0.001	-39	-58	55	4.92	0.022	-34	-55	37	5.84	<0.001
		-42	-49	43	7.03	<0.001	-42	-58	43	4.74	0.043					
	R	42	-58	46	7.48	<0.001	42	-52	49	5.95	<0.001	45	-49	46	7.57	<0.001
Dorsal anterior cingulum	L/ R	-3	20	46	5.57	<0.001	-5	26	43	5.58	<0.001	3	20	52	7.20	<0.001

Brodmann's area; p_{FWE}, whole brain family-wise error corrected; L, left; OCD, obsessive-compulsive disorder; R, right; Z, Z-score. Coordinates in Montreal Neurological Institute space.

Supplementary Table S6.2 Demographic and clinical characteristics of the OCD subgroups, after median-split on task performance accuracy.

	OCD high accuracy (*n* = 22)		**OCD low accuracy (*n* = 21)**		**Statistical Analysis**	
	Mean	SD	Mean	SD	*t* (*df* = 41)	*p*-value
Demographic measures						
Age (years)	37.9	9.6	38.2	10.0	0.11	0.91
Gender (female:male)	12:10		9:12		0.59[a]	0.44
Estimated verbal IQ (points)	101.9	12.1	92.0	10.6	-2.86	0.007
Clinical measures						
Y-BOCS (points)	20.7	5.3	22.0	7.0	0.70	0.48
MADRS (points)	8.3	7.3	14.2	7.7	2.59	0.013
BAI (points)	11.7	8.4	19.2	11.5	2.46	0.018
Current comorbidity (*n*)	9	40.9%	14	66.7%	2.9[a]	0.091
Comorbid mood disorder (*n*)	3	13.6%	7	33.3%	2.3[a]	0.13
Comorbid anxiety disorder (*n*)	8	36.4%	6	28.6%	0.3[a]	0.59
Comorbid other disorder (*n*)	1	4.5%	6	28.6%	4.6[a]	0.041
Accuracy (%)						
Accuracy at N1	97.3	3.1	84.8	17.2	384.5[b]	<0.001
Accuracy at N2	93.3	7.6	58.2	22.7	441.0[b]	<0.001
Accuracy at N3	77.8	14.8	42.5	15.6	-7.6	<0.001
Mean accuracy N1, N2, N3	89.5	5.7	61.8	14.2	-8.2 (*df* = 26)	<0.001

[a] chi square, [b] Mann-Whitney U. SD, standard deviation. Y-BOCS= Yale-Brown Obsessive-Compulsive Scale. MADRS= Montgomery Asberg Depression Rating Scale

Supplementary Table S6.3. Post-hoc 2 sample *t* tests between OCD high task performance accuracy (OCD high, *n* = 22), OCD low task performance accuracy (OCD low, *n* = 21), siblings (Sib, *n* = 17), comparison subjects (HC, *n* = 37). Comparisons between siblings and comparison subjects were not repeated.

Region of Interest	BA	Side	Interaction	Coordinates			k_e	Z	p_{FWE}	p_{uncorr}
				x	y	z				
Dorsolateral prefrontal cortex	46	L	Sib>OCD low	-36	41	28	2	2.82	0.058	0.002
			OCD high>OCD low	-36	38	25	2	2.81	0.053	0.002
			OCD high>HC	-33	32	28	20	3.42	0.010	
		R	No interactions							
Premotor cortex/pre-supplementary motor area	6	L	Sib>OCD low	-18	8	64	45	3.82	0.003	
			Sib>OCD high	-24	8	67	1	2.66	0.082	0.004
			OCD high>HC	-30	2	61	2	3.06	0.028	
		R	No interactions							
Precuneus	7	L	Sib>OCD low	-12	-70	58	4	3.16	0.024	
		R	OCD low>HC	15	-79	55	4	3.24	0.017	
Inferior parietal cortex	40	L	Sib>OCD low	-42	-46	46	4	3.03	0.034	
		R	No interactions							
Dorsal anterior cingulum	32/8		Sib>OCD low	0	11	52	65	3.67	0.005	
				-3	23	46		3.32	0.015	

BA, Brodmann's area; L, left; R, right; K_e, cluster size; p_{FWE}, *p*-value with family-wise error correction for the search volume; p_{uncorr}, *p*-value not corrected for the search volume

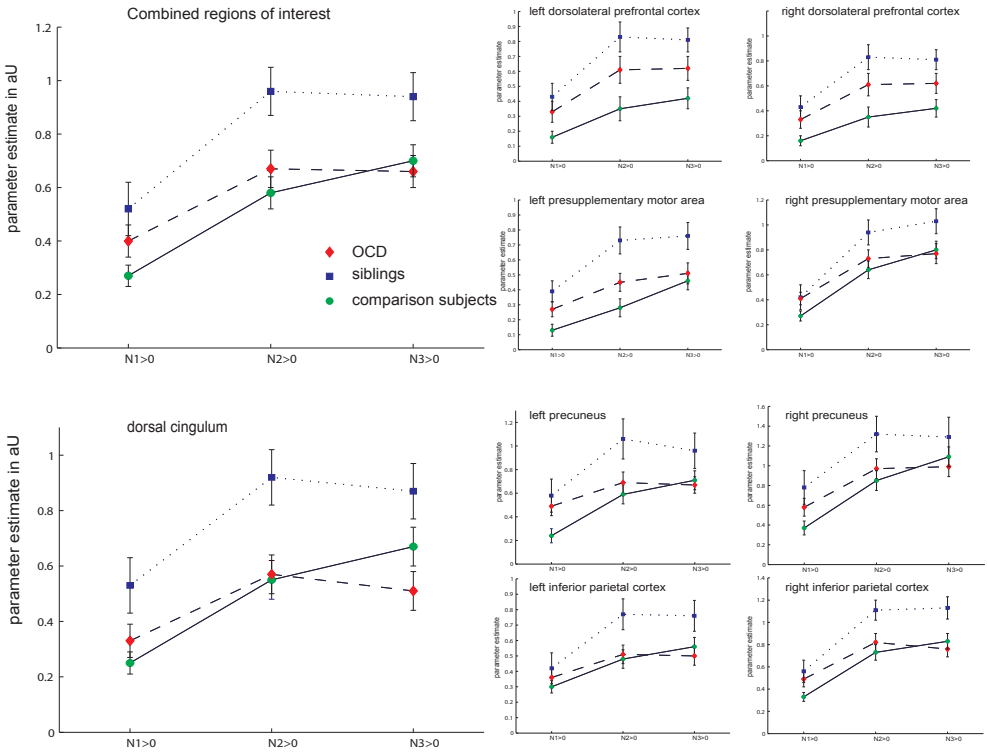


Figure S6.1. Parameter estimates in arbitrary Units (AU) for each task load-level for the individual regions-of-interest.